The value of vitamins after a colorectal cancer diagnosis

THE VALUE OF VITAMINS AFTER A COLORECTAL CANCER DIAGNOSIS

Associations of vitamin D, B-vitamins, and supplement use with fatigue and quality of life

The studies described within this thesis are part of the '*En*ergy for life after *C*olo*Re*ctal cancer (EnCoRe) study'.

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CHAPTER 1

General Introduction

Colorectal cancer patients commonly encounter health problems, such as fatigue and problems in physical functioning, during the years following completion of therapy.[1-3] Improvements in lifestyle behaviour, including adaptations in nutritional or exercise habits, may contribute to a reduction of these problems and are therefore considered important potential approaches to enhance the quality of life of colorectal cancer survivors.[4-6] The research conducted in this thesis is focused on the use of dietary supplements and on blood biomarkers of vitamin D and B-vitamin status in relation to fatigue, quality of life, and functioning among colorectal cancer survivors. It is specifically relevant to study the use of supplements among colorectal cancer survivors given the relatively large proportion of this population using supplements, ranging between 7% and 66% in previous studies,[7-10] even though use is discouraged in cancer-specific guidelines.[11-13] Moreover, from the colorectal cancer survivor's perspective, there is a need for more guidance and information provision on nutrition and supplement use in relation to symptoms such as fatigue during the post-treatment period.[14-16] Up to now, studies investigating associations of supplement use and vitamin status with health outcomes in colorectal cancer survivors are limited, and consequences for quality of life remain unclear.[17,18]

Colorectal cancer survivorship

A growing population

Colorectal cancer has evolved as the 3rd most commonly diagnosed cancer worldwide, predominantly as a result of population ageing and a shift in lifestyle behaviour, which occurred parallel to economic development and led to more obesity, higher consumption of meat, processed foods and alcohol, and an increasingly sedentary lifestyle.[19,20] In the coming decade, the global incidence of colorectal cancer is expected to rise further; from 1.8 million newly diagnosed cases in 2018 to an expected 2.2 million new cases in 2030.[21,22] In the Netherlands, almost 13,000 new patients were diagnosed with colorectal cancer in 2019.[23]

Advancements in anti-cancer therapy have led to an upward trend in survival after colorectal cancer.[24] Moreover, screening programmes result in earlier detection, consequently also improving chances for survival.[24] The average 5-year survival rate after colorectal cancer is currently 65% globally and 66% in the Netherlands, whereas this was still 54% in the period 1991-2000 in the Netherlands.[22,23,25] The survival rate is highly dependent on disease stage and currently 88% for stage I, 68% for stage II, 44% for stage III, and 3% for stage IV patients in the Netherlands.[23] In light of the increasing incidence and the improved survival rate, the total population of colorectal cancer survivors is expected to continue to grow in the forthcoming years.[19]

Defining a 'cancer survivor'

A plethora of definitions of a 'cancer survivor' and 'cancer survivorship' can be found.[26] Clinicians, researchers, and patients approach the concept from a different perspective and thus far, no generic definition has been established.[26-28] It is important to clearly delineate how the concept of cancer survivorship was defined in this thesis, in order to start from a uniform understanding of the concept and to make sure that the results of the research presented in this thesis are, eventually, translated to a clearly defined target population. In this thesis, an individual is considered a cancer survivor from the time of cancer diagnosis throughout his or her life, as adapted from the National Coalition for Cancer Survivorship.[29] In other words, any individual living with a current or past cancer diagnosis is considered a cancer survivor. This is the most commonly accepted definition of a cancer survivor in the literature.[12,30,31]

Health problems after colorectal cancer

The consequences of a colorectal cancer diagnosis and its treatment may be experienced throughout the rest of a cancer survivor's life.[32,33] A diverse array of health problems can be observed in the domains of physical, psychological, and/or social health and functioning.[1,34] Physical problems primarily occur due to major abdominal surgery or as adverse effects of chemo- and/or radiotherapy. Bowel complaints, urogenital dysfunctions, and insomnia are commonly experienced problems that have been reported up to 10 years after treatment for both colon and rectum cancer patients.[35-37] Furthermore, peripheral neuropathy can occur as a result of tumour compression or infiltration into neural structures[38] or, more often, as adverse effect of oxaliplatin-based chemotherapy, which is part of standard adjuvant therapy options for colorectal cancer stages II-III, and frequently causes pain and numbness in feet/hands.[39,40] Fatigue is another commonly reported symptom experienced by colorectal cancer patients that often persists after completion of therapy.[41] It is characterized by a lack of energy, tiredness, and general weakness, not primarily caused by physical or mental effort and profoundly impacting physical functioning and daily living.[37,41,42] Next to its physical manifestations, fatigue is also recognized as a psychological problem.[43] Other psychological issues after colorectal cancer include fear of recurrence and loss of control.[1,44] A significant proportion of colorectal cancer survivors encounter anxiety and depressive symptoms throughout the first 5 years following diagnosis.[45,46] Lastly, on a social level, colorectal cancer survivors may experience poorer social functioning as a result of reduced participation in social activities due to physical and/or psychological problems.[1] Restrictions in social participation are often reinforced by the presence of an ostomy.[47] All in all, the health problems that many colorectal cancer survivors face can lead to a reduction of overall quality of life up to many years after the end of treatment.[37]

Supportive care needs of colorectal cancer survivors

Colorectal cancer survivors have special needs with regard to supportive care in managing the physical and psychological problems they experience as a consequence of the cancer.[48] The identification of specific supportive care needs, as well as unmet needs, may help to recognize potential gaps in survivorship research and/or care, thereby providing important research directions for the eventual development of evidence-based follow-up care programs to improve the quality of life of this population. A systematic review from 2017 reported the most prominent individual needs of colorectal cancer survivors from a total of 54 articles.[49] The provision of information/education emerged as a major domain in which colorectal cancer survivors expressed needs. More information about diet and nutrition was ranked second in the top 20 of most prominent needs. Additionally, help with the long-term self-management of fatigue was an important aspect in the domains of information/education and physical/ cognitive needs. These results emphasize the importance of research that is conducted in the areas of nutrition, quality of life and fatigue, the focus points of this thesis.

Health-related quality of life

It is important to define a number of key terms related to the main outcomes of the analyses performed in this thesis. The concept of health-related quality of life is frequently used in relation to cancer survivorship yet its definition may vary in relation to specific diseases or populations. This paragraph provides some background on the terms health status, quality of life, and health-related quality of life, and their interpretation in this thesis.

The terms health status, quality of life, and health-related quality of life are multidimensional, largely interrelated, often used interchangeably, and therefore hard to distinguish. *Health* has been defined by the World Health Organisation (WHO, 1948) as "a state of complete physical, mental and social well-being, and not merely the absence of disease and infirmity".[50] Towards the end of the previous century, the WHO defined *quality of life* as "an individuals' perception of their position in life in the context of the culture and value systems in which they live and in relation to their goals, expectations, standards and concerns".[51] Quality of life is generally recognized as a comprehensive and subjective concept, whereas health covers only part of the construct of quality of life and is often approached from a clinical or functional perspective.[52] *Health-related quality of life* shows much overlap with the concepts health status and quality of life and is commonly defined as the health aspects of quality of life.[52]

The utilization of an existing framework can help in obtaining a comprehensive picture of the concept of health-related quality of life and its measurement. The

International Classification of Functioning, Disability and Health (ICF), developed by the WHO in 2001, is such a framework.[53] The ICF gives a classification of the functioning of an individual by defining a number of domains of functioning; body functions and structures, activities, and participation, in combination with contextual factors, including environmental and personal factors. While taking these factors into account, health-related quality of life can be defined as "the subjective perception of an individual's level of functioning in different domains within the context of the individual's health state and specific environmental and personal barriers and facilitators."[54] Health-related quality of life therefore not merely encompasses the absence of disease nor is it aimed at increasing longevity. It may involve a way of living *with* possible functioning problems (disability), yet being able to adapt to these disabilities and living a life of contentment.

Fatigue

Fatigue is included as a major outcome in this thesis as it is one of the most distressing and impactful symptoms experienced by colorectal cancer survivors and therefore strongly related to health-related quality of life.[41,55] Cancer-related fatigue can be defined as "a distressing, persistent, subjective sense of physical, emotional, and/or cognitive tiredness or exhaustion related to cancer or cancer treatment that is not proportional to recent activity and interferes with usual function", according to the National Comprehensive Cancer Network.[56] It is a complex symptom because of its multidimensional nature and its common occurrence in combination with other symptoms, such as emotional distress and decreased concentration or attention.[41] A single cause for fatigue has not been established, but inflammation seems to play a role in its development.[55] In practice, fatigue is not always recognized and acknowledged.[41,56] In addition to fatigue, depression and anxiety are part of the investigated outcomes in this thesis.

In this thesis, both generic as well as cancer-specific validated questionnaires are used to measure different aspects of health-related quality of life. The 30-item core questionnaire of the European Organization for Research and Treatment of Cancer (EORTC QLQ-C30) targets cancer patients and survivors and measures multiple aspects of quality of life, including global quality of life, fatigue, and five functioning domains, i.e. physical, role, cognitive, social, and emotional functioning.[57] The Checklist Individual Strength has originally been developed to assess subjective fatigue in patients with chronic fatigue syndrome and consists of an overall score and four subscales; subjective fatigue, concentration, motivation, and physical activity.[58] Lastly, the Hospital Anxiety and Depression Scale measures depression and anxiety, with the exclusion of physical complaints and symptoms of serious mental disorders, and includes a total score and separate scores for depression and anxiety.[59]

Nutritional factors and colorectal cancer survivorship

There is limited evidence on the role of nutritional factors, including dietary intake and supplement use, in colorectal cancer survivorship.[60] In contrast, a number of dietary factors seem to be important in the aetiology of colorectal cancer, such as the consumption of red and processed meat and alcohol.[13] Part of the rationale to investigate associations between nutritional factors and health outcomes in colorectal cancer survivors is the observed low adherence to dietary guidelines in this population, potentially because the unhealthy dietary habits that may have contributed to the onset of colorectal cancer persist after diagnosis.[61] Nonetheless, studies have also suggested that colorectal cancer survivors may be increasingly motivated to initiate changes in their lifestyle, since the major life event of a cancer diagnosis makes many survivors rethink and reflect on lifestyle habits.[62,63] However, it still remains an enormous challenge for cancer survivors to make sustainable and long-lasting changes in health behaviour.[64-66]

The small number of studies that have investigated nutritional factors in relation to colorectal cancer survivorship have mostly focused on cancer recurrence and survival, as reviewed previously.[6,67,68] Studies investigating quality of life as an outcome measure are underrepresented and usually focus on the same factors that have been associated with cancer risk, i.e. dietary habits associated with a 'Western style' diet.[69-71] Associations between nutritional factors and quality of life among colorectal cancer survivors have often been investigated by evaluating the adherence to a lifestyle guideline or a self-constructed lifestyle score, often not limited to nutritional factors alone.[61,72-75] The primary dietary habits that are suggested to be positively associated with quality of life in colorectal cancer survivors are the consumption of fruits, vegetables, and wholegrains, and the reduced consumption of energy-dense foods, red and processed meat, and alcohol.[61,69,71-75] Lifestyle interventions were found to be feasible and acceptable for short-term improvement of dietary habits in colorectal cancer survivors,[76] yet their impact on quality of life remains to be studied.[6,77] In addition, the effects of using single or combined vitamin supplements on quality of life of cancer survivors are still unclear.[18]

Current recommendations on diet and supplement use for cancer survivors

Several international organizations present lifestyle guidelines for cancer survivors, including guidelines on key dietary elements and the use of supplements. Guidelines are largely in line with each other, but primarily founded by evidence for the prevention of cancer because of the paucity of evidence from well-designed longitudinal and intervention studies in colorectal cancer survivors. Table 1 presents a summary of the current available guidelines on dietary intake (**D**) and supplement use (**S**) that are of interest to cancer survivors, including the Dutch national guideline on vitamin D supplementation.

Table 1. Currently available recommendations on diet and supplement use, of interest to colorectal cancer survivors.

	World Cancer Research Fund/American Institute for Cancer Research, 2018[13,78]
D	Cancer survivors are encouraged to follow the general advice for cancer prevention: • Eat a diet rich in wholegrains, vegetables, fruit and beans; • Limit consumption of 'fast foods' and other processed foods high in fat, starches or sugars; • Limit consumption of red and processed meat; • Limit consumption of sugar sweetened drinks; • Limit alcohol consumption.
S	• Do not use supplements for cancer prevention. Aim to meet nutritional needs through diet alone.
	American Cancer Society, 2012[11,79]
D	Follow the American Cancer Society Guidelines on Nutrition and Physical Activity for Cancer Prevention: • Limit how much processed meat and red meat you eat; • Eat at least 2½ cups of vegetables and fruits each day; • Choose wholegrains instead of refined grain products; • People who drink alcohol should limit their intake.
S	• Before supplements are prescribed or taken, all attempts should be made to obtain needed nutrients through dietary sources. • Supplements should be considered only if a nutrient deficiency is either biochemically or clinically demonstrated. • Supplements should be considered if nutrient intakes fall persistently below two-thirds of the recommended intake levels.
	National Comprehensive Cancer Network, 2020[12]
D	All survivors should be encouraged to: • Make informed choices about food to ensure variety and adequate nutrient intake; • Limit red meat intake to <18 ounces (510 grams) per week and avoid processed meat; • Limit refined sugars and processed food; • Eat a diet that is at least 50% plant-based; • Minimize alcohol intake.
S	• Supplement use is not recommended for most survivors, except in instances of documented deficiencies, inadequate diet, or comorbid indications. • Taking vitamin supplements does not replace the need for adhering to a healthy diet. All efforts should be made to obtain nutrients from dietary intake.
	Health Council of the Netherlands, 2008[80]
S	• Women aged over 50 are recommended an additional 10 micrograms of vitamin D daily. Women and men aged over 70 are recommended an additional 20 micrograms of vitamin D daily.[a]

D: recommendations on the diet. **S**: recommendations on supplement use.

[a] Vitamin D supplementation recommendations are based on research into the effects of vitamin D on bone quality, the risk of fracture, and the risk of falling in the elderly.

Supplement use among colorectal cancer survivors

The current recommendations, as presented in Table 1, discourage the use of supplements for cancer survivors. There is a lack of evidence supporting the advantages of supplement use, as well as uncertainty regarding the potential detrimental effects of supplement use, as they may interact with anti-cancer therapy and easily lead to intakes exceeding the tolerable upper intake level.[81,82] Nevertheless, between 7% and 66% of colorectal cancer survivors use supplements, often with the belief that it cannot cause any harm.[7-10] The prevalence of supplement use among cancer survivors was found to be comparable or higher than use in the general population.[8,10,17] The latest version of the Dutch national dietary survey (VCP 2012-2016) reported that 43% of the Dutch general population uses supplements.[83] Cancer survivors using supplements show similar characteristics as supplement users in the general population, i.e. female sex, older age, and higher educational level.[7,18,84] A prior study among colorectal cancer survivors reported that supplement use widely varied within individuals during the first 2 years after diagnosis.[8] Other studies reported that about half of colorectal cancer survivors start the use of a new supplement after a cancer diagnosis.[7,63] The most commonly used supplements among colorectal cancer survivors are multivitamins/multiminerals, vitamin D and/or calcium, vitamin C, and B-vitamin complexes.[7,8,17,85,86] Notably, between one third and two thirds of cancer survivors do not discuss the use of supplements with their health care provider.[10]

Vitamin D and B-vitamins in relation to quality of life

The use of supplements may be beneficial in acquiring adequate vitamin levels.[87] Colorectal cancer survivors, comprising a predominantly elderly population, may have difficulties in obtaining adequate vitamin levels due to a lower dietary intake, diminished absorption and utilization, and through the presence of comorbid conditions.[17,88] Supplement use might additionally alleviate potential symptoms that are caused by vitamin deficiencies.[87] This thesis specifically focuses on vitamin D and B-vitamins given their interest in relation to colorectal cancer and their involvement in pathways related to aspects of quality of life. When proven effective, supplement use can be a straightforward and uncomplicated option when it comes to lifestyle advice for colorectal cancer survivors because it requires relatively little effort in comparison to alternative strategies such as changing dietary intake.

Vitamin D deficiencies are common among the general population (around 40% in Europe)[89] and have been associated with increased risk for colorectal cancer.[90-93] Cancer survivors are susceptible for deficiencies because anti-cancer therapy may lead to a reduction in circulating vitamin D.[94-96] Vitamin D supplementation is generally regarded as safe and vitamin D is the only vitamin that is recommended for supplementation in populations aged >50 years in the Netherlands for the prevention of osteoporosis.[80] Vitamin D is involved in many

bodily processes as many cells and tissues have vitamin D receptors.[95] Vitamin D has previously been related to fatigue in various conditions,[97] cognitive functioning in older adults,[98,99] and depression in adults.[100,101] The vitamin may thus be able to contribute to a reduction of the health problems experienced by colorectal cancer survivors and has the potential to enhance quality of life.

B-vitamins are a second group of vitamins that are of interest in relation to colorectal cancer survivorship and quality of life. Folate, one of the B-vitamins, is known for its dual role in relation to colorectal cancer etiology because of its potential protective role in the onset of colorectal cancer and its promotion of the progression of existing colorectal neoplasms.[102] Folate and the vitamins B2, B6, and B12 are involved in DNA synthesis through folate-mediated one-carbon metabolism.[103] Through these and other pathways, B-vitamins and related biomarkers have been linked to several aspects of quality of life, including chronic fatigue syndrome,[104] cognition,[105] physical functioning,[106] and depression[107] in various populations. From this perspective, B-vitamins may be potentially beneficial, next to vitamin D, for the reduction of health problems among colorectal cancer survivors.

Measuring dietary vitamin intake

A large challenge of studies that aim to analyze associations between diet and a particular health outcome is to obtain a valid and reliable measurement of dietary intake, including the intake of vitamins. Measuring dietary intake is particularly challenging given the many possibilities for information bias, such as systematic over- or underreporting and the impact of socially desirable responses concerning foods that are believed to be healthy or unhealthy.[108-110] The resulting measurement error usually attenuates the estimate between the exposure and the outcome.[110] To reduce the risk of bias, it is essential to evaluate the validity of each dietary assessment method in the specific target population. The lack of a gold standard in dietary assessment requires validation studies to evaluate the relative validity in comparison to a second assessment method, preferably with independent measurement errors.[111] In this thesis, the validity of a food frequency questionnaire is evaluated against a 7-day dietary record in a population of colorectal cancer survivors.

Rationale and aims of this thesis

As delineated in this introduction, many colorectal cancer survivors are exposed to treatment-related health problems and express the need for support in dealing with those problems. Research is often aimed at strategies to increase the capability of survivors to manage their symptoms themselves, such as the initiation of lifestyle changes, so that individuals are empowered to regain a sense of control over their own health. Up to date, there is insufficient evidence to develop evidence-based nutritional advice that can contribute to a reduction of health problems among colorectal cancer survivors. Colorectal cancer survivors frequently use dietary supplements without considering the potential harmful consequences and assuming that it will positively affect their health, while no studies have corroborated the benefits of supplement use for cancer survivors. The research in this thesis will contribute to the building of evidence for developing new and improving existing treatment strategies aimed at improving quality of life by investigating the use of supplements and biomarkers of vitamin status in relation to aspects of quality of life. Results will ultimately, together with evidence from other studies, contribute to the development of clear-cut recommendations and the delivery of tailored support for colorectal cancer survivors. Besides, it will provide insight in the measurement of dietary intake, including vitamin intake, in cancer survivors by evaluating the agreement between a food frequency questionnaire and a 7-day dietary record.

The main aims of the research presented in this thesis are the following:

I. To examine the association of supplement use in general, and the use of supplements containing vitamin D and B-vitamins, with quality of life and fatigue in colorectal cancer survivors (chapters 2, 3, and 4).
II. To investigate whether blood biomarkers of vitamin D and B-vitamins are associated with quality of life and fatigue in colorectal cancer survivors (chapters 3 and 4).
III. To evaluate the validity of a food frequency questionnaire in comparison to a 7-day dietary record to measure dietary intake in colorectal cancer survivors (chapter 5).

Study designs

The findings presented in this thesis are based on observational data deriving from a prospective study in colorectal cancer survivors at Maastricht University Medical Center+, the EnCoRe study, and from an international consortium of colorectal cancer survivorship studies, the FOCUS consortium, in which the EnCoRe study participates.

EnCoRe study The EnCoRe study (Energy for Life after ColoRectal Cancer) is an ongoing prospective cohort study that was initiated in 2012, aiming to evaluate longitudinal associations between lifestyle factors and health-related quality of life, functioning, and prognostic outcomes in colorectal cancer survivors.[54] Stage I-III colorectal cancer patients are recruited upon diagnosis in three Dutch hospitals in the southeast of the Netherlands; Maastricht University Medical Center+, VieCuri Medical Center, and Zuyderland Medical Center. Data are collected by research dietitians during home visits. A first visit took place prior to start of treatment and four follow-up visits were planned at 6 weeks, 6 months, 1 year, and 2 years after treatment end date. Each measurement included, amongst others, the collection of blood samples, the performance of a number of anthropometric measurements, and the collection of questionnaires to assess quality of life and fatigue. In addition, a food frequency questionnaire was used at diagnosis to assess dietary intake in the preceding year, whereas 7-day dietary records were collected at the 4 post-treatment follow-up measurements.

FOCUS consortium The FOCUS consortium (Biomarkers related to Folate-dependent One-carbon metabolism in colorectal Cancer recUrrence and Survival) started in 2013 and consists of four cohort studies; the COLON study from Wageningen University & Research (the Netherlands), three sites of the ColoCare study from the University of Heidelberg (Germany), the Huntsman Cancer Institute (Salt Lake City, Utah, USA), and the Fred Hutchinson Cancer Research Center (Seattle, Washington, USA), the CORSA study from the Medical University of Vienna (Austria), and the EnCoRe study.[112] All studies recruit colorectal cancer patients >18 years of age upon diagnosis. The consortium is able to comprehensively study the role of folate in relation to a state-of-the art set of one-carbon metabolism biomarkers, and their joint influence on clinical outcomes, including recurrence, survival, treatment toxicity, and quality of life. The main objective of the consortium is to study associations of circulating folate and related biomarkers with prognosis in colorectal cancer survivors. Demographic, clinical, and lifestyle characteristics, including supplement use, and patient-reported outcomes are collected at baseline and approximately 6 months after baseline. Data are harmonized across study sites. Blood-based biomarkers are analyzed at one expert laboratory in Bergen, Norway.

Outline of chapters

A schematic overview of the chapters of this thesis can be found in Figure 1. The current chapter, **Chapter 1**, has given a general introduction to the research conducted in this thesis. It has introduced important concepts and terminology and provides the rationale for this thesis. In **Chapter 2,** longitudinal associations are assessed for overall supplement use and fatigue, from 6 weeks to 2 years post-treatment. Associations between- and within individuals over time are disentangled, giving important insights into the nature of the associations.

Chapter 3 is focused on vitamin D. The chapter investigates the longitudinal association between vitamin D and global quality of life, fatigue, cognition, depression, and anxiety. The analyses are performed for 25-hydroxyvitamin D_3, a marker of vitamin D status, as well as for vitamin D intake through supplements and the diet. In **Chapter 4,** the role of B-vitamins in relation to aspects of quality of life is evaluated using data from the FOCUS consortium. Analyses are performed for circulating concentrations of nine biomarkers and for B-vitamin supplement use. **Chapter 5** reports on the validation of the food frequency questionnaire that is being used in the EnCoRe study, in comparison to a 7-day dietary record. Its relative validity is assessed by comparing the intake of a selection of nutrients and food groups, and by comparing scoring of adherence to the World Cancer Research Fund/American Institute for Cancer Research recommendations between the two dietary assessment methods. The final chapter, **Chapter 6**, summarizes and discusses the findings from the preceding chapters. It elaborates on possible methodological issues, provides implications for practice, and gives a direction for future studies in this field.

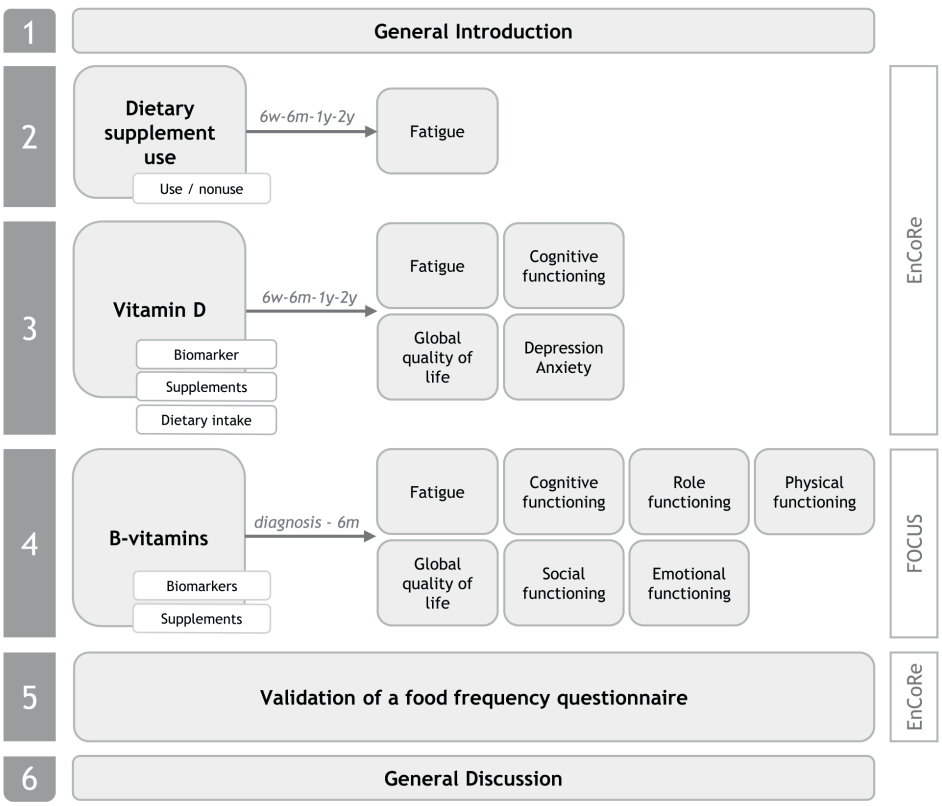

Figure 1. Schematic overview of chapters of this thesis.

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CHAPTER 2

Is dietary supplement use longitudinally associated with fatigue in stage I-III colorectal cancer survivors?

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Abstract

Supplement use among colorectal cancer (CRC) survivors is common, yet evidence supporting its beneficial health effects is mostly lacking and cancer-specific lifestyle guidelines advise against the use of supplements. We aimed to describe the use of supplements by CRC survivors from diagnosis to 2 years post-treatment and investigate how overall supplement use is longitudinally associated with fatigue.

In a prospective cohort study of stage I-III CRC survivors (n=325), information on supplement use was collected during repeated home visits at diagnosis and at 6 weeks, 6, 12, and 24 months post-treatment. Fatigue was assessed using the Checklist Individual Strength (score range 20-140) at all post-treatment time points. Linear mixed-models were applied to analyze longitudinal associations of overall supplement use with fatigue, adjusted for sex, age, comorbidities, chemotherapy, and physical activity.

At all time points, about 40% of participants used supplements. Multivitamins/multiminerals were the most frequently used supplements at all time points. Of participants with at least two available measurements, 28% were consistent users, 45% consistent nonusers, and 27% inconsistent users (i.e. reported both use and nonuse). Reported fatigue levels declined significantly after treatment. Overall, no statistically significant differences in fatigue score over time were observed between supplement users and nonusers. Likewise, no intra-individual associations of supplement use and fatigue were found. However, in inter-individual analyses, supplement users reported to experience more fatigue compared to nonusers (β 7.0, 95% CI 0.3;13.7).

No overall association between supplement use and fatigue was found. Results of the current study do therefore not imply that supplement use alleviates complaints of fatigue among CRC survivors. However, increased levels of fatigue may be a reason for supplement use among CRC survivors.

Introduction

Dietary supplement use has increased substantially over the past decades.[1,2] Previous studies among colorectal cancer (CRC) survivors observed supplement use estimates ranging from 7% to 66% across different countries.[3-6] Although cancer survivors live beyond cancer, the aftermath of the tumor and its treatment can have an enduring negative impact on their health and wellbeing.[7] Fatigue is commonly experienced by cancer survivors; previous research reported complaints of fatigue by up to 67% of survivors.[8-10] Its combined physical and emotional burden, and the persistence over multiple years, make fatigue one of the most severe long-term symptoms reported by cancer survivors.[10] Given the relatively high survival rate after cancer as a result of early diagnosis and improved treatment options, the number of cancer survivors living with fatigue is expected to rise.[7,11]

The diagnosis of cancer may lead to the implementation of important individual lifestyle changes.[12] These can involve the use of supplements, which may therefore be perceived as a self-managing or coping strategy to deal with chronic disease and other setbacks in life.[13,14] It remains largely unknown, however, to what extent supplement use can be beneficial after CRC. Cancer-specific guidelines of the National Comprehensive Cancer Network and the World Cancer Research Fund/American Institute for Cancer Research state that cancer survivors should aim to ensure adequate nutrient intake by the diet alone and should use supplements only in case of specific deficiencies.[15,16] Moreover, supplement use is controversial because of the largely unknown interrelation with chemo- and radiotherapy.[17] In addition, studies investigating the use of supplements after cancer therapy are scarce and often do not reach clear-cut conclusions. A longitudinal study among 160 Dutch CRC survivors found substantial variability over time in supplement use after diagnosis.[6]

Within the current study, we firstly aimed to describe the use of supplements by CRC survivors from the moment of diagnosis up to 2 years after the end of cancer treatment. Secondly, we aimed to study how overall supplement use is longitudinally associated with fatigue in CRC survivors.

Methods

Study design and population

Data from an ongoing prospective cohort study of CRC survivors, the 'Energy for Life after ColoRectal Cancer' (EnCoRe) study, were used (Netherlands Trial Register number NTR7099).[18] From April 2012 onwards, CRC patients were recruited at diagnosis in three Dutch hospitals. Patients with an established diagnosis of stage I, II, or III CRC, including recurrent CRC, were eligible for inclusion when above 18 years of age. Stage IV patients were excluded, as well

as patients with a current home address outside of the Netherlands, patients unable to understand the Dutch language in speech as well as in writing, and patients with comorbidities obstructing successful participation (e.g. Alzheimer's disease). The study was approved by the Medical Ethics Committee of the University Hospital Maastricht and Maastricht University, the Netherlands, and all participants signed informed consent.

Newly diagnosed CRC patients eligible to participate were informed about the study. A first home visit was planned prior to the start of treatment whereas four additional follow-up visits took place 6 weeks, 6 months, 12 months, and 24 months post-treatment. Home visits were performed by trained research dieticians. The data in this paper are based on 4.5 years of follow-up until November 1st, 2016. This is the most recent available dataset, for which the data have been processed and cleaned. The response rate was 46% at diagnosis and response rates for follow-up visits were all above 90% (Figure 1). The decline in the number of participants during follow-up time points, as visible in Figure 1, is due to the fact that participants had not yet reached their follow-up visit(s) on November 1st, 2016.

Supplement use

Information about the use of supplements was collected by research dieticians at every time point during the study. Pre-treatment, participants were asked to retrospectively report supplement use during the prior year, including (brand) name of the supplement, dose and frequency of use, months that the supplement was used, start date, stop date, ingredients and motivations for use. Post-treatment, participants were asked to report changes in supplement use since the prior visit. Changes in use were defined as the initiation of use of a new supplement, a change in frequency or dose of a currently used supplement, or the discontinuation of a previously used supplement. When available, participants were asked to show the original package of the supplement(s) they used, after which all required information was listed on standardized registration forms.

Supplements were defined according to the definition of the European Food Safety Authority (EFSA): *"Food supplements are concentrated sources of nutrients or other substances with a nutritional or physiological effect, whose purpose is to supplement the normal diet. Food supplements are marketed 'in dose' form, for example as pills, tablets, capsules or liquids in measured doses etc. Supplements may be used to correct nutritional deficiencies or maintain an adequate intake of certain nutrients"*.[19] Supplements prescribed by a physician were considered supplements and not medication (e.g. combined calcium/vitamin D supplements), while vitamins or minerals given as an injection or when administered intravenously (e.g. vitamin B_{12}) were considered medication and not supplements. Supplement use was defined as the regular use of a supplement during the recalled period, with regular use being defined as use at

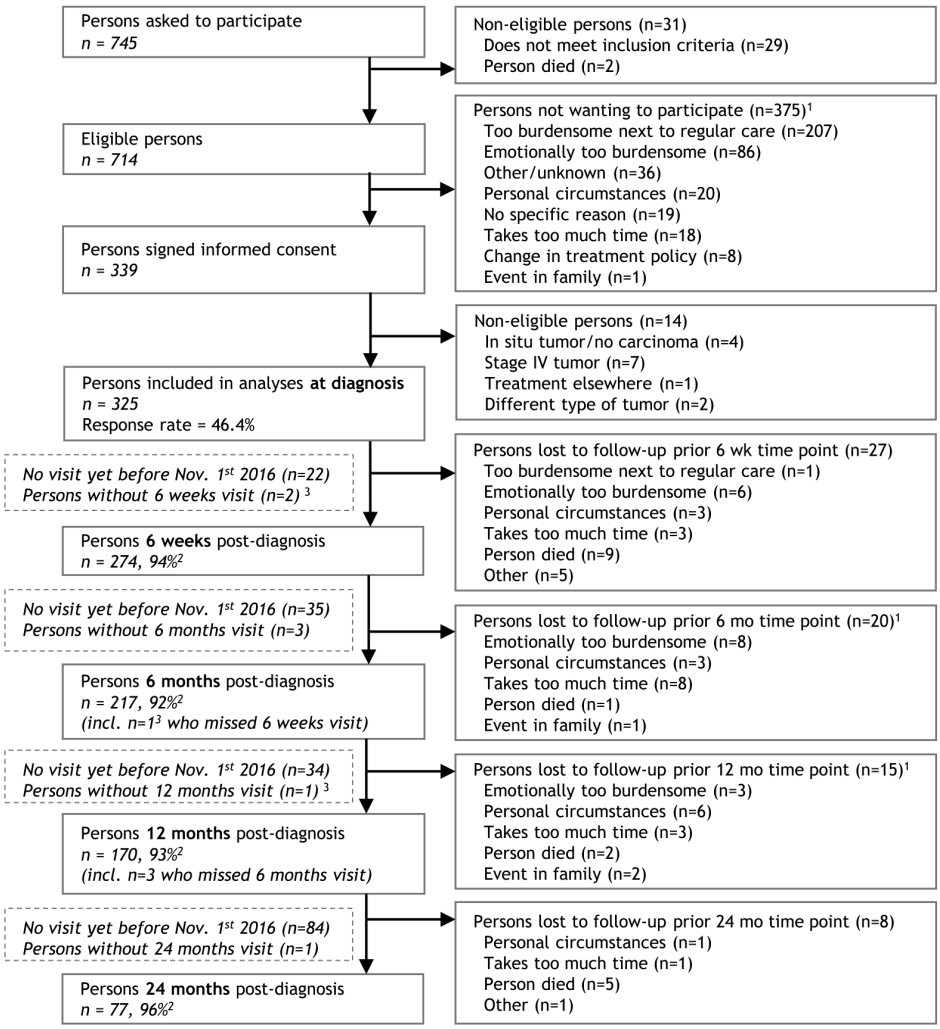

Figure 1. Flow-diagram of inclusion of individuals within the EnCoRe study and included in the analyses presented in this paper. Data of home visits performed before Nov. 1st 2016 were included in the analyses.

[1] Totals do not add up because some individuals reported multiple reasons for non-participation.

[2] Response rate post-treatment = (persons included) / (persons included + persons lost to follow-up – persons died).

[3] Of the two persons without 6 weeks follow-up visits, one person did not have a 6 weeks follow-up visit before Nov. 1st 2016. The person without the 12 months follow-up visit did not have a 24 months follow-up visit before Nov. 1st 2016.

least once per week for a consecutive period of at least one month. Vitamins or minerals that were used less than once a week, but contained a high dose to cover the intake for a longer period of time (e.g. vitamin D), were also considered supplements.

Participants with at least two available longitudinal measurements were classified into three categories of supplement users. Consistent users were participants using supplements at all time points measured, whereas consistent nonusers were participants not using supplements at all time points measured; the remaining participants reported both use and nonuse and were classified as inconsistent users.

Fatigue

Fatigue was assessed using the Checklist Individual Strength (CIS)[20], a validated 20-item questionnaire that has been used in CRC survivors before.[21] A total sum score can be calculated, with higher scores indicating more fatigue. The CIS consists of four subscales reflecting different aspects of fatigue: subjective feelings of fatigue (8 items, score range: 8-56), concentration problems (5 items, score range: 5-35), reduced motivation (4 items, score range: 4-28), and reduced physical activity (3 items, score range: 3-21). Fatigue severity levels were calculated based on the subscale of subjective fatigue using previously published cutoff scores: normal levels of fatigue (8-26), elevated levels of fatigue (27-35), and severe levels of fatigue (36-56).[22] The CIS had a high internal consistency in the current population for both the total score (Cronbach's $\alpha \geq 0.93$) and the subscales (Cronbach's $\alpha \geq 0.72$).

Other variables

Data on demographic factors and educational level were retrieved from a questionnaire administered at recruitment, and smoking was part of a questionnaire administered at every time point. Information about comorbidities pre-treatment was obtained from hospital records using the Charlson Comorbidity Index[23] while information post-treatment was collected during home visits using the Self-Administered Comorbidity Questionnaire.[24] Anthropometric measurements were performed at each time point by trained dieticians and included measurements of height and weight. Medical data on e.g. tumor location and therapy was retrieved from clinical records. Physical activity was assessed at every time point by the Short QUestionnaire to ASsess Health-enhancing physical activity (SQUASH).[25] Time spent in light-intensity and moderate-to-vigorous physical activity (hours/week) was calculated by summing self-reported hours/week of activities classified as ≤1.5 metabolic equivalent of task (MET) and ≥3 METs, respectively.[26,27] A 253-item food frequency questionnaire was administered pre-treatment to retrospectively assess dietary intake over the preceding year.

Statistical analyses

A linear mixed-model approach was used to analyze longitudinal associations between supplement use and fatigue. This analysis can deal with missing data of patients who have not yet reached follow-up time points. These data are assumed to be missing at random. Assumptions for the analyses were met as the residuals were normally distributed, linear, and had a constant variance. Time was defined as the time since the date of the first home visit in units of 6 months. A random intercept for each subject was added to all models. The use of random slopes was tested according to the change in log-restricted likelihood values. Fatigue scores were modeled over time to examine changes in fatigue score from 6 weeks to 24 months post-treatment. Inter- and intra-individual associations were disaggregated by adding centered person-mean values to the model to estimate inter-individual associations and individual deviations from the person-mean value to estimate intra-individual associations.[28]

Potential confounders that were preselected based on the literature and included in all models as covariates were: sex, age, number of comorbidities at all time points (0, 1, ≥2), and treatment with chemotherapy (yes/no). A forward selection procedure based on a 10% change in the beta-coefficient was applied next to evaluate the influence of possible additional confounders, including supplement use, energy intake (kcal/day), and therapy (chemotherapy/radiotherapy) pre-treatment, and moderate-to-vigorous physical activity (hours/week) and body mass index pre- and post-treatment. Sex-stratified analyses were performed to evaluate differences in associations between men and women. Sensitivity analyses were performed to obtain more insight in the possible direction of the association between supplement use and fatigue by applying a time-lag model, in which supplement use was associated with fatigue at the next time point.

Statistical analyses were performed with Stata 15.0 (StataCorp. 2017. College Station, TX) and $P<0.05$ (two-sided) was considered statistically significant.

Results

Participant characteristics

Table 1 presents the characteristics of the study participants at diagnosis. The proportion of men (n=218, 67%) was twice as high as the proportion of women (n=107, 33%). Participants had a mean ±SD age of 67 ±9 years and 27% of the participants were diagnosed with stage I CRC, 22% with stage II CRC, and 47% with stage III CRC. Forty-two percent of participants received chemotherapy and 27% received radiotherapy. Of the participants who received pre-operative chemotherapy, 97% were treated with 5-fluorouracil (5-FU), and of the participants who received post-operative chemotherapy, 83% were treated with capecitabine and oxaliplatin (CAPOX) and 11% with 5-FU. Of all participants, 39% reported to use supplements within the year preceding CRC treatment.

Table 1. Demographic, clinical, and lifestyle characteristics at colorectal cancer diagnosis of EnCoRe study participants included in the current analyses.

	Total population	Supplement use at diagnosis[a]			
		Yes (n=127)		No (n=193)	
		Men	Women	Men	Women
Total population, n (%)	325 (100.0)	73 (57.5)	54 (42.5)	141 (73.1)	52 (26.9)
Age, mean (SD)	67 (9)	67 (9)	69 (10)	66 (9)	65 (10)
Education level, n (%)					
Low	90 (27.7)	15 (20.6)	20 (37.0)	33 (23.4)	22 (42.3)
Medium	122 (37.5)	29 (39.7)	20 (37.0)	54 (38.3)	15 (28.9)
High	105 (32.3)	27 (37.0)	12 (22.2)	51 (36.2)	14 (26.9)
Cancer stage[b], n (%)					
I	89 (27.4)	18 (24.7)	16 (29.6)	40 (28.4)	11 (21.2)
II	72 (22.2)	17 (23.3)	10 (18.5)	29 (20.6)	16 (30.8)
III	152 (46.8)	36 (49.3)	27 (50.0)	65 (46.1)	23 (44.2)
Type of cancer, n (%)					
Colon	198 (60.9)	43 (58.9)	36 (66.7)	81 (57.5)	35 (67.3)
Rectum	127 (39.1)	30 (41.1)	19 (33.3)	60 (42.6)	17 (32.7)
Chemotherapy, n (%)					
Yes	137 (42.2)	29 (39.7)	21 (38.9)	62 (44.0)	24 (46.2)
No	188 (57.9)	44 (60.3)	33 (61.1)	79 (56.0)	28 (53.9)
Radiotherapy, n (%)					
Yes	89 (27.4)	22 (30.1)	13 (24.1)	44 (31.2)	10 (19.2)
No	236 (72.6)	51 (69.9)	41 (75.9)	97 (68.8)	42 (80.8)
Comorbidities, n (%)					
None	60 (18.5)	13 (17.8)	9 (16.7)	29 (20.6)	9 (17.3)
1	67 (20.6)	18 (24.7)	12 (22.2)	25 (17.7)	11 (21.2)
≥2	198 (60.9)	42 (57.5)	33 (61.1)	87 (61.7)	32 (61.5)
BMI in kg/m^2, mean (SD)	28.2 (4.6)	28.6 (4.0)	28.4 (5.8)	27.9 (4.5)	28.1 (4.5)
Smoking status, n (%)					
Current	42 (12.9)	12 (16.4)	6 (11.1)	18 (12.8)	5 (9.6)
Former	174 (53.5)	39 (53.4)	19 (35.2)	84 (59.6)	29 (55.8)
Never	101 (31.1)	20 (27.4)	27 (50.0)	36 (25.5)	17 (32.7)
Energy intake in kcal/day, mean (SD)	2243 (649)	2372 (630)	1867 (424)	2393 (656)	2046 (682)
Alcohol intake in g/day, mean (SD)	13.1 (17.0)	17.2 (17.6)	5.8 (9.1)	16.2 (19.6)	5.6 (7.3)
Physical activity, mean (SD)					
LPA (hours/week)	15.9 (16.4)	11.7 (15.4)	18.8 (15.4)	14.5 (17.3)	23.2 (14.0)
MVPA (hours/week)	14.4 (14.5)	18.1 (18.7)	8.4 (7.5)	16.7 (15.0)	9.7 (8.5)

Abbreviations: body mass index (BMI), light-intensity physical activity (LPA), moderate-to-vigorous intensity physical activity (MVPA).

Percentages may not add up to 100% as a result of missing data.

[a] Supplement use at diagnosis was unknown for 5 participants.

[b] For 12 participants it was unknown whether they had stage I, II, or III CRC.

Supplement use

After treatment, supplement use remained relatively stable and the lowest frequency of use was reported at 6 weeks post-treatment (Table 2). Multivitamins/multiminerals were the most frequently used supplement at all time points. Use of other types of supplements, such as B-vitamin complexes, single supplement vitamin D, combined vitamin D and calcium, single supplement iron, and folic acid, ranged between 0-11% over the entire recalled period and no major changes over time were observed (data not shown). The consistent users represented 28% (n=75) of the population, the inconsistent users 27% (n=74), and the consistent nonusers 45% (n=123).

Reported motivations for using supplements are illustrated in Figure 2a. The most frequently reported motivation concerned specific complaints not related to the bowel. No major differences were observed in reported motivations before and after CRC treatment. Motivations to start using a new supplement after CRC treatment were comparable to the overall motivations for supplement use (Figure 2a). Figure 2b shows reported motivations to discontinue the use of supplements; the most frequently reported reason for discontinuation was that participants felt like supplement use was no longer necessary.

Fatigue

The total fatigue score followed a linear, significant decline over the course of 6 weeks to 24 months post-treatment (β -3.3, 95% CI -4.3;-2.3) (Table 2, Figure 3a). The subscales subjective fatigue (β -1.7, 95% CI -2.2;-1.2), reduced motivation (β -0.6, 95% CI -0.8;-0.3), and reduced physical activity (β -0.8, 95% CI -1.0;-0.5) also significantly decreased over time (Table 2, Figure 3b). Severe fatigue was most frequently reported at 6 weeks post-treatment (29%) and the largest decline was observed between the 6-week and 6-month post-treatment time points (Table 2). Decreasing fatigue scores were seen for all categories of supplement users (Figure 3a). At 6 weeks post-treatment, the consistent nonusers had an almost 9-point lower fatigue score compared to the consistent and inconsistent users (Figure 3a). The consistent users reported increasing levels of fatigue after the time point 12 months post-treatment, whereas the decline in fatigue was maintained for the inconsistent users and consistent nonusers.

Table 2. Prevalence of supplement use pre- and post-colorectal cancer treatment and fatigue levels post-colorectal cancer treatment.

	Pre-treatment *(n=325)*	Time post-treatment			
		6 wk	6 mo	12 mo	24 mo
Prevalence of supplement use, n (%)		*n=274*	*n=217*	*n=170*	*n=77*
Overall supplement users	127 (39.1)	98 (35.8)	82 (37.8)	69 (40.6)	29 (37.7)
Users of supplements with vitamins/minerals[a]	116 (91.3)	81 (82.7)	68 (82.9)	54 (78.3)	22 (75.9)
Users of multivitamins/ multiminerals[b]	61 (48.0)	39 (39.8)	32 (39.0)	20 (29.0)	14 (48.3)
Users of supplements without vitamins/minerals[a]	40 (31.5)	35 (35.7)	31 (37.8)	26 (37.7)	8 (27.6)
Changes in supplement use, n (%)					
Started use of new supplement[c]		25 (9.1)	34 (15.7)	21 (12.4)	12 (15.6)
Changed dose of supplement[c]		1 (0.4)	3 (1.4)	3 (1.8)	1 (1.2)
Stopped use of supplement[c]		41 (15.0)	17 (7.8)	10 (5.9)	7 (9.0)
Fatigue scores, mean (SD)		*n=264*	*n=213*	*n=168*	*n=72*
Total fatigue score (range 20-140)		62.9 (26.6)	59.2 (27.4)	54.4 (26.2)	51.5 (25.2)
Subjective fatigue (range 8-56)		27.1 (13.4)	24.8 (12.8)	22.7 (12.4)	21.3 (12.8)
Reduced motivation (range 4-28)		12.3 (6.1)	12.0 (6.1)	10.9 (6.1)	10.5 (5.9)
Reduced physical activity (range 3-21)		10.6 (5.1)	9.7 (5.2)	8.7 (5.1)	8.1 (4.8)
Concentration problems (range 5-35)		12.9 (7.4)	12.9 (7.4)	12.0 (6.7)	11.6 (6.5)
Total fatigue scores for types of supplement users[d], mean (SD)					
Consistent users (n=75)		66.7 (29.2)	59.0 (30.0)	56.7 (28.1)	61.5 (28.2)
Inconsistent users (n=74)		66.6 (26.1)	60.7 (30.5)	56.2 (29.2)	48.2 (24.3)
Consistent nonusers (n=123)		58.0 (24.7)	58.2 (24.1)	51.6 (23.0)	49.6 (24.2)
Categories of fatigue severity[e], n (%)		*n=266*	*n=215*	*n=169*	*n=72*
Normal levels of fatigue (range 8-26)		131 (49.4)	116 (54.0)	108 (63.9)	49 (68.1)
Elevated levels of fatigue (range 27-35)		58 (21.9)	55 (25.6)	30 (17.8)	8 (11.1)
Severe levels of fatigue (range 36-56)		76 (28.7)	44 (20.5)	31 (18.3)	15 (20.8)

[a] Reported supplements were divided into two groups: supplements that contained vitamins and/or minerals (e.g. multivitamins/multiminerals or single folic acid supplements) and supplements that did not contain vitamins and minerals (e.g. herbal supplements). Percentages are taken from the number of overall supplement users. Totals do not add up as some participants used both supplements with vitamins/minerals and supplements without vitamins/minerals.

[b] Multivitamins/multiminerals: supplements that contained at least two vitamins and/or minerals, excluding B-vitamin complexes and combined calcium and vitamin D supplements. Percentages are taken from the number of overall supplement users.

[c] Numbers are based on the number of participants who started, changed or stopped the use of one or more supplements. Numbers overlap, as participants could have started one supplement and stopped another supplement during the same recall period.

[d] Participants with at least two available longitudinal measurements were classified into three categories of supplement users. Consistent users were participants using supplements at all time points measured, whereas consistent nonusers were participants not using supplements at all time points measured; the remaining participants reported both use and nonuse and were classified as inconsistent users.

[e] Based on previously published cutoff scores for the subscale of subjective fatigue.[22]

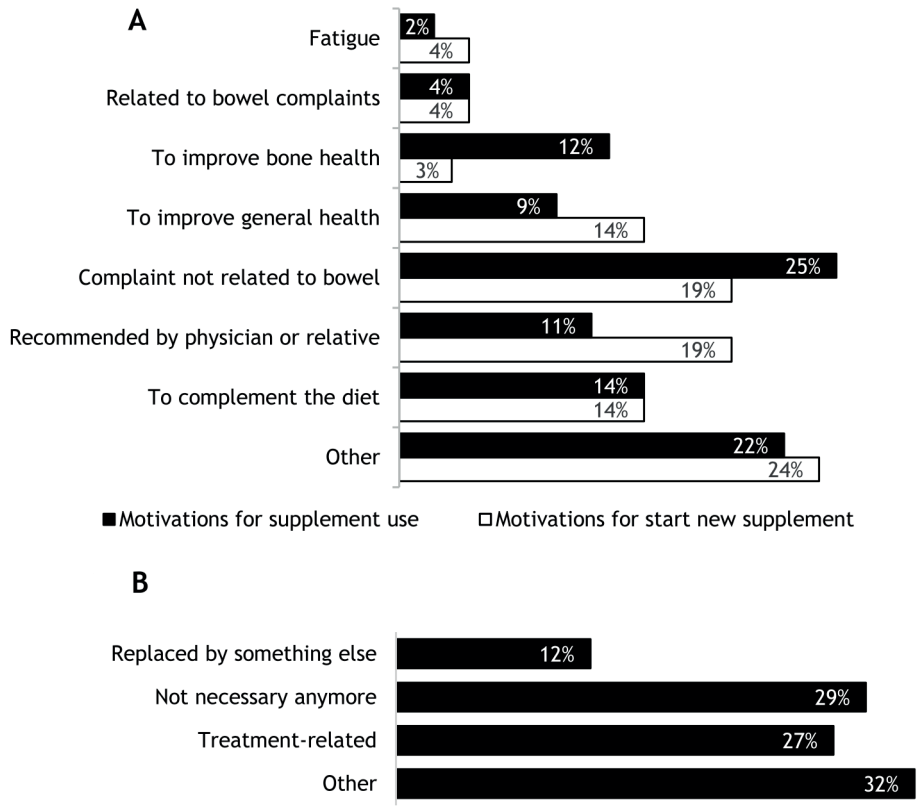

Figure 2. Reported motivations for supplement use (**A**) and discontinuation of supplement use (**B**) as reported by participants included in the current analysis within the EnCoRe study. Complaints not related to the bowel were for instance: neuropathy, allergies, or memory loss. Reasons included in the 'other' category for supplement use were for instance: to lower cholesterol levels or to help weight loss. Reasons included in the 'other' category for discontinuation of supplement use were for instance: experienced no results or finished the package.

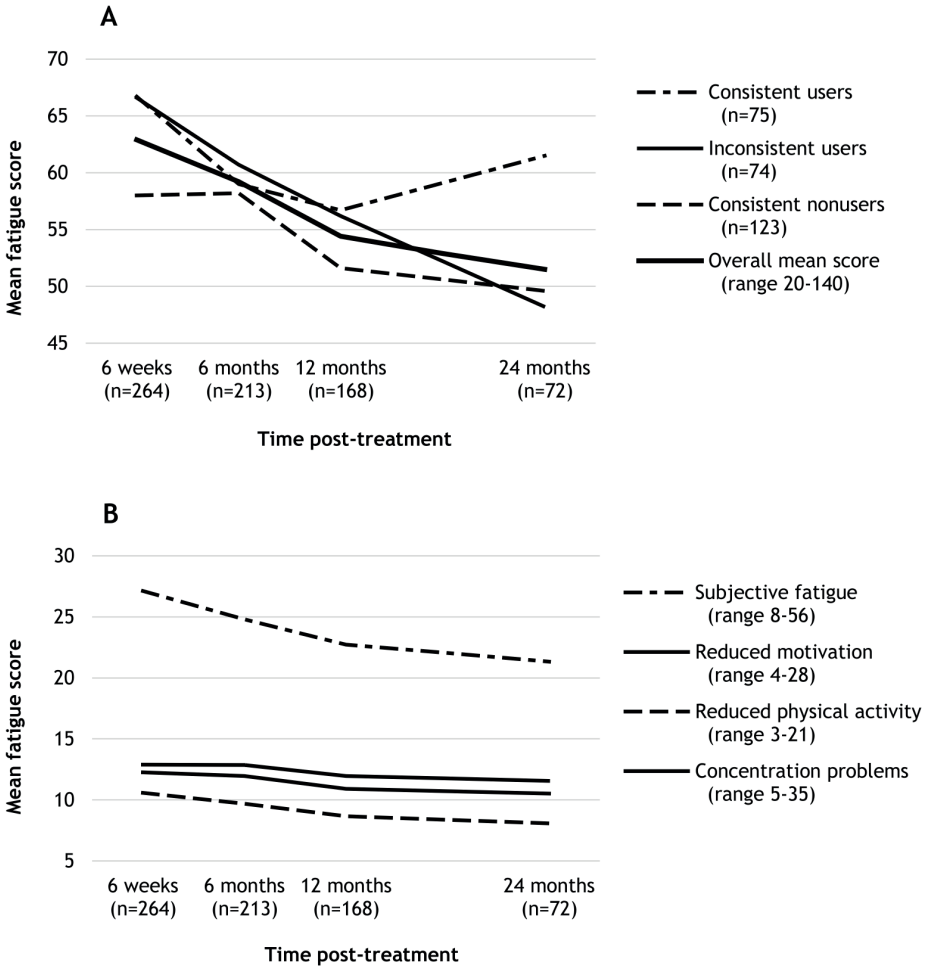

Figure 3. Overall fatigue score and fatigue scores for the three categories of supplement users over time (**A**), and mean scores on the subscales of fatigue over time (**B**) within the EnCoRe study.

Longitudinal associations of supplement use and fatigue

No significant longitudinal associations between supplement use and fatigue were observed in the overall confounder-adjusted model including both inter- and intra-individual associations (ß 4.0, 95% CI -0.7;8.7, Table 3). However, when inter- and intra-individual associations were disaggregated, the inter-individual analyses showed that supplement users had a significantly higher fatigue score over time compared to nonusers (ß 7.0, 95% CI 0.3;13.7). No significant intra-individual associations were found (ß 1.3, 95% CI -5.2;7.7). The sex-stratified analyses showed no difference between men and women in the overall longitudinal associations of supplement use with fatigue. Furthermore, no associations between supplement use and the separate subscales of fatigue were found (Table 3). In comparison to results of the main analysis, the overall longitudinal association of supplement use with fatigue was attenuated within time-lag analyses (ß 0.4, 95% CI -4.7;5.5). The inter-individual association was not significant anymore (ß 2.9, 95% CI -4.7;10.4), and the intra-individual association was reversed but still non-significant (ß -1.4, 95% CI -7.7;4.9).

Table 3. Results of mixed-model regression analyses on longitudinal associations between supplement use and fatigue.

	Total fatigue[a]		Subscales of fatigue[b]							
			Subjective fatigue		Reduced motivation		Reduced physical activity		Concentration problems	
	β	95% CI	β	95% CI	β	95% CI	β	95% CI	β	95% CI
Un-adjusted[c]	4.9	0.1; 9.8	2.1	-0.2; 4.4	0.9	-0.2; 2.0	0.8	-0.1; 1.7	1.1	-0.1; 2.3
Adjusted model I[c,d]	4.6	-0.3; 9.5	1.9	-0.5; 4.2	0.7	-0.4; 1.8	0.8	-0.1; 1.7	1.1	-0.1; 2.4
Adjusted model II[c,e]	4.0	-0.7; 8.7	1.6	-0.6; 3.9	0.5	-0.6; 1.6	0.7	-0.2; 1.6	1.1	-0.1; 2.3
Intra-individual[f]	1.3	-5.2; 7.7	0.2	-2.8; 3.3	0.2	-1.4; 1.8	0.5	-0.8; 1.9	0.5	-1.1; 2.1
Inter-individual[g]	7.0	0.3; 13.7	3.1	-0.1; 6.4	0.7	-0.7; 2.2	0.8	-0.5; 2.0	1.8	0.0; 3.7

Abbreviations: beta-coefficient (ß), confidence interval (CI).

[a] Total score range, 20-140.

[b] Subscale ranges: subjective fatigue, 8-56; reduced motivation, 4-28; reduced physical activity, 3-21; concentration problems, 5-35.

[c] The beta-coefficient represents the overall longitudinal difference in fatigue score between supplement users and nonusers, including both inter- and intra-individual associations.

[d] Adjusted for sex and age.

[e] Adjusted for sex, age, number of comorbidities (0, 1, ≥2), chemotherapy (yes/no), moderate-to-vigorous-intensity physical activity (hours/week).

[f] The beta-coefficient represents the difference in fatigue score over time within individuals when changes in supplement use were made (from nonuser to user).

[g] The beta-coefficient represents the difference in fatigue score over time between supplement users and nonusers.

Discussion

Within this study, we described the use of supplements by CRC survivors from the moment of diagnosis up to 2 years after the end of cancer treatment and studied how supplement use was longitudinally associated with fatigue. The frequency of supplement use was relatively stable (~40% at all time points) and the majority of the participants were consistent in their (non)use of supplements. No significant overall longitudinal association of supplement use with fatigue over time was observed. However, when distinguishing inter- from intra-individual relations over time, a significant inter-individual association was observed, indicating that supplement users had a higher fatigue score over time compared to nonusers.

The proportion of supplement users in this sample of CRC survivors was similar to the general Dutch population aged 51-79 years. Among men, supplement use was 33% compared to 26% in the general population, and among women, supplement use was 50% compared to 52% in the general population.[29] Percentages also correspond to the observations of another prospective study of Dutch CRC survivors, where 38% of the population used supplements at diagnosis (39% in our population).[6] In the Netherlands, supplements are only recommended for certain population subgroups or in case of diagnosed deficiencies of e.g. vitamin B_{12}. A large part of the colorectal cancer patients under study is recommended to use vitamin D supplements as all Dutch women aged 50+ and men aged 70+ are recommended to daily use 10-20µg vitamin D. The numbers of consistent users, inconsistent users, and consistent nonusers in the study by Heine-Bröring *et al*.[6] slightly differ from our observations, and could be due to differences between the study populations, such as the inclusion of stage IV CRC patients, the number of time points that supplement use was assessed (3 vs. 5 in our study), and the assessment method (self-report vs. interview-based in our study). Furthermore, wide ranges in supplement use have been observed in different studies among CRC survivors[3-5], which may be explained by dissimilarities in the definition of a supplement (user) and to different periods after diagnosis that supplement use was assessed. Despite the existing definitions of dietary supplements, e.g. the definition of the EFSA used in our study, the distinction between supplements, medication, and food products can be arbitrary, thereby resulting in discrepancies among studies.

The large diversity that was observed in the types of supplements used as well as in reported motivations for use may indicate a sense of ambivalence about the use of (specific types) of supplements among CRC survivors. A study among Dutch oncology nurses reported that 43% of nurses felt unable to provide advice on nutrition, including the use of supplements, due to lacking knowledge on the subject.[30] When the health effects of supplement use after cancer and associations of supplement use with fatigue would be unraveled further, this could potentially improve the competence of health professionals to provide cancer survivors with tailored and well-founded advice about using supplements.

In addition, guidance on nutrition and diet should be interwoven with advice on supplement use as the diet is the primary source of vitamins and minerals.

The inter-individual associations observed in our study showed that supplement users were experiencing more fatigue over time compared to nonusers. Although no firm conclusions on the direction of this association can be drawn based on the observational nature of our data, the time-lag analyses showed an attenuated, non-significant inter-individual association and a reversed intra-individual association. This suggests an association in the opposite direction, i.e. fatigue associated with supplement use, indicating that individuals with more fatigue might be inclined to start using supplements as a consequence of their fatigue. This would not be unlikely given that fatigue is often accompanied by other feelings of ill-health and cancer survivors are known to be searching for ways to alleviate these senses of discomfort.[31] As supplement use was defined as use at least once per week for a consecutive period of at least one month, the dosage of some supplements may have been inadequate for beneficial health effects to occur. This might therefore be a reason for the insignificant findings of the longitudinal analyses.

An important strength of the current study is the prospective nature and repeated-measures design. In addition, a high quality dataset was obtained by collecting all data during home visits by trained research dieticians who maintained close contact with the participants, resulting in high response rates during post-treatment follow-up and a low percentage of missing data. Furthermore, detailed information on supplement use was obtained during home-based interviews, instead of relying on participant self-report. Despite the above strengths, the observational nature of our study limits the ability to draw conclusions regarding causality. Another limitation concerns the possibility that the study population might have consisted of relatively healthy cancer survivors, as participants with more fatigue may have been less inclined to participate in the study. The results on fatigue might therefore give a more positive outlook than reality and the association between supplement use and fatigue might be attenuated. Furthermore, reduced statistical power in the stratified analyses may, on the one hand, have contributed to insignificant findings in the intra-individual analyses and, on the other hand, explain the increased fatigue score for consistent users 24 months post-treatment (Figure 3a).

Concluding, although supplement use is discouraged in cancer-specific guidelines, prevalence of supplement use is high among Dutch stage I-III CRC survivors. The overall association between supplement use and fatigue was not significant, but the results of our study suggest that supplement users were experiencing higher levels of fatigue compared to nonusers. CRC survivors may introduce lifestyle changes, including the initiation of supplement use, to take charge of their health and to alleviate complaints such as fatigue. Results of the current study imply no beneficial effects of overall supplement use on fatigue

and the recommendation for cancer survivors to not use supplements therefore coheres with our results. Future research should aim to gain deeper insight in the needs of CRC survivors regarding modifiable lifestyle factors including supplement use and other dietary factors, and how these are associated with health outcomes, in order to provide them with well-founded advice on how to deal with commonly experienced complaints such as fatigue.

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CHAPTER 3

Higher serum vitamin D concentrations are longitudinally associated with better global quality of life and less fatigue in colorectal cancer survivors up to 2 years after treatment

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ABSTRACT

Vitamin D status may be an important determinant of health-related quality of life of colorectal cancer (CRC) survivors. The current study investigated longitudinal associations between serum 25-hydroxyvitamin D_3 ($25OHD_3$) concentrations and quality of life in stage I-III CRC survivors up to 2 years post-treatment.

CRC patients (n=261) were included upon diagnosis. Home visits (including blood sampling) were performed at diagnosis and at 6 weeks, 6 months, 1 and 2 years post-treatment. Serum $25OHD_3$ concentrations were measured using liquid chromatography-tandem mass spectrometry and adjusted for season. Validated questionnaires were used to assess global quality of life and cognitive functioning (EORTC QLQ-C30), fatigue (EORTC QLQ-C30 and Checklist Individual Strength, CIS), and depression and anxiety (Hospital Anxiety and Depression Scale). Statistical analyses were performed using linear mixed-models and adjusted for sex, age, time since diagnosis, therapy, comorbidities, physical activity, and BMI.

At diagnosis, 45% of patients were vitamin D deficient (<50 nmol/L). After treatment, $25OHD_3$ concentrations increased on average with 3.1 nmol/L every 6 months. In confounder-adjusted models, 20 nmol/L increments in $25OHD_3$ were longitudinally associated with increased global quality of life (β 2.9; 95%CI 1.5,4.3) and reduced fatigue (EORTC QLQ-C30 subscale: β -3.5; 95%CI -5.3,-1.8 and CIS: β -2.8; 95%CI -4.7,-0.9). Observed associations were present both within and between individuals over time.

Higher concentrations of $25OHD_3$ were longitudinally associated with better global quality of life and less fatigue in CRC survivors. This study suggests that higher $25OHD_3$ concentrations may be beneficial for CRC survivors. Future intervention studies are needed to corroborate these findings.

Introduction

Colorectal cancer (CRC) survivors are susceptible to enduring physical and psychological distress as a result of the tumor and therapy. A growing number of individuals are living with a history of CRC given the improving 5-year survival rate after CRC, currently 65% in the Netherlands.[1,2] Previous studies reported complaints of fatigue by more than one third of Dutch CRC survivors, especially within 5 years post-diagnosis.[3-5] In addition, CRC survivors are at increased risk of impaired cognitive functioning and mental health problems such as depression.[6-8]

Vitamin D is a potentially important determinant of health-related quality of life (HRQoL) of CRC survivors given its involvement in many cellular processes related to cognition[9,10], depression[11,12], and fatigue.[13] Exposure of the skin to ultraviolet B radiation contributes to about two-third of vitamin D supply, whilst about one-third originates from vitamin D intake.[14] Vitamin D deficiencies have become a concern of public health[15,16] and the Dutch Health Council recommends women aged 50-70 (10 mcg) and men and women aged >70 (20 mcg) to daily supplement vitamin D.[14] No specific guidelines for cancer survivors are available. To maintain adequate vitamin D concentrations, 15-30 minutes of daily sunlight exposure from March to November is generally sufficient for the general population.[17] CRC survivors are at risk of low vitamin D because of the negative impact of the cancer and chemotherapy on circulating concentrations.[18-20] Moreover, the association between low vitamin D and increased CRC risk[21-24] makes CRC survivors particularly susceptible as low concentrations may be sustained after therapy. Inflammation may be an important confounder in the association between vitamin D and HRQoL. Inflammatory processes and vitamin D interact through 1,25-dihydroxyvitamin D, the biologically active metabolite also known as calcitriol, which is engaged in different intracellular inflammatory reactions.[25] In addition, as inflammatory markers have been associated with increased fatigue after cancer, a potential observed association between vitamin D and HRQoL may be altered by inflammation.[26]

Few studies have investigated associations between vitamin D and HRQoL after CRC. An observational study among 453 stage II CRC survivors reported better symptom-related quality of life among participants using vitamin D supplements over 2 years of follow-up.[27] Cancer survivors are known to be highly motivated to intentionally alter their lifestyle in order to make health improvements.[28] Therefore, lifestyle recommendations that focus on the enhancement of vitamin D concentrations may be an opportunity to prevent or reduce problems of diminished HRQoL after CRC.

The objective of the present study was to investigate longitudinal associations of serum 25-hydroxyvitamin D_3 ($25OHD_3$) concentrations with HRQoL in CRC survivors from 6 weeks to 2 years after treatment, including global quality of life, fatigue, depression, anxiety, and cognitive functioning.

Methods

Study design and population

The EnCoRe-study (Energy for Life after ColoRectal Cancer) is an ongoing prospective cohort study that was initiated in 2012 (Netherlands Trial Register no. NL6904).[5,29] The purpose of the EnCoRe study is to evaluate longitudinal associations between lifestyle factors and HRQoL, functioning and prognosis after colorectal cancer. Stage I-III CRC patients ≥18 years of age were recruited at diagnosis. Research dietitians performed home visits at diagnosis (prior to treatment), and at 6 weeks, 6 months, 1 and 2 years after the end of treatment. The study was approved by the Medical Ethics Committee of the University Hospital Maastricht and Maastricht University, the Netherlands. All participants signed informed consent. Exclusion criteria were: diagnosis with stage IV CRC, no home address in the Netherlands, inability to understand the Dutch language, and presence of comorbidities obstructing successful study participation. Data used in the current analyses were based on the first 4.5 years of follow-up until November 1st, 2016. Participants with at least 1 follow-up visit with available data on both $25OHD_3$ and HRQoL were included in the current analyses. The final analyses contained 261 participants at diagnosis, 260 at 6 weeks, 213 at 6 months, 168 at 12 months, and 77 at 24 months post-treatment. Response rate for inclusion was 46% and >90% for follow-up visits.

Measurement of $25OHD_3$

At diagnosis, blood samples were drawn either during the first home visit or at the hospital, and were mostly non-fasting. At follow-up, fasting blood samples were drawn during home visits. Samples were collected in 8.5 mL serum tubes (BD Vacutainer SST II Advance) and pipetted into aliquots after centrifugation. Aliquots were stored at -80°C within 4 hours after blood draw until analysis. Serum $25OHD_3$ concentrations were measured using liquid chromatography-tandem mass spectrometry (LC-MS/MS) at the Canisius-Wilhelmina Hospital in Nijmegen, the Netherlands.[30] Inter-assay coefficients of variation were 5.3%, 3.1%, and 2.9% at $25OHD_3$ concentrations of 39.0, 92.5, and 127.0 nmol/L, respectively. $25OHD_3$ concentrations are a robust indicator and the most commonly used marker of vitamin D status.[31]

Health-related quality of life

HRQoL was assessed during post-treatment time points. Global quality of life, cognitive functioning, and fatigue were assessed by the Quality of Life Questionnaire of the European Organisation for Research and Treatment of Cancer (EORTC QLQ-C30, version 3.0).[32,33] Higher scores reflect better global quality of life, better cognitive functioning, and more fatigue (range 0-100). Fatigue was also measured by the Checklist Individual Strength (CIS), a validated 20-item questionnaire that has been used in CRC survivors before.[34,35]

Higher scores indicate more fatigue (range 20-140). Depression and anxiety were assessed using the Hospital Anxiety and Depression Scale (HADS).[36,37] The HADS includes separate depression and anxiety scales, higher scores indicate more depression and anxiety (range 0-42).

Socio-demographic, lifestyle and clinical data

Socio-demographic information was collected based on self-report. Cancer stage, type of therapy and other clinical information were obtained from medical records. Information on comorbidities at diagnosis was retrieved from medical records by the Charlson Comorbidity Index[38] and during follow-up from the Self-Administered Comorbidity Questionnaire, which included heart condition; stroke; high blood pressure; asthma, chronic bronchitis or chronic obstructive pulmonary disease; diabetes; stomach ulcer; kidney disease; liver disease; aneamia or other disease of the blood; thyroid gland disease; depression; osteoarthritis; back pain; and rheumatoid arthritis.[39]

Information on dietary supplement use, including vitamin D supplements, was collected during home visits. Information on (brand) name, dosage, frequency, duration, and ingredients was listed on standardized forms. Vitamin D intake from supplements was calculated in micrograms per day by multiplying daily frequency and dosage. At diagnosis, participants completed a semi-quantitative 253-item food frequency questionnaire to retrospectively assess dietary vitamin D intake during the preceding year.[40] Participants completed a 7-day dietary record as part of each post-treatment follow-up visit. Vitamin D levels in food products for both methods were obtained from the 2011 Dutch Food Composition Database.[41] Methods were described in more detail previously.[5,42]

Measurements of height and weight were performed during home visits to determine body mass index (BMI). Physical activity was self-reported using the Short QUestionnaire to ASsess Health enhancing physical activity (SQUASH). Time spent in moderate-to-vigorous physical activity (≥3 metabolic equivalents of task) was calculated in hours/week.[43] Sun exposure was self-reported at each time point by the number of days per week that participants had spent at least 15 minutes outside (in daylight), on average over the preceding month. The variable was dichotomized for the analyses in order to reflect adherence to the Dutch recommendations regarding sun exposure (spent 15 minutes outside on 7 or <7 days/week).

Plasma concentrations of inflammation markers interleukin-6, interleukin-8, interleukin-10, and tumor necrosis factor-alpha (TNF-alpha) were measured for all time points. The selection of this set of inflammation markers was based on a review that evaluated inflammation markers that interact with vitamin D and that are specifically relevant in relation to colorectal cancer.[25] Samples were collected in 6.0 mL EDTA plasma tubes (BD Vacutainer K2E) and pipetted into aliquots after centrifugation. Aliquots were stored at -80°C within 4

hours after blood draw until analysis. Measurements were performed using a custom-made multiplex assay and electrochemiluminescence (Meso Scale Diagnostics, Rockville, Maryland, USA) at Wageningen University & Research, the Netherlands. Assay plates were analyzed on a QuickPlex SQ 120 plate reader (Meso Scale Diagnostics).

Statistical analyses

Descriptive analyses were performed to calculate means and standard deviations (SD) for normally distributed variables and medians and interquartile ranges (IQR) for skewed variables. $25OHD_3$ concentrations at each time point were adjusted for season using the week (1-52) of blood collection. Locally weighted regression analyses (LOWESS) were performed in order to account for the variation caused by seasonal differences.[44,45] For post-treatment time points, longitudinal associations between $25OHD_3$ concentrations and HRQoL were investigated using linear mixed-model analyses. Each model contained a random intercept for subject . The addition of a random slope for vitamin D was evaluated in each model according to the change in log-restricted likelihood values. A separate model was used to disentangle inter- and intra-individual associations.[46] Inter-individual associations were estimated by the centered person-mean value, indicating the difference between participants' mean $25OHD_3$ concentrations over time. Intra-individual associations were estimated by the individual deviations from the person-mean, indicating individual changes in vitamin D concentration over time. Vitamin D was modeled as a continuous variable in units of 20 nmol/L, and as a dichotomous variable (vitamin D concentration <50 nmol/L; yes/no).[20] In addition, scoring on the outcomes was compared for $25OHD_3$ concentrations of <30 nmol/L, 30-50 nmol/L, and >50 nmol/L. Vitamin D supplement use (yes/no), total vitamin D intake from diet and supplements (µg/day), and vitamin D intake only from supplements (µg/day) were modeled separately as the independent variable in multivariable adjusted models to obtain more insight into the role of vitamin D supplement use. A summary score for the inflammatory markers was calculated by summing z-scores of natural-log transformed concentrations of interleukin-6, interleukin-8, and TNF-alpha, and subtracting interleukin-10, for each time point.[47] Higher scores are indicative of higher inflammation.

Relevant confounders were pre-selected according to the literature and included sex, age at diagnosis, time since diagnosis (units of 6 months), cancer treatment (neither chemotherapy nor radiotherapy, chemotherapy only, radiotherapy only, both), number of comorbidities (0, 1, ≥2), MVPA (h/wk), and BMI (kg/m^2). All time-dependent variables, including confounders, were included in the model as the repeated measurements. Other potential covariates that did not change the beta-coefficients for the relationship of main exposures with outcomes, and therefore not included in the models, were: inflammatory markers (z-score), $25OHD_3$ at diagnosis, season, vitamin D intake from supplements (µg/d), and sun exposure (dichotomous: spent 15 minutes outside on 7 or <7 days/week).

Subgroup analyses were performed for sex (men/women), age at diagnosis (<70 and ≥70 years), vitamin D supplement use (users/nonusers), and (neo-)adjuvant chemotherapy (yes/no).

Statistical analyses were performed using Stata15 (StataCorp. 2017. College Station, TX). *P*-values <0.05 (two-sided) were considered as statistically significant.

Results

Participant characteristics

The 261 participants who enrolled in the study (31% women) had a mean ±SD age of 67 ±9 years (Table 1). Median $25OHD_3$ concentrations decreased after diagnosis and subsequently increased during follow-up (Table 2, Figure 1). Almost half (45%) of participants were vitamin D deficient (<50nmol/L) at diagnosis. Regarding the subgroups advised to use extra vitamin D; 24% of men and women aged ≥70 years (n=98) were vitamin D supplement users at diagnosis. Additionally, 33% of women aged 50-70 years (n=39) used vitamin D supplements at diagnosis. Users of vitamin D supplements had higher serum concentrations compared to nonusers (Figure 1, panel B). Participants who received chemotherapy had consistently lower concentrations compared to the group who did not receive chemotherapy (Figure 1, panel C).

Vitamin D and health-related quality of life

With every 6 months, global quality of life scores improved on average with 1.3 points (95% CI 0.4, 2.1) and fatigue levels decreased with 2.8 points (95% CI -3.8, -1.8; EORTC) and 3.3 points (95% CI -4.3, -2.2; CIS) (Table 3, Supplemental Figure 1).

$25OHD_3$ was longitudinally associated with better global quality of life (β 2.9; 95% CI 1.5, 4.3) and reduced fatigue (EORTC: β -3.5; 95% CI -5.3, -1.8 and CIS: β -2.8; 95% CI -4.7, -0.9). An increase of 20 nmol/L $25OHD_3$ within an individual over time was associated with an average 3.7 point (95% CI 1.7, 5.6) higher global quality of life score. Additionally, a 20 nmol/L higher mean $25OHD_3$ concentration between individuals over time was associated with an average 2.1 point (95% CI 0.2, 4.1) higher global quality of life score. Intra- and inter-individual associations of $25OHD_3$ with fatigue were of similar degree when measured by the CIS (β's -2.5 and -3.2, respectively), whereas for fatigue as measured by the EORTC, the association for intra-individual changes was stronger compared to inter-individual differences (β's -4.4 and -2.6, respectively). Not being vitamin D deficient was longitudinally associated with better global quality of life (β 4.4; 95% CI 1.6, 7.2) and less fatigue (EORTC: β -5.2; 95% CI -8.6, -1.8 and CIS: β -5.4; 95% CI -9.0, -1.8), both within and between individuals (Table

3). Dose-response relations were observed for the outcomes global quality of life and fatigue when comparing the scoring of participants having severely deficient (<30 nmol/L) and deficient (30-50 nmol/L) $25OHD_3$ concentrations, with participants having adequate concentrations (>50 nmol/L) (Figure 2).

Although not statistically significant, better global quality of life and less fatigue (EORTC QLQ-C30) were observed for individuals who changed from being nonuser to user of vitamin D supplements over time, whereas inter-individual differences suggested lower global quality of life and more fatigue for supplement users compared to nonusers (Table 4). No longitudinal associations were found between the sum of dietary and supplemental vitamin D intake with global quality of life and fatigue, and between vitamin D intake from supplements only and these outcomes. In subgroup analyses, longitudinal associations of $25OHD_3$ concentrations with global quality of life and fatigue were only present in men (Supplemental Table 1).

Table 1. Socio-demographic and clinical characteristics of study participants at time of inclusion (colorectal cancer diagnosis), included in the EnCoRe study.

	Total population (n=261)[a]	Vitamin D deficiency (<50 nmol/L)[b]	
		Yes (n=111)	No (n=136)
Age, mean (SD)	67 (9)	67 (9)	66 (9)
Sex, n (%)			
Men	179 (69)	78 (70)	91 (67)
Women	82 (31)	33 (30)	45 (33)
Education level, n (%)			
Low	66 (25)	26 (23)	35 (26)
Medium	105 (40)	48 (43)	53 (39)
High	89 (34)	37 (33)	47 (35)
Cancer type, n (%)			
Colon	159 (61)	72 (65)	79 (58)
Rectum	102 (39)	39 (35)	57 (42)
Cancer stage[c], n (%)			
I	77 (30)	32 (30)	43 (34)
II	60 (23)	26 (24)	27 (21)
III	113 (43)	50 (46)	58 (45)
Treatment, n (%)			
Neither chemotherapy nor radiotherapy	143 (55)	60 (54)	77 (57)
Chemotherapy only	46 (18)	25 (23)	18 (13)
Radiotherapy only	20 (8)	6 (5)	10 (7)
Both chemotherapy and radiotherapy	52 (20)	20 (18)	31 (23)
Inflammatory markers, median (IQR)			
Interleukin-6 in pg/ml	1.1 (0.7-1.7)	1.2 (0.8-1.8)	1.0 (0.6-1.6)
Interleukin-8 in pg/ml	5.5 (4.4-8.0)	5.7 (4.4-8.0)	5.5 (4.5-7.9)
Interleukin-10 in pg/ml	0.3 (0.2-0.4)	0.3 (0.2-0.4)	0.3 (0.2-0.4)
TNF-alpha in pg/ml	2.2 (1.8-2.8)	2.2 (1.9-2.7)	2.2 (1.7-2.8)
Summary z-score	-0.1 (-1.4, 1.1)	-0.1 (-1.0, 1.2)	-0.1 (-1.6, 1.1)
Sun exposure[d], n (%)			
<7 days	87 (33)	41 (37)	41 (30)
7 days	173 (67)	70 (63)	94 (70)
Number of comorbidities, n (%)			
None	49 (19)	18 (16)	29 (21)
1	57 (22)	21 (19)	36 (26)
≥2	155 (59)	72 (65)	71 (52)
Body mass index in kg/m^2, mean (SD)	28.4 (4.5)	29.2 (5.0)	27.6 (4.1)
Physical activity, median (IQR)			
LPA (hours/week)	11 (4-23)	11 (4-21)	12 (4-30)
MVPA (hours/week)	11 (5-20)	11 (4-19)	12 (6-21)
Current smoking, n (%)			
Yes	33 (13)	15 (14)	16 (12)
No	227 (87)	96 (86)	119 (88)

Abbreviations: standard deviation (SD), interquartile range (IQR), light-intensity physical activity (LPA), moderate-to-vigorous intensity physical activity (MVPA).

[a] The number of participants may slightly vary for the different variables given a small percentage of missing data.

[b] Fourteen participants had unknown 25OHD$_3$ status at time of inclusion.

[c] Eleven participants had missing stage I, II, or III CRC.

[d] Number of days per week spent at least 15 minutes outside (in daylight), on average over the preceding 4 weeks.

Table 2. Descriptive statistics of $25OHD_3$ concentrations, vitamin D supplement use and dietary intake, health-related quality of life outcomes, and other important characteristics of patients with colorectal cancer from the EnCoRe study, from inclusion (colorectal cancer diagnosis) to 24 months after treatment.

	At diagnosis	Post-treatment follow-up measurements			
		6 weeks	6 months	12 months	24 months
	n=261[a]	*n=260*	*n=213*	*n=168*	*n=77*
$25OHD_3$ concentration in nmol/L, median (IQR)	53.1 (40.6-67.6)	48.0 (35.2-66.1)	53.4 (41.8-69.4)	57.0 (42.5-70.5)	62.9 (46.5-82.4)
Vitamin D deficiency, n (%)					
Yes (<50 nmol/L)	111 (45)	133 (53)	87 (42)	60 (36)	22 (30)
No (≥ 50 nmol/L)	136 (55)	120 (47)	120 (58)	106 (64)	52 (70)
Vitamin D supplement use, n (%)					
Yes	62 (24)	49 (19)	44 (21)	38 (23)	17 (22)
No	194 (76)	207 (81)	163 (79)	129 (77)	60 (78)
Vitamin D intake from supplements in µg/d, median (IQR)	5 (5-10)	5 (5-10)	6 (5-15)	7 (5-20)	10 (5-20)
Dietary vitamin D intake[b] in µg/d, median (IQR)	3.5 (2.5-4.7)	4.1 (2.9-5.6)	3.7 (2.8-4.7)	3.8 (2.9-5.0)	3.7 (2.6-4.6)
EORTC QLQ-C30[c]					
Global quality of life, mean (SD)		74.1 (18.3)	76.9 (19.0)	77.8 (18.0)	79.6 (18.5)
Range (min-max)		16.7-100	0-100	0-100	33.3-100
Cognitive functioning, mean (SD)		86.3 (20.8)	85.9 (19.1)	87.6 (19.4)	87.7 (15.1)
Range (min-max)		0-100	16.7-100	16.7-100	33.3-100
Fatigue, mean (SD)		29.1 (23.0)	23.6 (21.6)	21.6 (23.0)	19.3 (21.5)
Range (min-max)		0-100	0-100	0-100	0-88.9
Checklist Individual Strength (incl. 4 subscales)[d]					
Total fatigue, mean (SD)		62.9 (26.4)	59.3 (27.4)	54.0 (25.9)	51.5 (25.2)
Range (min-max)		20-127	20-132	20-134	20-101
Subjective fatigue, mean (SD)		27.2 (13.3)	24.9 (12.8)	22.6 (12.3)	21.3 (12.8)
Range (min-max)		(8-56)	(8-56)	(8-56)	(8-54)
Reduced motivation, mean (SD)		12.3 (6.1)	12.0 (6.2)	10.9 (6.1)	10.5 (5.9)
Range (min-max)		(4-28)	(4-27)	(4-28)	(4-27)
Reduced physical activity, mean (SD)		10.6 (5.1)	9.6 (5.1)	8.6 (5.0)	8.1 (4.8)
Range (min-max)		(3-21)	(3-21)	(3-21)	(3-21)
Concentration problems, mean (SD)		12.9 (7.3)	12.8 (7.3)	11.9 (6.7)	11.6 (6.5)
Range (min-max)		(5-34)	(5-33)	(5-31)	(5-28)
Hospital Anxiety and Depression Scale (incl. 2 subscales)[e]					
Total depression and anxiety, mean (SD)		7.4 (6.4)	7.4 (6.7)	6.8 (6.2)	5.6 (5.5)
Range (min-max)		0-30	0-32	0-33	0-28
Depression, mean (SD)		3.8 (3.6)	3.9 (3.9)	3.4 (3.4)	2.7 (3.1)
Range (min-max)		(0-15)	(0-19)	(0-17)	(0-14)
Anxiety, mean (SD)		3.6 (3.4)	3.5 (3.4)	3.4 (3.5)	2.9 (3.1)
Range (min-max)		(0-16)	(0-15)	(0-16)	(0-16)

Table 2 continued.

	At diagnosis	Post-treatment follow-up measurements			
		6 weeks	6 months	12 months	24 months
	n=261[a]	*n=260*	*n=213*	*n=168*	*n=77*
Inflammatory markers, median (IQR)					
Interleukin-6, pg/ml	1.1 (0.7-1.7)	1.5 (0.8-2.2)	1.2 (0.8-2.0)	0.9 (0.6-1.4)	0.9 (0.5-1.5)
Interleukin-8, pg/ml	5.5 (4.4-8.0)	5.6 (4.4-7.3)	5.2 (4.4-7.0)	3.9 (3.1-4.8)	4.8 (3.8-6.2)
Interleukin-10, pg/ml	0.3 (0.2-0.4)	0.4 (0.3-0.5)	0.4 (0.2-0.5)	0.2 (0.2-0.4)	0.2 (0.1-0.3)
TNF-alpha, pg/ml	2.2 (1.8-2.8)	2.9 (2.4-3.8)	2.8 (2.3-3.6)	2.0 (1.6-2.5)	2.0 (1.6-2.9)
Summary z-score	-0.1 (-1.4, 1.1)	-0.1 (-1.5, 1.1)	-0.2 (-1.1, 1.1)	-0.3 (-1.4, 1.0)	0.1 (-1.5, 1.3)
Sun exposure[f], n (%)					
<7 days	87 (33)	119 (46)	87 (41)	65 (39)	32 (44)
7 days	173 (67)	141 (54)	124 (59)	102 (61)	40 (56)
Number of comorbidities, n (%)					
None	49 (19)	55 (21)	49 (21)	41 (24)	16 (21)
1	57 (22)	64 (25)	51 (24)	39 (23)	17 (22)
≥2	155 (59)	141 (54)	113 (53)	87 (52)	43 (57)
Body mass index in kg/m^2, mean (SD)	28.4 (4.5)	27.7 (4.4)	28.2 (4.4)	28.5 (4.5)	28.5 (5.0)
Physical activity, median (IQR)					
LPA (hours/week)	11 (4-23)	8 (2-16)	11 (4-21)	11 (3-22)	11 (5-24)
MVPA (hours/week)	11 (5-20)	7 (3-14)	9 (4-15)	9 (4-18)	8 (3-18)
Current smoking, n (%)					
Yes	33 (13)	24 (9)	17 (8)	17 (10)	6 (8)
No	227 (87)	235 (91)	194 (92)	150 (90)	66 (92)

Abbreviations: interquartile range (IQR), standard deviation (SD), TNF (tumor necrosis factor), light physical activity (LPA), moderate-to-vigorous physical activity (MVPA).

[a] Participants with at least 1 follow-up measurement with available data on both vitamin D and quality of life were included in the analyses. The numbers of included participants decrease during follow-up because data collected until November 1st, 2016 were used and participants had not yet reached their follow-up measurement at this time. The number of participants may slightly vary for the different variables given a small percentage of missing data.

[b] Dietary intake of vitamin D was measured by a semi-quantitative 253-item food frequency questionnaire at diagnosis, and by a 7-day dietary record at each follow-up measurement.

[c] Ranges EORTC QLQ-C30 subscales: 0-100.

[d] Ranges Checklist Individual Strength: total score, 20-140; subjective fatigue, 8-56; reduced motivation, 4-28; reduced physical activity, 3-21; concentration problems, 5-35.

[e] Ranges Hospital Anxiety and Depression Scale: total score, 0-42; depression, 0-21; anxiety, 0-21.

[f] Number of days per week spent at least 15 minutes outside (in daylight), on average over the preceding 4 weeks.

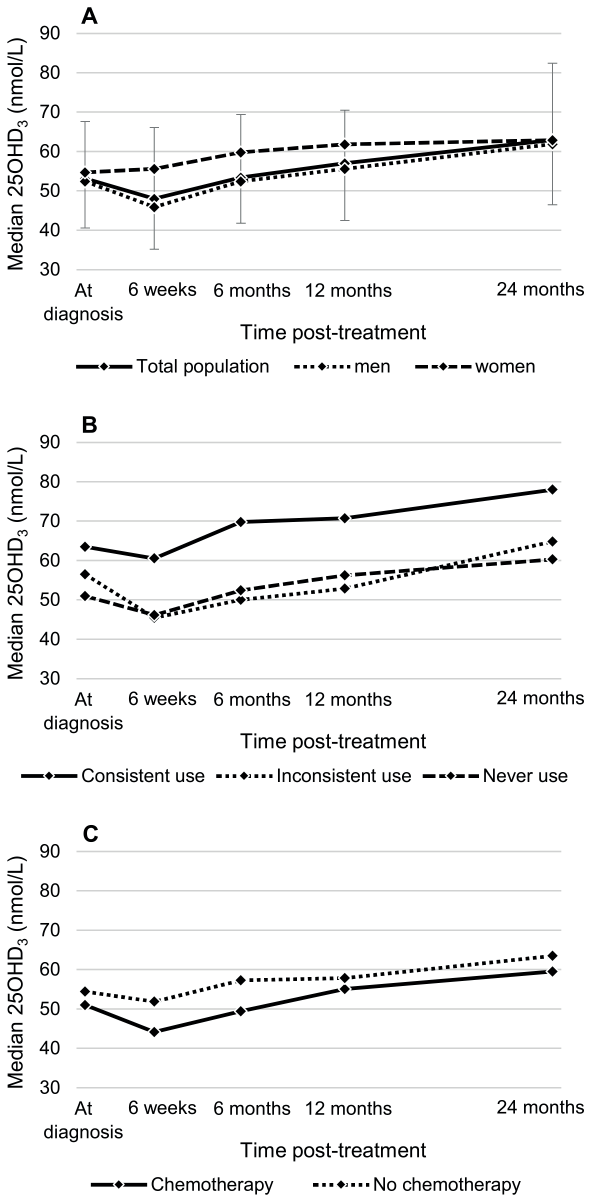

Figure 1. Median concentrations of $25OHD_3$ over time from colorectal cancer diagnosis (study inclusion) to 24 months after treatment for patients with colorectal cancer included in the EnCoRe study, in the total population (with interquartile range) and stratified by sex (panel A), stratified by vitamin D supplement user type (panel B), and stratified by chemotherapy (panel C). The actual time between the measurement at diagnosis and the measurement 6 weeks post-treatment can vary between individual patients due to differences in treatment duration.

Table 3. Results of mixed-model analyses on changes of health-related quality of life outcomes over time and longitudinal associations of vitamin D with health-related quality of life outcomes in colorectal cancer patients from the EnCoRe study followed up from diagnosis to 2 years post-treatment.

	Change in quality of life outcome over time[a]	Longitudinal associations of $25OHD_3$ concentrations (per 20 nmol/L)				Longitudinal associations of vitamin D deficiency (yes/no)			
		Adjusted model I[b]	Adjusted model II[c]			Adjusted model I[b]	Adjusted model II[c]		
			Overall	Intra-individual	Inter-individual		Overall	Intra-individual	Inter-individual
	ß (95% CI)	ß (95% CI)	ß (95% CI)	ß (95% CI)	ß (95% CI)	ß (95% CI)	ß (95% CI)	ß (95% CI)	ß (95% CI)
EORTC QLQ-C30[d]									
Global Quality of Life	**1.3 (0.4, 2.1)**	**3.6 (2.2, 5.0)**	**2.9 (1.5, 4.3)**	**3.7 (1.7, 5.6)**	**2.1 (0.2, 4.1)**	**5.7 (3.0, 8.4)**	**4.4 (1.6, 7.2)**	**4.4 (0.9, 7.9)**	4.4 (-0.03, 8.9)
Cognitive function	-0.1 (-0.9, 0.7)	0.3 (-1.1, 1.7)	0.3 (-1.1, 1.8)	0.1 (-1.9, 2.0)	0.8 (-1.6, 3.1)	0.9 (-1.9, 3.6)	1.1 (-1.7, 3.9)	0.2 (-3.2, 3.5)	3.2 (-2.0, 8.5)
Fatigue	**-2.8 (-3.8, -1.8)**	**-4.8 (-6.5, -3.1)**	**-3.5 (-5.3, -1.8)**	**-4.4 (-6.9, -2.0)**	**-2.6 (-5.1, -0.2)**	**-7.4 (-10.8, -4.1)**	**-5.2 (-8.6, -1.8)**	**-5.0 (-9.3, -0.7)**	**-5.7 (-11.2, -0.1)**
Checklist Individual Strength (incl. 4 subscales)[e]									
Total fatigue	**-3.3 (-4.3, -2.2)**	**-4.5 (-6.4, -2.7)**	**-2.8 (-4.7, -0.9)**	**-2.5 (-4.9, -0.1)**	**-3.2 (-6.1, -0.2)**	**-8.1 (-11.7, -4.5)**	**-5.4 (-9.0, -1.8)**	**-4.3 (-8.6, -0.1)**	**-7.9 (-14.6, -1.3)**
Subjective fatigue	**-1.7 (-2.2, -1.2)**	**-2.5 (-3.4, -1.5)**	**-1.5 (-2.4, -0.6)**	**-1.7 (-2.9, -0.4)**	-1.3 (-2.8, 0.2)	**-4.4 (-6.3, -2.6)**	**-2.9 (-4.7, -1.2)**	**-2.8 (-4.9, -0.7)**	**-3.3 (-6.5, -0.03)**
Reduced motivation	**-0.6 (-0.8, -0.3)**	**-0.5 (-0.9, -0.1)**	-0.2 (-0.6, 0.3)	0.0 (-0.6, 0.6)	-0.4 (-1.0, 0.3)	**-1.5 (-2.3, -0.6)**	**-1.0 (-1.9, -0.1)**	-0.8 (-1.9, 0.3)	-1.4 (-2.9, 0.1)
Reduced physical activity	**-0.8 (-1.0, -0.5)**	**-0.9 (-1.3, -0.5)**	**-0.5 (-0.9, -0.1)**	-0.4 (-0.9, 0.2)	**-0.6 (-1.2, -0.1)**	**-1.6 (-2.4, -0.8)**	**-0.9 (-1.7, -0.2)**	-0.7 (-1.6, 0.3)	**-1.4 (-2.6, -0.2)**
Concentration problems	-0.3 (-0.6, 0.1)	**-0.7 (-1.2, -0.1)**	**-0.6 (-1.2, -0.1)**	-0.5 (-1.2, 0.3)	**-0.8 (-1.7, -0.1)**	-0.8 (-1.8, 0.2)	-0.7 (-1.7, 0.4)	-0.1 (-1.4, 1.2)	**-1.9 (-3.7, -0.1)**

Table continues on next page.

Table 3 continued.

	Change in quality of life outcome over time[a]	Longitudinal associations of $25OHD_3$ concentrations (per 20 nmol/L)				Longitudinal associations of vitamin D deficiency (yes/no)			
		Adjusted model I[b]	Adjusted model II[c]			Adjusted model I[b]	Adjusted model II[c]		
			Overall	Intra-individual	Inter-individual		Overall	Intra-individual	Inter-individual
	ß (95% CI)	ß (95% CI)	ß (95% CI)	ß (95% CI)	ß (95% CI)	ß (95% CI)	ß (95% CI)	ß (95% CI)	ß (95% CI)
Hospital Anxiety and Depression Scale (incl. 2 subscales)[f]									
Total anxiety/ depression	-0.2 (-0.4, 0.1)	-0.1 (-0.5, 0.4)	-0.0 (-0.5, 0.4)	0.1 (-0.5, 0.6)	-0.2 (-1.0, 0.5)	-0.1 (-0.9, 0.7)	-0.1 (-0.9, 0.7)	-0.1 (-0.9, 1.0)	-0.6 (-2.3, 1.1)
Depression	**-0.1 (-0.3, -0.0)**	-0.1 (-0.4, 0.1)	-0.1 (-0.3, 0.2)	0.0 (-0.3, 0.3)	-0.2 (-0.6, 0.2)	-0.2 (-0.7, 0.3)	-0.1 (-0.6, 0.4)	-0.04 (-0.6, 0.5)	-0.4 (-1.3, 0.7)
Anxiety	-0.1 (-0.2, 0.1)	0.1 (-0.2, 0.3)	0.1 (-0.2, 0.3)	0.1 (-0.2, 0.4)	0.0 (-0.4, 0.4)	0.05 (-0.4, 0.5)	0.03 (-0.4, 0.5)	0.1 (-0.4, 0.7)	-0.2 (-1.1, 0.7)

Abbreviations: beta-coefficient (ß), confidence interval (CI).

[a] Changes in quality of life scores over time were measured from 6 weeks to 24 months after treatment, in units of 6 months.

[b] Model I: adjusted for sex, age at diagnosis.

[c] Model II: adjusted for sex, age at diagnosis, time since diagnosis, cancer treatment (neither chemotherapy nor radiotherapy, chemotherapy only, radiotherapy only, both chemotherapy and radiotherapy), no. of comorbidities (0, 1, ≥2), moderate-to-vigorous physical activity (hours/week), body mass index (kg/m^2).

[d] Ranges EORTC QLQ-C30 subscales: 0-100.

[e] Ranges Checklist Individual Strength: total score, 20-140; subjective fatigue, 8-56; reduced motivation, 4-28; reduced physical activity, 3-21; concentration problems, 5-35.

[f] Ranges Hospital Anxiety and Depression Scale: total score, 0-42; depression, 0-21; anxiety, 0-21.

Table 4. Longitudinal associations of vitamin D supplement use, vitamin D intake from diet and supplements, and vitamin D intake from supplements only, with health-related quality of life outcomes in colorectal cancer patients from the EnCoRe study followed up from diagnosis to 2 years post-treatment.

	Vitamin D supplement use (no/yes)[a]			Sum vitamin D intake from diet and supplements (µg/d)[b]			Vitamin D intake from supplements (µg/d)[c]		
	Overall	Intra-individual	Inter-individual	Overall	Intra-individual	Inter-individual	Overall	Intra-individual	Inter-individual
	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)
EORTC QLQ-C30									
Global Quality of Life	-1.5 (-6.2, 3.2)	2.7 (-3.5, 8.8)	-5.2 (-11.0, 0.7)	-0.1 (-0.4, 0.1)	0.2 (-0.1, 0.6)	**-0.4 (-0.7, -0.1)**	-0.05 (-0.4, 0.3)	0.2 (-0.2, 0.7)	-0.3 (-0.7, 0.1)
Fatigue	0.3 (-5.5, 6.0)	-5.6 (-12.9, 1.8)	5.9 (-1.4, 13.1)	0.1 (-0.2, 0.4)	-0.2 (-0.6, 0.2)	0.3 (-0.1, 0.7)	-0.01 (-0.4, 0.3)	-0.3 (-0.8, 0.2)	0.2 (-0.3, 0.7)
Checklist Individual Strength									
Total fatigue	5.3 (-1.0, 11.6)	4.2 (-3.2, 11.7)	6.8 (-1.8, 15.5)	0.2 (-0.1, 0.5)	0.2 (-0.3, 0.6)	0.3 (-0.2, 0.7)	0.4 (-0.0, 0.8)	**0.5 (0.01, 1.0)**	0.2 (-0.4, 0.8)

[a] Adjusted for sex, age at diagnosis, overall supplement use (y/n), time since diagnosis, cancer treatment (neither chemotherapy nor radiotherapy, chemotherapy only, radiotherapy only, both chemotherapy and radiotherapy), no. of comorbidities (0, 1, ≥2), moderate-to-vigorous physical activity (hours/week), body mass index (kg/m^2).

[b] Adjusted for sex, age at diagnosis, overall supplement use (y/n), total energy intake (kcal/d), time since diagnosis, cancer treatment (neither chemotherapy nor radiotherapy, chemotherapy only, radiotherapy only, both chemotherapy and radiotherapy), no. of comorbidities (0, 1, ≥2), moderate-to-vigorous physical activity (hours/week), body mass index (kg/m^2).

[c] Adjusted for sex, age at diagnosis, time since diagnosis, cancer treatment (neither chemotherapy nor radiotherapy, chemotherapy only, radiotherapy only, both chemotherapy and radiotherapy), no. of comorbidities (0, 1, ≥2), moderate-to-vigorous physical activity (hours/week), body mass index (kg/m^2).

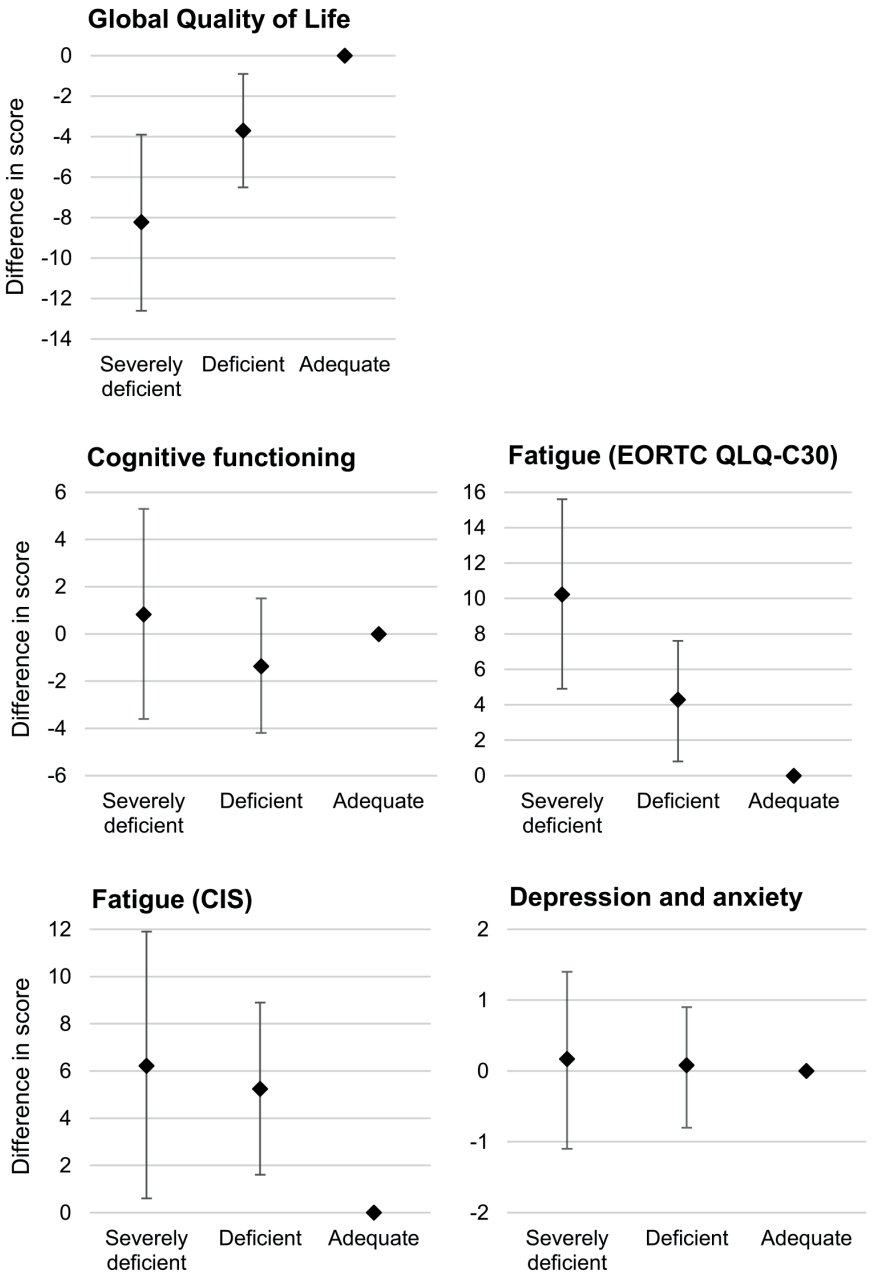

Figure 2. Comparison of scoring on quality of life outcomes over time for participants having severely deficient (<30 nmol/L) and deficient (30-50 nmol/L) $25OHD_3$ concentrations, with participants having adequate concentrations (>50 nmol/L), from 6 weeks to 24 months after treatment in colorectal cancer patients from the EnCoRe study.

Discussion

To our knowledge, the current study is the first to investigate longitudinal associations between serum $25OHD_3$ concentrations and HRQoL outcomes in CRC survivors. Higher concentrations of $25OHD_3$ were longitudinally associated with better global quality of life and less fatigue from 6 weeks up to 2 years after CRC treatment, both within and between individuals. In addition, having a vitamin D deficiency was associated with lower global quality of life and more fatigue. Intra-individual associations suggested better global quality of life and less fatigue for individuals who started using vitamin D supplements during follow-up.

The prevalence of vitamin D deficiency increased from 45% at diagnosis to 53% 6 weeks post-treatment (cut-off 50 nmol/L). The latter is slightly higher compared to the general Dutch population aged 60+ with prevalences of 34-51%.[48] A study among 2910 stage I-IV CRC patients from Germany observed deficiencies in 84% of participants, yet samples were assessed approximately 3 weeks after diagnosis.[49] Another study among 1598 Scottish stage I-III CRC patients found concentrations <25 nmol/L among 50% of participants approximately 15 weeks post-treatment.[50] This compares to only 10% of participants when using the cut-off point of 25 nmol/L in our population. Differences may be due to vitamin D supplement use, latitude, and time of sampling as concentrations in our study steadily increased after treatment. Nevertheless, the important health concern of vitamin D deficiency among this group of patients is indisputable.

Previous studies examining the potential relation between vitamin D and aspects of HRQoL are still inconclusive and scarce among population subgroups such as cancer survivors. A systematic literature review concluded a moderate positive effect of short-term vitamin D supplementation on HRQoL in clinical populations, not including cancer survivors.[51] Despite the large heterogeneity between studies included in this review, results correspond to the observed associations of the current study. Regarding fatigue, contradicting results were found in two intervention studies. A randomized placebo-controlled trial among patients with chronic fatigue syndrome found no effect of vitamin D supplementation,[52] whereas another trial for treating fatigue among otherwise healthy individuals observed lower fatigue among vitamin D supplement users compared to a placebo group.[53] A retrospective study among 100 ambulatory advanced cancer patients, including gastrointestinal cancer patients, found no association between vitamin D concentrations and symptoms of depression, anxiety, and fatigue.[54] Our study neither found associations between serum $25OHD_3$ concentrations and depression, anxiety, and cognitive functioning, potentially caused by the lack of variation over time in these outcomes. In summary, results from the current study are largely in line with the literature, yet comparing results is difficult given the large differences in methodology and study parameters between studies.

The observed effect sizes for associations with global quality of life and fatigue were small and could raise questions about clinical relevance. Minimally important differences for global quality of life and fatigue (EORTC QLQ-C30) were defined as 10 and 9 points[55,56], respectively, and for overall fatigue (CIS) as 10 points.[57] Increments of 20 nmol/L 25OHD$_3$ were used as clinically relevant contrast as it resembled 1 SD and represented realistic fluctuations in intra-individual 25OHD$_3$ concentrations. Although effect sizes were larger in dichotomous analyses (vitamin D deficiency yes/no), none of the effect sizes reached the level of clinical relevance. Nevertheless, observed beta-coefficients suggest the presence of a longitudinal association between higher 25OHD$_3$ and better HRQoL.

An important strength of the current study was the prospective character with repeated measurements of 25OHD$_3$ and HRQoL outcomes. Further, follow-up response rates were high and the percentage of missing data was low. Another strength was the use of mixed-model analysis techniques that enabled disentangling of inter- and intra-individual associations, thereby providing additional insights into the nature of the associations. A limitation of the current study concerns the inability to draw conclusions on causality. Observed associations could be due to the fact that CRC survivors with poorer HRQoL spend more time indoors, resulting in lower 25OHD$_3$ concentrations. However, adjustment for sun exposure did not change the associations. Additionally, despite adjustment for important variables such as therapy, residual confounding may have occurred, as observed associations could be caused by unmeasured underlying factors related to the cancer that also influenced biological mechanisms related to 25OHD$_3$ and HRQoL.

The use of vitamin D supplements was low despite the national recommendations that applied to about half of the study population. Although vitamin D supplement users on average had higher serum 25OHD$_3$ concentrations compared to nonusers, no direct beneficial association of vitamin D supplement use with HRQoL was found. Additionally, associations of 25OHD$_3$ with global quality of life and fatigue were also present in participants not using vitamin D supplements. In fact, vitamin D supplement use itself seemed associated with poorer HRQoL and more fatigue in inter-individual associations. An alternative explanation, also hypothesized by Patterson et al.,[58] is that supplement use may be a coping strategy rather than actually improving health of cancer survivors. Associations with supplement use should therefore be interpreted with caution, since individuals with poorer HRQoL and more fatigue may begin to use (vitamin D) supplements as a way to alleviate complaints.[5] Intra-individual associations contrarily suggested that individuals who started using supplements during follow-up were generally likely to report better global quality of life and less fatigue. Whether this association is causal needs to be addressed in intervention studies.

Our study does not provide strong enough evidence to formulate recommendations on the necessity of vitamin D supplementation for CRC survivors. However, higher $25OHD_3$ concentrations seem beneficial, and CRC survivors are advised to follow the guidelines for the general Dutch population since no specific guidelines on vitamin D for cancer survivors are available. According to the national guidelines, women aged >50 and women and men aged >70 are recommended to use vitamin D supplements (10 and 20 mcg, respectively) to ensure adequate blood concentrations for the prevention of osteoporosis.[14] In addition, all CRC survivors should spend the recommended time outdoors to sufficiently expose their skin to sunlight.[17] In practice, however, many CRC survivors seem unaware of the prevailing recommendations, potentially as a consequence of the lack of proper information provision.

In conclusion, our results suggest that higher $25OHD_3$ concentrations are longitudinally associated with better global quality of life and reduced fatigue in CRC survivors. It is important for CRC survivors to become aware of the national guidelines regarding sun exposure and vitamin D supplementation, and individual $25OHD_3$ status should be monitored by medical professionals. Placebo-controlled randomized trials are needed to examine the potential advantage of the use of vitamin D supplements in CRC survivors for improvement of HRQoL and fatigue to clarify questions on cause and effect and to deepen the understanding of possible underlying mechanisms.

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Supplemental Table 1. Results of subgroup analyses of $25OHD_3$ concentrations (per 20 nmol/L) with health-related quality of life outcomes by sex, age, vitamin D supplement use and (neo-)adjuvant chemotherapy.

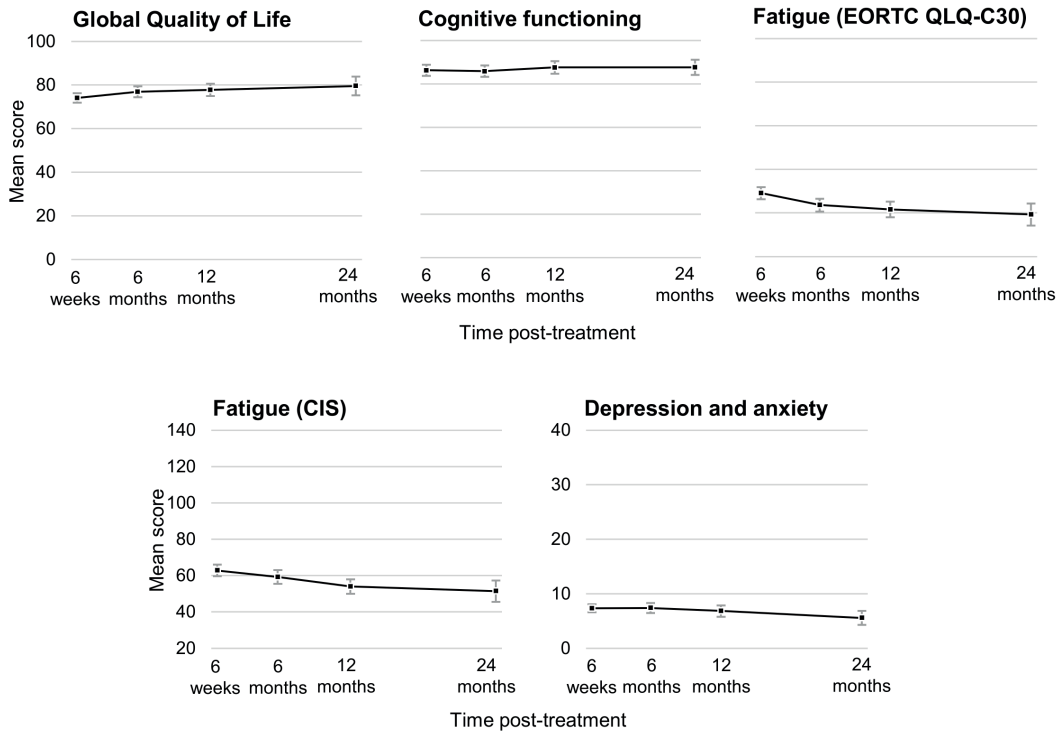
	$25OHD_3$ concentrations (per 20 nmol/L)							
	Sex[a]		Age at diagnosis[b]		Vitamin D supplement use[c]		(Neo-)adjuvant chemotherapy[d]	
	Men *n=179*	Women *n=81*	<70 *n=164*	≥70 *n=96*	Users *n=80*	Nonusers *n=180*	Yes *n=98*	No *n=162*
	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)
EORTC-QLQ-C30								
Global Quality of Life	**4.1 (2.2, 6.0)**	1.4 (-0.7, 3.5)	**3.1 (1.3, 4.9)**	**2.6 (0.3, 4.9)**	2.2 (-0.1, 4.5)	**3.9 (2.0, 5.8)**	**3.4 (1.1, 5.7)**	**2.7 (0.9, 4.5)**
Fatigue	**-4.8 (-7.3, -2.4)**	-1.7 (-4.3, 0.8)	**-3.5 (-5.9, -1.2)**	**-3.3 (-6.0, -0.7)**	**-2.9 (-5.5, -0.3)**	**-4.5 (-7.0, -2.1)**	**-3.6 (-6.5, -0.6)**	**-3.6 (-5.8, -1.5)**
Checklist Individual Strength								
Total fatigue	**-4.3 (-6.7, -1.8)**	-0.5 (-3.6, 2.6)	-2.1 (-4.6, 0.3)	**-3.3 (-6.3, -0.3)**	-0.8 (-3.7, 2.0)	**-4.8 (-7.4, -2.1)**	-1.7 (-4.6, 1.3)	**-3.5 (-6.0, -1.1)**

[a] Adjusted for age at diagnosis, time since diagnosis, cancer treatment (neither chemotherapy nor radiotherapy, chemotherapy only, radiotherapy only, both chemotherapy and radiotherapy), no. of comorbidities (0, 1, ≥2), moderate-to-vigorous physical activity (hours/week), body mass index (kg/m^2).

[b] Adjusted for sex, time since diagnosis, cancer treatment (neither chemotherapy nor radiotherapy, chemotherapy only, radiotherapy only, both chemotherapy and radiotherapy), no. of comorbidities (0, 1, ≥2), moderate-to-vigorous physical activity (hours/week), body mass index (kg/m^2).

[c] Adjusted for sex, age at diagnosis, time since diagnosis, cancer treatment (neither chemotherapy nor radiotherapy, chemotherapy only, radiotherapy only, both chemotherapy and radiotherapy), no. of comorbidities (0, 1, ≥2), moderate-to-vigorous physical activity (hours/week), body mass index (kg/m^2).

[d] Adjusted for sex, age at diagnosis, time since diagnosis, radiotherapy (y/n), no. of comorbidities (0, 1, ≥2), moderate-to-vigorous physical activity (hours/week), body mass index (kg/m^2).



Supplemental Figure 1. Mean quality of life outcome scores over time, including 95% confidence intervals, from 6 weeks to 24 months after treatment in colorectal cancer patients from the EnCoRe study.

CHAPTER 4

Circulating B-vitamin biomarkers and B-vitamin supplement use in relation to quality of life in patients with colorectal cancer: results from the FOCUS consortium

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ABSTRACT

B-vitamins have been associated with colorectal cancer (CRC) risk and progression given their central roles in nucleotide synthesis and methylation, yet their association with quality of life in established CRC is unclear. This study aimed to investigate whether: 1) circulating concentrations of B-vitamins and related biomarkers 6 months post-diagnosis, 2) changes in these concentrations between diagnosis and 6 months post-diagnosis, 3) B-vitamin supplement use 6 months post-diagnosis, and 4) changes in B-vitamin supplement use between diagnosis and 6 months post-diagnosis, are associated with quality of life at 6 months post-diagnosis.

We included 1676 newly-diagnosed stage I-III CRC patients from three prospective European cohorts. Circulating concentrations of nine biomarkers related to the B-vitamins folate, riboflavin, vitamin B6, and cobalamin were measured at diagnosis and 6 months post-diagnosis. Information on dietary supplement use was collected at both time points. Health-related quality of life (including global quality of life, functioning scales, and fatigue) was assessed by the EORTC QLQ-C30 questionnaire 6 months post-diagnosis. Confounder-adjusted linear regression analyses were performed, adjusted for multiple testing.

Higher pyridoxal 5'-phosphate (PLP) concentrations were cross-sectionally associated with better physical, role, and social functioning, and reduced fatigue at 6 months post-diagnosis. Higher HKr, an inverse marker of vitamin B6 status, was cross-sectionally associated with worse global quality of life, and lower physical and role functioning. Dose-response relations were observed for PLP with global quality of life, physical, role, and social functioning. No associations were observed for changes in biomarker concentrations between diagnosis and 6 months. Participants who stopped using B-vitamin supplements after diagnosis reported higher fatigue than nonusers.

Higher vitamin B6 status was associated with better quality of life, yet limited associations were observed for the use of B-vitamin supplements. Vitamin B6 needs further study to clarify its role in relation to quality of life post-CRC diagnosis.

Introduction

The diagnosis of colorectal cancer (CRC) imposes a major long-term burden on a patient's physical, emotional, and social well-being.[1] On average, 65% of CRC patients are alive 5 years after diagnosis, and the number of CRC survivors is expected to rise substantially in the coming decade.[2-4]

Folates are of particular interest in relation to CRC. Folate may have a protective effect regarding the development of CRC, whereas this vitamin could promote progression of already existing colorectal neoplasms.[5,6] Folate together with other B-vitamins, i.e. vitamin B2 (riboflavin), vitamin B6 (pyridoxal 5'-phosphate, PLP), and vitamin B12 (cobalamin), are important factors in DNA synthesis and methylation reactions through a network of interdependent metabolic pathways that mediate the transfer of one-carbon units.[5,6] It is unknown whether B-vitamins may play a role in reducing health problems among CRC survivors.

Fatigue is highly prevalent during anti-cancer therapy and generally decreases after treatment completion.[7] However, between 40 and 50% of CRC survivors still experience fatigue in the first year following diagnosis.[8,9] Folic acid and vitamin B12 supplementation may be effective in treating chronic fatigue syndrome in case of deficiencies,[10] although an intervention study in patients with irritable bowel syndrome or inflammatory bowel disease found no effect of vitamin B12 supplementation on fatigue,[11] B-vitamins may further be effective in improving cognitive functioning, as elevated homocysteine – a marker of folate and B12 deficiency – is associated with neuropsychiatric diseases.[12] Nonetheless, intervention studies investigating the effect of folic acid, and vitamin B6 and B12 supplement use, alone or combined, on cognitive functioning in elderly populations were not conclusive.[12-14] Circulating unmetabolized folic acid has been associated with adverse effects, including impaired cognitive function in the elderly.[15,16] Further, higher homocysteine concentrations have prospectively been associated with a decline in physical functioning in older adults.[17,18] Elevated homocysteine and low folate concentrations have been associated with the etiology of psychiatric problems.[19]

Dietary supplements are used by 20-85% of cancer survivors, yet benefits remain uncertain.[9,20,21] Investigating whether B-vitamin status and the use of B-vitamin supplements are associated with quality of life after CRC diagnosis is a first step in evaluating whether B-vitamins may be beneficial for quality of life. Additional insight may be obtained by investigating whether an increase or decrease in biomarker concentrations between diagnosis and 6 months post-diagnosis and the initiation or discontinuation of B-vitamin supplement use could influence quality of life.

The current study aims to investigate whether: 1) circulating concentrations of B-vitamins and related biomarkers 6 months post-diagnosis, 2) changes

in these concentrations between diagnosis and 6 months post-diagnosis, 3) B-vitamin supplement use 6 months post-diagnosis, and 4) changes in B-vitamin supplement use between diagnosis and 6 months post-diagnosis, are associated with quality of life at 6 months post-diagnosis.

Methods

The FOCUS consortium

Data were analyzed from three prospective cohort studies participating in the *'Biomarkers related to Folate-dependent One-carbon metabolism in colorectal Cancer recUrrence and Survival'* (FOCUS) consortium. The primary objective of the FOCUS consortium is to evaluate whether folate status at diagnosis and changes during and after treatment are related to prognosis of stage I-III CRC patients. Studies included in the current analyses are the COLON study from Wageningen University & Research, the Netherlands (ClinicalTrials.gov identifier: NCT03191110),[22] the EnCoRe study from Maastricht University, the Netherlands (Netherlands Trial Register number: NL6904),[23] and the Heidelberg site of the ColoCare study, from the National Center for Tumor Diseases (NCT) in Heidelberg, Germany (ClinicalTrials.gov Identifier: NCT02328677).[24] Newly diagnosed CRC patients aged >18 years were recruited upon diagnosis. Each study was approved by local ethics committees and all participants signed informed consent.

The current analyses included only patients with cancer stage I-III. A first baseline measurement took place shortly after diagnosis, prior to the start of treatment (COLON and EnCoRe) or prior to undergoing surgery (ColoCare). Patients (n=135) were excluded from the analyses on biomarker concentrations when baseline blood draw took place after the start of neo-adjuvant therapy. A follow-up measurement took place 6 months after baseline (COLON and ColoCare) or after treatment end date (EnCoRe). As the EnCoRe study includes an additional follow-up measurement at 6 weeks after treatment end date, either the 6 week or 6 month post-treatment measurement was selected for these analyses, based on the measurement that was closest to the time point 6 months post-diagnosis. In this way, timing of the measurements was comparable between studies.

Participants were included in the current analyses based on the availability of data (Figure 1). Information on the independent variables, i.e. biomarker concentrations or B-vitamin supplement use, was required to be available at diagnosis *and* 6 months after diagnosis in order to adjust for baseline biomarker concentrations and calculate changes between the time points. Ultimately, a total of 1,089 participants were included for the analyses on biomarker concentrations and 1,676 patients were included for the analyses on B-vitamin supplement use.

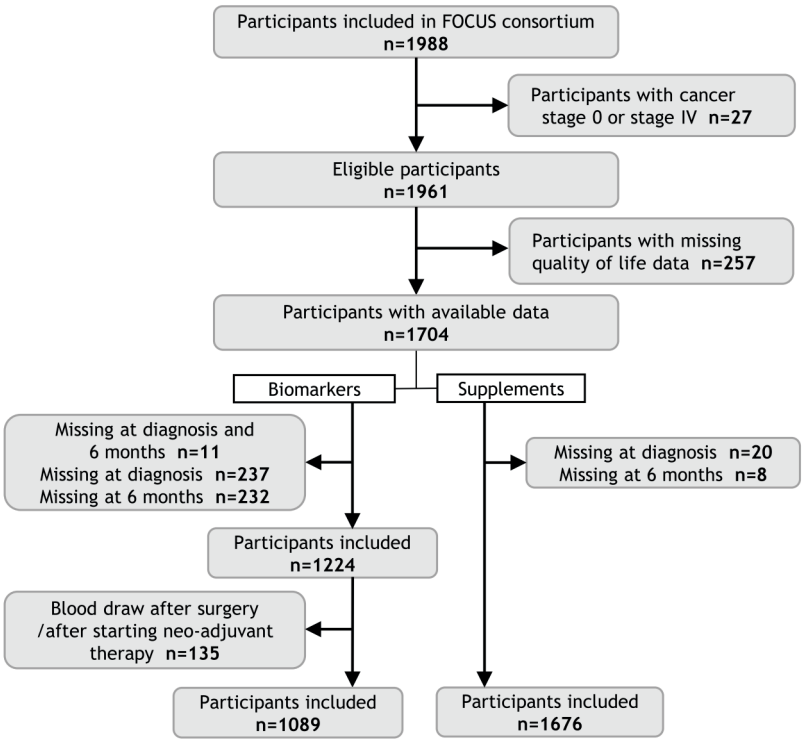


Figure 1. Flow diagram of the inclusion of participants in the analyses for (a) biomarker concentrations and (b) B-vitamin supplement use with health-related quality of life in colorectal cancer patients from the FOCUS consortium, for the cohorts with data relevant for the research question of this paper. Selections were made based on the availability of blood samples to measure biomarker concentrations and the availability of information on B-vitamin supplement use. The overlap between both datasets was 1077 participants (64%).

Blood biomarkers

EDTA plasma (EnCoRe and COLON) or serum (ColoCare) samples were collected, divided into aliquots, and stored at -80°C until analyses. Samples were shipped on dry ice to the laboratory of Bevital in Bergen, Norway (www.bevital.no). Available samples were shipped in 2 batches with a 1-year time interval from 2016-2018 to increase the final number of samples to be included for the analyses. ColoCare sample volumes were limited and therefore diluted (1:2). The dilution factor was taken into account by Bevital when analyzing the samples.

Nine principal biomarkers related to the folate-dependent one-carbon metabolism were examined. Total folate was analyzed as the sum of 5mTHF

and 4-α-hydroxy-5-methyltetrahydrofolate (hmTHF).[25] Folic acid, the synthetic form of folate, was also analyzed. Total homocysteine (tHcy) was included as a marker of folate and vitamin B12 status. Methylmalonic acid (MMA) was analyzed alongside cobalamin as a functional marker of vitamin B12 status. Methionine was included given its close connection with homocysteine and cobalamin in the methylation cycle. Riboflavin was included as marker of vitamin B2 status. PLP was included as the most commonly used marker of vitamin B6 status along with the HK ratio (HKr), a recently described functional marker of vitamin B6 status calculated as the ratio of 3-hydroxykynurenine (HK) to the sum of kynurenic acid (KA), xanthurenic acid (XA), 3'-hydroxyanthranilic acid (HAA), and anthranilic acid (AA).[26]

Liquid chromatography-tandem mass spectroscopy was used to analyze the folate species mTHF, hmTHF, and folic acid,[27] the tryptophan metabolites HK, KA, XA, HAA, and AA, and riboflavin, PLP, cotinine, and creatinine.[28,29] The biomarkers kynurenine, tryptophan, tHcy, methionine, and MMA were analysed by gas chromatography-tandem mass spectroscopy.[30] Cobalamin was measured by a microbiological assay,[31] and C-reactive protein (CRP) was measured by an immuno-MALDI-MS approach.[32]

Duplicate samples with known biomarker concentrations, as well as calibrator samples and a blank vial were added to each assay tray by Bevital to control for quality of the samples. Additionally, each cohort provided duplicate samples to be included on every plate. The coefficients of variation from a total of 160 duplicate samples ranged from 1.8% for cobalamin to 12.2% for hmTHF.

Use of supplements containing B-vitamins

Information on the use of supplements containing B-vitamins was collected by self-administered questionnaires (COLON and ColoCare) or interviews by research dieticians during home visits (EnCoRe). The COLON questionnaire contained questions on the intake of multivitamins, B-vitamin complexes, and single vitamin B12 and folic acid use and was developed by the Division of Human Nutrition and Health, Wageningen University & Research.[22] When multivitamins were used, participants were considered to be using B-vitamins. Within the EnCoRe study, participants were asked about the supplements they were using, and to show the original package when available. All details on e.g. (brand) name and frequency were listed on standardized registration forms. In ColoCare, detailed self-reported questionnaires were used to collect data on regular dietary supplement use. The intake of multivitamins, eleven single vitamins, six minerals, and 14 other ingredients (e.g. herbal supplements) as well as frequency of intake were recorded prior to surgery and six months thereafter. The reference period for supplement use at diagnosis was either the preceding year (COLON and EnCoRe) or the preceding month (ColoCare). At 6 months post-diagnosis, reference periods were the preceding 6 months (COLON and ColoCare), or the period since the previous measurement (EnCoRe) that

took place either at diagnosis (mean of 6.5 preceding months) or at 6 weeks post-treatment (mean of 4.5 preceding months) because the EnCoRe study included an additional follow-up measurement at 6 weeks after treatment end date.

For the current analyses, frequency of B-vitamin supplement use was defined as use of any dietary supplement (including multivitamins) containing at least one of the B-vitamins folate/folic acid, vitamin B2, vitamin B6 or vitamin B12, at least once per week, for a consecutive period of one month or more. Details on the dosages of B-vitamin intake were not considered in the current analyses. B-vitamin supplement use at diagnosis and 6 months after diagnosis was categorized into 4 mutually exclusive classes: 1) participants who started use after diagnosis, 2) participants who stopped use after diagnosis, 3) nonusers at both time points, and 4) users at both time points.

Health-related quality of life

At 6 months after diagnosis, all studies assessed health-related quality of life by the Quality of Life Questionnaire of the 'European Organisation for Research and Treatment of Cancer' (EORTC QLQ-C30, version 3.0), a 30-item validated questionnaire.[33,34] The subscale global quality of life, as well as 5 functional scales, i.e. physical, role, emotional, cognitive, and social functioning, and the symptom scale fatigue were analyzed. All scales range from 0-100 and a higher score indicates better quality of life and functioning, except for fatigue, where higher scores are indicative of more fatigue. Previously published thresholds for clinical importance were used to interpret the scores.[35]

Measurement of covariates

Information on cancer stage, therapy and other clinical variables were derived from medical records or clinical registries. In order to calculate body mass index (BMI; kg/m^2), height and weight were measured during home visits (EnCoRe), self-reported (COLON), or from anesthesia protocols (ColoCare). Adherence to the physical activity guideline to spend at least 150 minutes per week in moderate-to-vigorous physical activity was assessed based on self-report; using the SQUASH questionnaire (COLON and EnCoRe)[22,23] or the VITAL questionnaire (ColoCare).[24] Dietary information at 6 months post-diagnosis (i.e. total energy and alcohol intake) was assessed using a food frequency questionnaire by COLON and ColoCare, and by a 7-day dietary record in the EnCoRe cohort. Cotinine and creatinine concentrations were included in the models to adjust for nicotin exposure and kidney function, respectively, and were part of the biomarker analyses as measured at Bevital.

Statistical methods

Descriptive statistics were presented for the characteristics of the total study population and the individual cohorts, using means and standard deviations for normally distributed variables, medians and interquartile ranges for skewed variables, and frequencies for categorical variables. Four main analyses were performed, in line with the four objectives of the study. 1) Multivariable linear regression models were used to investigate the associations of biomarkers with health-related quality of life, in cross-sectional analyses at 6 months post-diagnosis. Biomarker concentrations were log2-transformed and the regression coefficients can therefore be interpreted as effects of a doubling in concentrations. 2) Multivariable linear regression analysis was used to analyze changes in biomarker concentrations between diagnosis and 6 months with health-related quality of life at 6 months post-diagnosis. Changes were calculated by subtracting the concentration at diagnosis from the concentration at 6 months. Subsequently, individual change scores were Z-standardized by subtracting the population mean change and dividing by the population standard deviation of change scores, in order to be able to compare the magnitudes of changes across the biomarkers. Change scores were normally distributed and therefore not transformed. 3) B-vitamin supplement use was modeled as a binary variable in cross-sectional analyses (use at 6 months: yes/no). 4) Changes in B-vitamin supplement use were modeled for the four different classes of supplement users and performed with three indicator variables with nonuse at every time point as the reference category.

An *a priori* selected set of confounders was used, based on the literature and their hypothesized relation with the exposure and outcome variables. The analyses on biomarker concentrations were adjusted for the confounders sex, age, cohort (COLON, EnCoRe, ColoCare), neo-adjuvant therapy (yes/no), adjuvant therapy (yes/no), cancer stage (I,II,III), any supplement use (yes/no), total energy intake (kcal/day), alcohol intake (g/day), BMI (kg/m^2), physical activity (adherence to international guidelines yes/no), cotinine (nmol/L), and creatinine (μmol/L). Adjustment for any supplement use was considered necessary because the use of supplements in general has previously been associated with low quality of life and increased fatigue.[9,36] Analyses of changes in biomarker concentrations were additionally adjusted for the concentration at diagnosis.

Analyses on B-vitamin supplement use were adjusted for sex, age, cohort (COLON, EnCoRe, ColoCare), neo-adjuvant therapy (yes/no), adjuvant therapy (yes/no), cancer stage (I,II,III), total energy intake (kcal/day), BMI (kg/m^2), physical activity (adherence to national guidelines yes/no), and any supplement use (yes/no). Any supplement use was added to the model for the aforementioned reason.

The cross-sectional analyses of biomarkers and supplement use at 6 months after diagnosis were adjusted for all above-mentioned covariates measured at 6 months after diagnosis. In the analyses of changes, covariates were added as the change between diagnosis and 6 months for BMI, cotinine, and creatinine. Other covariates were either not available at diagnosis for all cohorts, e.g. dietary variables, or not considered relevant to include as changes, e.g. age, and therefore added as measured at 6 months after diagnosis. Since many different exposures were associated with many different outcomes, the analyses for each of the four objectives were separately adjusted for multiple testing using the false discovery rate (FDR) method.[37]

Sub-analyses were performed to evaluate the influence of outliers on the analyses. Log2-transformed biomarker concentrations with values 3 standard deviations below or above the mean within each cohort were excluded and compared to the original analyses to evaluate their influence. Sensitivity analyses were done by stratification by cohort to evaluate associations within each cohort separately. Further, given the relation between B-vitamins and inflammation, the inflammatory markers CRP and KTR (kynurenine/tryptophan ratio) were used to evaluate potential confounding by inflammation when statistically significant associations were observed.[38,39] In addition, B-vitamin concentrations were modeled as index variables by categories in relation to quality of life to explore potential dose-response relationships, only performed when associations were still statistically significant after adjustment for multiple testing. Deficient, suboptimal, and adequate levels of vitamin B6 were defined as serum/plasma PLP concentrations of <20 nmol/L, 20-30 nmol/L, and >30 nmol/L, respectively.[40] Analyses were performed in R, version 3.6.1. *P* values below 0.05 after adjustment for multiple testing were considered statistically significant.

Results

Table 1 presents the characteristics of study participants (n=1,676) included in the analyses for the associations between B-vitamin supplement use and health-related quality of life. In general, characteristics of the 1,089 participants included in the analyses on biomarker concentrations were comparable to the characteristics presented in Table 1; the overlap between both datasets was 1,077 participants (64%). On average, 62% of the study population were colon cancer patients and 36% rectum/rectosigmoid cancer patients. ColoCare included a larger proportion of rectum/rectosigmoid cancer patients as compared to COLON and EnCoRe. One-fourth (26%) of participants were diagnosed with stage I CRC, 30% with stage II CRC, and 41% with stage III CRC. ColoCare participants were more commonly diagnosed with stage II CRC (41%) as compared to EnCoRe (23%) and COLON (29%) participants. In total, 96% of patients received surgery, 24% received neo-adjuvant therapy, and 26% received adjuvant therapy.

Table 1. Characteristics of study participants included in the FOCUS consortium, for the total population and stratified by cohort.

Participant characteristics[a]	Total population[b]	COLON	EnCoRe	ColoCare
Number of participants, n (%)	1676	1200 (71.6)	274 (16.4)	202 (12.1)
Men, n (%)	1068 (63.8)	758 (63.2)	184 (67.7)	126 (62.4)
Age at diagnosis in years, mean (SD)	65.6 (9)	65.8 (8.6)	66.5 (9.4)	63.3 (11.8)
Clinical characteristics				
Tumor location, n (%)				
Colon	1037 (61.9)	779 (64.9)	163 (59.5)	95 (47.0)
Rectum/Rectosigmoid	606 (36.2)	390 (32.5)	109 (39.8)	107 (53.0)
Unknown/Missing	33 (2.0)	31 (2.6)	2 (0.7)	0
Cancer stage, n (%)				
I	429 (25.6)	294 (24.5)	79 (28.8)	56 (27.7)
II	496 (29.6)	350 (29.2)	63 (23.0)	83 (41.1)
III	684 (40.8)	502 (41.8)	119 (43.4)	63 (31.2)
Unspecified/Unknown	67 (4.0)	54 (4.5)	13 (4.7)	0
Surgery, n (%)				
Yes	1606 (95.8)	1159 (96.6)	245 (89.4)	202 (100.0)
No	35 (2.1)	8 (0.7)	27 (9.9)	0
Unknown/Missing	35 (2.1)	33 (2.8)	2 (0.7)	0
Neo-adjuvant therapy, n (%)				
Yes	399 (23.8)	269 (22.4)	76 (27.4)	54 (26.7)
No	1242 (74.1)	899 (74.9)	196 (71.5)	147 (72.8)
Unknown/Missing	35 (2.1)	32 (2.7)	2 (0.7)	1 (0.5)
Adjuvant therapy, n (%)				
Yes	434 (25.9)	284 (23.7)	78 (28.5)	72 (35.6)
No	1147 (68.4)	828 (69.0)	194 (70.8)	125 (61.9)
Unknown/Missing	95 (5.7)	88 (7.3)	2 (0.7)	5 (2.5)
Lifestyle characteristics				
Body mass index in kg/m^2, mean (SD)	26.5 (4.0)	26.3 (3.9)	28.1 (4.5)	25.6 (3.8)
Adherence to physical activity guidelines[c], n (%)	990 (60.6)	766 (65.6)	169 (63.3)	55 (27.4)
Any supplement use, n (%)	625 (37.3)	487 (40.6)	90 (32.9)	48 (23.8)
Total energy intake[d] in kcal/day, mean (SD)	1900 (565)	1816 (501)	2062 (487)	2317 (888)
Alcohol intake[d] in gr/day, median (IQR)	5.2 (0.3-17.5)	4.8 (0.1-16.5)	5.7 (0.0-21.0)	9.0 (1.9-24.3)
Cotinine[e] in nmol/L, mean (SD)	90 (320)	84 (300)	101 (366)	117 (378)
Creatinine[e] in µmol/L, mean (SD)	82.4 (18.9)	81.1 (17.4)	85.2 (21.6)	87.2 (23.8)
Use of B-vitamin containing supplements[f], n (%)				
At diagnosis	402 (24.0)	318 (26.5)	63 (23.0)	21 (10.4)
6 months post-diagnosis	323 (19.3)	259 (21.6)	54 (19.7)	10 (5.0)

Table 1 continued.

Participant characteristics[a]	Total population[b]	COLON	EnCoRe	ColoCare
Users at diagnosis and 6 months	232 (13.8)	186 (15.5)	42 (15.3)	4 (2.0)
Participants who started use after diagnosis	91 (5.4)	73 (6.1)	12 (4.4)	6 (3.0)
Participants who stopped use after diagnosis	167 (10.0)	129 (10.8)	21 (7.7)	17 (8.4)
Nonusers at diagnosis and 6 months	1173 (70.0)	801 (66.8)	197 (71.9)	175 (86.6)
Health-related quality of life[g]				
Global quality of life[h]				
Scoring, mean (SD)	72.8 (20.0)	74.6 (19.0)	75.5 (19.2)	58.2 (20.5)
Physical functioning				
Scoring, mean (SD)	80.7 (18.9)	82.2 (18.0)	79.3 (19.0)	74.1 (22.0)
TCI ≤83, n (%)	742 (44.3)	497 (41.4)	129 (47.1)	116 (57.4)
Role functioning				
Scoring, mean (SD)	73.3 (29.6)	74.9 (29.2)	75.2 (27.6)	61.3 (31.7)
TCI ≤58, n (%)	418 (24.9)	282 (23.5)	56 (20.4)	80 (39.6)
Emotional functioning				
Scoring, mean (SD)	83.3 (20.2)	85.8 (17.5)	87.3 (18.1)	63.1 (26.0)
TCI ≤71, n (%)	389 (23.2)	230 (19.2)	45 (16.4)	114 (56.4)
Cognitive functioning				
Scoring, mean (SD)	84.7 (20.0)	85.4 (19.0)	85.8 (20.1)	78.6 (24.0)
TCI ≤75, n (%)	422 (25.2)	288 (24.0)	58 (21.2)	76 (37.6)
Social functioning				
Scoring, mean (SD)	80.8 (24.1)	82.6 (22.3)	84.4 (21.6)	65.5 (31.0)
TCI ≤58, n (%)	268 (16.0)	159 (13.3)	35 (12.8)	74 (36.6)
Fatigue				
Scoring, mean (SD)	29.0 (25.7)	27.6 (25.2)	26.1 (22.4)	41.3 (29.0)
TCI ≥39, n (%)	477 (28.5)	319 (26.6)	61 (22.3)	97 (48.0)

Abbreviations: interquartile range (IQR), standard deviation (SD), threshold for clinical importance (TCI).

[a] Characteristics are given for the time point 6 months post-treatment, except when stated otherwise.

[b] Data are given of the 1676 participants included in the analyses for B-vitamin supplement use and health-related quality of life. Participant characteristics of the 1089 participants included in the analyses on biomarker concentrations were comparable to the characteristics as presented here. The overlap between both datasets was 1077 participants (64%).

[c] Moderate-to-vigorous physical activity of at least 150 minutes per week.

[d] Total energy and alcohol intake were assessed using a food frequency questionnaire by COLON and ColoCare, and by 7-day dietary records by the EnCoRe cohort.

[e] Cotinine and creatinine concentrations were included as markers of nicotin exposure and kidney function, respectively, and were measured in plasma for EnCoRe and COLON, and in serum for ColoCare.

[f] B-vitamin supplement use was defined as the use of dietary supplements containing at least one of the B-vitamins folic acid, vitamin B2, vitamin B6 or vitamin B12.

[g] Ranges EORTC QLQ-C30 subscales: 0-100. Higher scores are indicative of better health-related quality of life, except for fatigue where higher scoring is indicative of more fatigue.

[h] No threshold for clinical importance available.

B-vitamin containing supplements were used by 24% of all participants at diagnosis and by 19% of participants after 6 months (Table 1). B-vitamin supplement use was considerably lower for ColoCare participants as compared to the Dutch cohorts. Further differences between the cohorts could be observed in lifestyle-related variables such as BMI, adherence to physical activity guidelines, and total energy and alcohol intake. Scoring on all subscales of health-related quality of life was lower for ColoCare as compared to EnCoRe and COLON (Table 1). Furthermore, 44% of all participants experienced clinically important impairment in physical functioning, between 20% and 30% in role, emotional, cognitive functioning and fatigue, and 16% in social functioning.

Minimal changes were observed in biomarker concentrations between baseline and 6 months after diagnosis in the total population (Table 2). Spearman correlations between the separate biomarkers were similar at the two times points, and demonstrated expected correlations, including (at diagnosis) of -0.46 for PLP with HKr, -0.30 for folate with tHcy, -0.29 for cobalamin with tHcy, and -0.27 for cobalamin with MMA (Supplemental Figure 1).

When biomarker concentrations were stratified by the four types of supplement users, the highest concentrations of folate, riboflavin, PLP, and cobalamin were observed at each time point for users of B-vitamin supplements and the lowest concentrations for nonusers (Supplemental Table 1). As could be anticipated, mean concentrations of participants who started using B-vitamin supplements showed an increase from diagnosis to 6 months post-diagnosis, whereas mean concentrations of participants who stopped using B-vitamin supplements after diagnosis showed a decrease towards the 6 month time point.

Table 2. Biomarker concentrations (median and interquartile range) at diagnosis and 6 months after diagnosis, and standardized changes (mean and standard deviation) of concentrations between diagnosis and 6 months, of colorectal cancer patients included in the FOCUS consortium, for the total population and stratified by cohort.

	Total folate[c] (nmol/L)	Folic acid[d] (nmol/L)	Total homocysteine (µmol/L)	Methionine (µmol/L)	Riboflavin (nmol/L)	Pyridoxal 5'-phosphate (nmol/L)	HKr[e]	Cobalamin (pmol/L)	Methyl-malonic acid (µmol/L)
Total population[a] (n=1089)									
At diagnosis	13.26 [9.15-20.03]	0.00 [0.00-0.00]	11.63 [9.71-14.41]	25.57 [21.38-31.25]	13.65 [8.84-23.23]	41.65 [29.59-61.98]	0.35 [0.29-0.43]	395 [316-490]	0.21 [0.17-0.26]
6 months	12.76 [8.69-19.04]	0.00 [0.00-0.00]	11.75 [9.77-14.63]	28.44 [24.42-33.46]	14.06 [9.05-23.25]	42.30 [29.25-62.85]	0.36 [0.28-0.44]	381 [302-482]	0.20 [0.17-0.26]
z-score of change[b]	-0.05 (0.81)	-0.01 (1.01)	-0.04 (0.72)	+0.01 (0.86)	-0.03 (0.83)	-0.03 (0.92)	+0.02 (0.95)	+0.00 (0.94)	+0.02 (0.44)
COLON (n=774)									
At diagnosis	12.20 [8.50-18.35]	0.00 [0.00-0.00]	11.32 [9.54-14.10]	25.48 [21.23-31.14]	13.70 [8.76-21.80]	42.10 [30.70-63.0]	0.36 [0.28-0.44]	405 [326-499]	0.20 [0.17-0.26]
6 months	12.16 [8.51-18.01]	0.00 [0.00-0.00]	11.51 [9.60-14.13]	28.62 [24.06-34.12]	14.25 [9.20-24.00]	43.20 [29.58-64.70]	0.34 [0.28-0.43]	395 [316-498]	0.20 [0.17-0.26]
z-score of change[b]	-0.05 (0.73)	+0.01 (0.75)	-0.07 (0.61)	+0.06 (0.89)	-0.01 (0.87)	-0.03 (1.00)	-0.04 (0.95)	+0.01 (0.94)	+0.02 (0.47)
EnCoRe (n=232)									
At diagnosis	13.81 [9.89-18.94]	0.00 [0.00-0.00]	12.41 [10.39-14.89]	25.84 [22.22-32.17]	11.80 [8.27-23.45]	43.05 [28.95-60.90]	0.34 [0.29-0.40]	383 [311-469]	0.22 [0.18-0.28]
6 months	12.22 [8.29-17.76]	0.00 [0.00-0.00]	12.25 [10.26-15.26]	27.71 [25.04-30.50]	11.40 [7.64-19.73]	41.70 [28.85-58.78]	0.37 [0.31-0.47]	355 [282-431]	0.21 [0.16-0.28]
z-score of change[b]	-0.19 (0.43)	-0.10 (1.65)	-0.03 (0.71)	-0.21 (0.71)	-0.12 (0.69)	-0.07 (0.66)	+0.23 (0.99)	-0.14 (0.88)	-0.01 (0.36)

Table continues on next page.

Table 2 continued.

	Total folate[c] (nmol/L)	Folic acid[d] (nmol/L)	Total homocysteine (µmol/L)	Methionine (µmol/L)	Riboflavin (nmol/L)	Pyridoxal 5'-phosphate (nmol/L)	HKr[e]	Cobalamin (pmol/L)	Methyl-malonic acid (µmol/L)
ColoCare (n=83)									
At diagnosis	23.87 [19.04-32.70]	0.00 [0.00-0.00]	12.26 [10.46-16.62]	24.78 [21.62-29.81]	18.88 [12.69-29.20]	31.80 [22.10-46.10]	0.38 [0.32-0.46]	318 [243-455]	0.20 [0.17-0.24]
6 months	23.94 [16.54-44.79]	0.00 [0.00-0.00]	13.50 [10.67-18.01]	31.08 [26.92-36.04]	22.60 [14.15-29.70]	35.20 [25.30-58.20]	0.38 [0.31-0.46]	334 [277-451]	0.21 [0.18-0.27]
z-score of change[b]	+0.30 (1.70)	+0.08 (0.74)	+0.20 (1.39)	+0.18 (0.78)	+0.09 (0.79)	+0.08 (0.84)	+0.03 (0.77)	+0.28 (1.08)	+0.06 (0.32)

[a] Data are given of the 1089 participants included in the analyses for B-vitamin biomarkers and health-related quality of life.

[b] Changes were calculated by subtracting biomarker concentrations at diagnosis from concentrations at 6 months and standardized by subtracting the mean change and dividing by the population standard deviation of change scores.

[c] Total folate was analyzed as the sum of 5-methyltetrahydrofolate and 4-α-hydroxy-5-methyltetrahydrofolate.

[d] Folic acid is only detectable after intake of dietary supplements or fortified foods and may end up in the circulation in case of excessive intake of folic acid and dihydrofolate reductase inactivity.

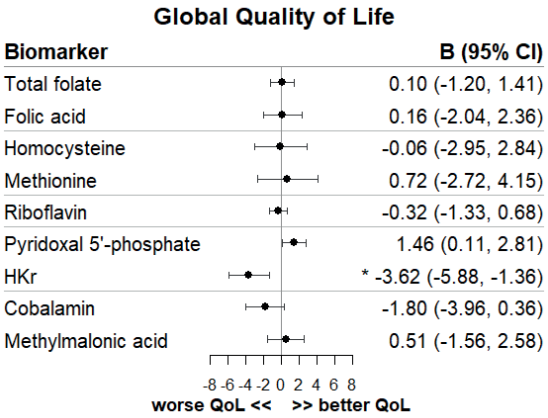
[e] HKr was analyzed as a marker of functional vitamin B6 status and calculated as 3-hydroxykynurenine : (kynurenic acid + xanthurenic acid + 3-hydroxyanthranilic acid + anthranilic acid).

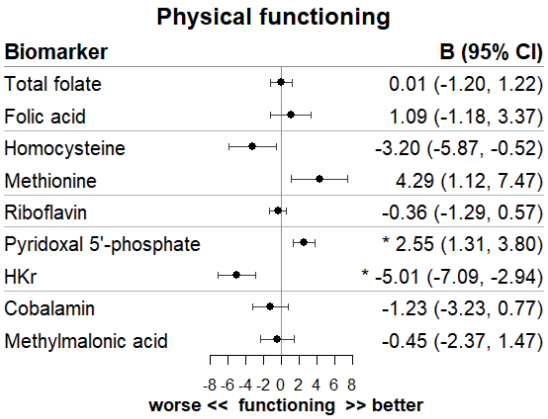
Multivariable regression analyses

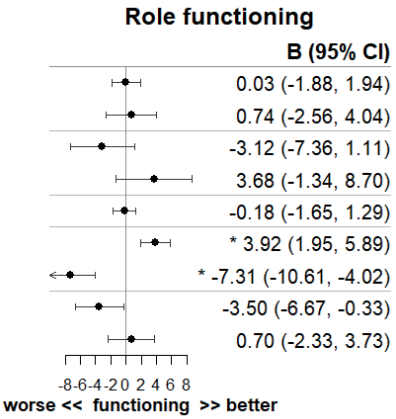
Six months after diagnosis, a doubling in PLP concentration was statistically significantly associated with better physical functioning (β 2.55, 95% CI 1.31; 3.80), role functioning (β 3.92, 95% CI 1.95; 5.89), and social functioning (β 2.69, 95% CI 1.11; 4.26), and decreased fatigue (β -2.58, 95% CI -4.31; -0.85), as analyzed in the cross-sectional confounder-adjusted models after adjustment for multiple testing (Figure 2A). Furthermore, statistically significant associations were observed for a doubling in HKr concentration, the inverse marker of vitamin B6, and reduced global quality of life (β -3.62, 95% CI -5.88; -1.36), and worse physical functioning (β -5.01, 95% CI -7.09; -2.94) and role functioning (β -7.31, 95% CI -10.61; -4.02). No statistically significant associations were observed for the biomarkers folate, folic acid, tHcy, methionine, riboflavin, cobalamin, and MMA with quality of life. In addition, no statistically significant associations were noted when modeling changes in biomarker concentrations between diagnosis and 6 months post-diagnosis (Figure 2B). Comparable patterns were observed in minimally adjusted models, only adjusted for sex and age (Supplemental Tables 2A and 2B).

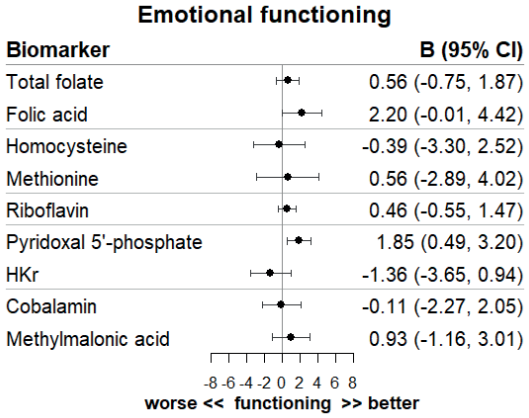
The use of B-vitamin supplements was not associated with health-related quality of life 6 months post-diagnosis in the cross-sectional confounder-adjusted model (Table 3). Notably, participants who stopped using B-vitamin supplements after diagnosis reported higher fatigue (β 7.1, 95% CI 2.9; 11.2) in comparison to nonusers.

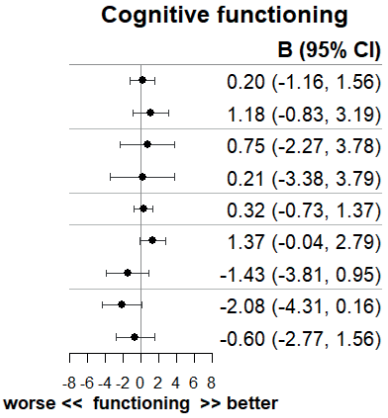
When outliers were excluded from the analyses, regression coefficients of PLP and HKr in relation to the quality of life outcomes slightly attenuated, yet conclusions remained the same as no notable changes were observed in the above-mentioned associations (data not shown). In stratified analyses, individual cohorts showed associations that were in the same direction as the overall association, and associations within ColoCare were more often non-statistically significant compared to COLON and EnCoRe (data not shown). Further, when the inflammatory markers CRP and KTR were added to the models of PLP and HKr with quality of life, regression coefficients were slightly attenuated (data not shown). Lastly, dose-response relationships were observed for the associations of deficient (PLP <20 nmol/L, n=92), suboptimal (PLP 20-30 nmol/L, n=204), and adequate (PLP >30 nmol/L, n=791) levels of vitamin B6 with global quality of life and physical, role, and social functioning (Figure 3).


Global Quality of Life
Biomarker
B (95% CI)
Total folate
0.10 (-1.20, 1.41)
Folic acid
0.16 (-2.04, 2.36)
Homocysteine
-0.06 (-2.95, 2.84)
Methionine
0.72 (-2.72, 4.15)
Riboflavin
-0.32 (-1.33, 0.68)
Pyridoxal 5'-phosphate
1.46 (0.11, 2.81)
HKr
* -3.62 (-5.88, -1.36)
Cobalamin
-1.80 (-3.96, 0.36)
Methylmalonic acid
0.51 (-1.56, 2.58)
-8 -6 -4 -2 0 2 4 6 8
worse QoL << >> better QoL


Physical functioning
Biomarker
B (95% CI)
Total folate
0.01 (-1.20, 1.22)
Folic acid
1.09 (-1.18, 3.37)
Homocysteine
-3.20 (-5.87, -0.52)
Methionine
4.29 (1.12, 7.47)
Riboflavin
-0.36 (-1.29, 0.57)
Pyridoxal 5'-phosphate
* 2.55 (1.31, 3.80)
HKr
* -5.01 (-7.09, -2.94)
Cobalamin
-1.23 (-3.23, 0.77)
Methylmalonic acid
-0.45 (-2.37, 1.47)
-8 -6 -4 -2 0 2 4 6 8
worse << functioning >> better


Role functioning
B (95% CI)
0.03 (-1.88, 1.94)
0.74 (-2.56, 4.04)
-3.12 (-7.36, 1.11)
3.68 (-1.34, 8.70)
-0.18 (-1.65, 1.29)
* 3.92 (1.95, 5.89)
* -7.31 (-10.61, -4.02)
-3.50 (-6.67, -0.33)
0.70 (-2.33, 3.73)
-8 -6 -4 -2 0 2 4 6 8
worse << functioning >> better


Emotional functioning
Biomarker
B (95% CI)
Total folate
0.56 (-0.75, 1.87)
Folic acid
2.20 (-0.01, 4.42)
Homocysteine
-0.39 (-3.30, 2.52)
Methionine
0.56 (-2.89, 4.02)
Riboflavin
0.46 (-0.55, 1.47)
Pyridoxal 5'-phosphate
1.85 (0.49, 3.20)
HKr
-1.36 (-3.65, 0.94)
Cobalamin
-0.11 (-2.27, 2.05)
Methylmalonic acid
0.93 (-1.16, 3.01)
-8 -6 -4 -2 0 2 4 6 8
worse << functioning >> better


Cognitive functioning
B (95% CI)
0.20 (-1.16, 1.56)
1.18 (-0.83, 3.19)
0.75 (-2.27, 3.78)
0.21 (-3.38, 3.79)
0.32 (-0.73, 1.37)
1.37 (-0.04, 2.79)
-1.43 (-3.81, 0.95)
-2.08 (-4.31, 0.16)
-0.60 (-2.77, 1.56)
-8 -6 -4 -2 0 2 4 6 8
worse << functioning >> better

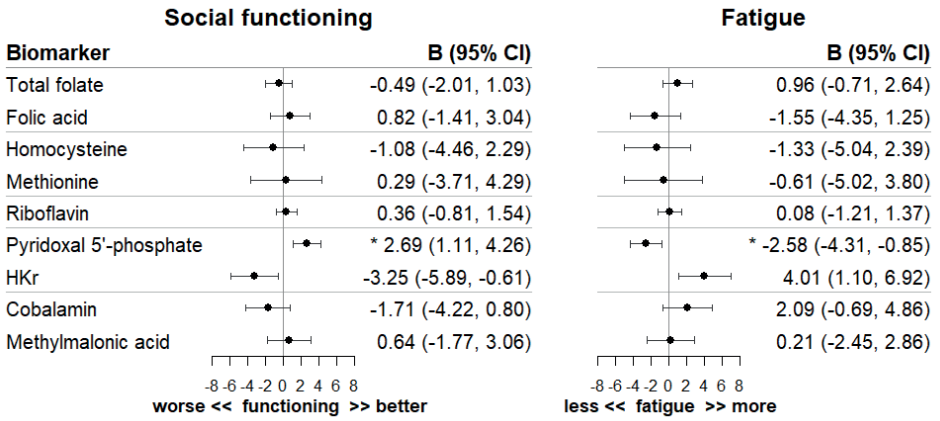
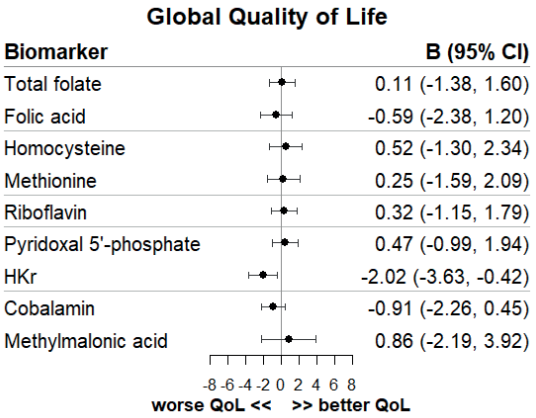
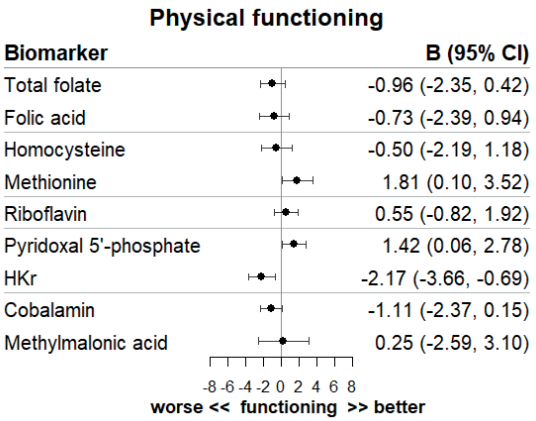


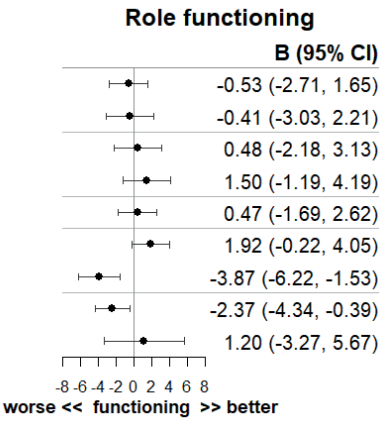
Figure 2, panel A. Regression coefficients (B) and 95% confidence intervals (95% CI) of cross-sectional linear regression analyses of log2-transformed biomarker concentrations with aspects of health-related quality of life 6 months after colorectal cancer diagnosis.

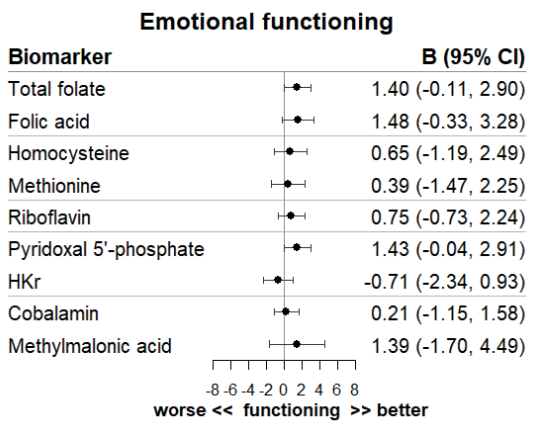
Analyses were adjusted for sex, age, cohort (COLON, EnCoRe, ColoCare), neo-adjuvant therapy (yes/no), adjuvant therapy (yes/no), cancer stage (I,II,III), any supplement use (yes/no), total energy intake (kcal/day), alcohol intake (gr/day), body mass index (kg/m^2), adherence to physical activity guidelines (yes/no), creatinine (µmol/L), cotinine (nmol/L).

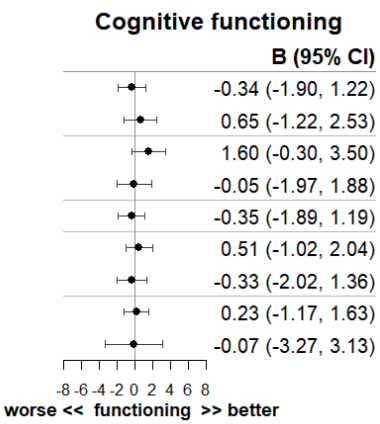
*Statistically significant after adjustment for multiple testing.


Global Quality of Life
Biomarker
B (95% CI)
Total folate
0.11 (-1.38, 1.60)
Folic acid
-0.59 (-2.38, 1.20)
Homocysteine
0.52 (-1.30, 2.34)
Methionine
0.25 (-1.59, 2.09)
Riboflavin
0.32 (-1.15, 1.79)
Pyridoxal 5'-phosphate
0.47 (-0.99, 1.94)
HKr
-2.02 (-3.63, -0.42)
Cobalamin
-0.91 (-2.26, 0.45)
Methylmalonic acid
0.86 (-2.19, 3.92)
-8 -6 -4 -2 0 2 4 6 8
worse QoL << >> better QoL


Physical functioning
Biomarker
B (95% CI)
Total folate
-0.96 (-2.35, 0.42)
Folic acid
-0.73 (-2.39, 0.94)
Homocysteine
-0.50 (-2.19, 1.18)
Methionine
1.81 (0.10, 3.52)
Riboflavin
0.55 (-0.82, 1.92)
Pyridoxal 5'-phosphate
1.42 (0.06, 2.78)
HKr
-2.17 (-3.66, -0.69)
Cobalamin
-1.11 (-2.37, 0.15)
Methylmalonic acid
0.25 (-2.59, 3.10)
-8 -6 -4 -2 0 2 4 6 8
worse << functioning >> better


Role functioning
B (95% CI)
-0.53 (-2.71, 1.65)
-0.41 (-3.03, 2.21)
0.48 (-2.18, 3.13)
1.50 (-1.19, 4.19)
0.47 (-1.69, 2.62)
1.92 (-0.22, 4.05)
-3.87 (-6.22, -1.53)
-2.37 (-4.34, -0.39)
1.20 (-3.27, 5.67)
-8 -6 -4 -2 0 2 4 6 8
worse << functioning >> better


Emotional functioning
Biomarker
B (95% CI)
Total folate
1.40 (-0.11, 2.90)
Folic acid
1.48 (-0.33, 3.28)
Homocysteine
0.65 (-1.19, 2.49)
Methionine
0.39 (-1.47, 2.25)
Riboflavin
0.75 (-0.73, 2.24)
Pyridoxal 5'-phosphate
1.43 (-0.04, 2.91)
HKr
-0.71 (-2.34, 0.93)
Cobalamin
0.21 (-1.15, 1.58)
Methylmalonic acid
1.39 (-1.70, 4.49)
-8 -6 -4 -2 0 2 4 6 8
worse << functioning >> better


Cognitive functioning
B (95% CI)
-0.34 (-1.90, 1.22)
0.65 (-1.22, 2.53)
1.60 (-0.30, 3.50)
-0.05 (-1.97, 1.88)
-0.35 (-1.89, 1.19)
0.51 (-1.02, 2.04)
-0.33 (-2.02, 1.36)
0.23 (-1.17, 1.63)
-0.07 (-3.27, 3.13)
-8 -6 -4 -2 0 2 4 6 8
worse << functioning >> better

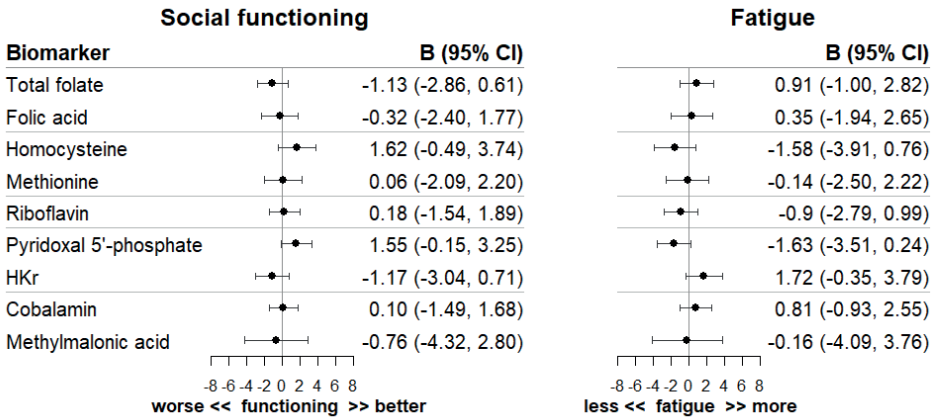


Figure 2, panel B. Regression coefficients (B) and 95% confidence intervals (95% CI) of linear regression analyses of standardized changes in biomarker concentrations (concentration at 6 months – concentration at diagnosis) with aspects of health-related quality of life 6 months after colorectal cancer diagnosis.

Analyses were adjusted for sex, age, cohort (COLON, EnCoRe, ColoCare), neo-adjuvant therapy (yes/no), adjuvant therapy (yes/no), cancer stage (I,II,III), any supplement use (yes/no), total energy intake (kcal/day), alcohol intake (gr/day), body mass index (kg/m^2), adherence to physical activity guidelines (yes/no), creatinine (µmol/L), cotinine (nmol/L).

Table 3. Results of cross-sectional linear regression analyses of B-vitamin[a] supplement use 6 months after diagnosis and of associations of changes in B-vitamin supplement use between diagnosis and 6 months after diagnosis with health-related quality of life.

	Cross-sectional analysis of B-vitamin use at 6 months (y/n)	Analysis of changes in B-vitamin use			
		Nonuse	Use at diagnosis and 6 months	Started use after diagnosis	Stopped use after diagnosis
	n=323 ß (95% CI)	*n=1173*	*n=232* ß (95% CI)	*n=91* ß (95% CI)	*n=167* ß (95% CI)
Global quality of life					
Sex and age adjusted	0.8 (-1.6, 3.2)	REF	2.2 (-0.6, 5.0)	-3.5 (-7.7, 0.8)	-1.5 (-4.8, 1.7)
Fully adjusted[b]	0.6 (-2.6, 3.8)		0.5 (-3.0, 4.0)	-2.4 (-7.3, 2.4)	-3.0 (-6.3, 0.2)
Physical functioning					
Sex and age adjusted	-1.2 (-3.5, 1.0)	REF	-0.1 (-2.7, 2.6)	-4.5 (-8.5, -0.6)	-0.6 (-3.7, 2.4)
Fully adjusted[b]	0.5 (-2.4, 3.5)		0.6 (-2.7, 3.9)	-2.3 (-6.9, 2.2)	-1.1 (-4.2, 1.9)
Role functioning					
Sex and age adjusted	0.1 (-3.5, 3.7)	REF	1.7 (-2.5, 5.8)	-4.0 (-10.3, 2.2)	-0.4 (-5.2, 4.4)
Fully adjusted[b]	1.7 (-3.1, 6.4)		1.6 (-3.7, 6.8)	-1.8 (-9.0, 5.4)	-2.1 (-7.0, 2.7)
Emotional functioning					
Sex and age adjusted	1.3 (-1.2, 3.7)	REF	2.9 (0.1, 5.7)	-2.9 (-7.1, 1.3)	-0.3 (-3.6, 2.9)
Fully adjusted[b]	0.4 (-2.7, 3.5)		1.4 (-2.1, 4.8)	-3.5 (-8.3, 1.2)	-0.9 (-4.1, 2.3)
Cognitive functioning					
Sex and age adjusted	-2.2 (-4.7, 0.2)	REF	-1.8 (-4.7, 1.0)	-5.1 (-9.4, -0.9)	-3.7 (-6.9, -0.5)
Fully adjusted[b]	-1.6 (-4.9, 1.8)		-2.3 (-6.0, 1.4)	-4.2 (-9.3, 0.9)	-4.7 (-8.1, -1.3)
Social functioning					
Sex and age adjusted	0.8 (-2.1, 3.7)	REF	2.5 (-0.9, 5.9)	-3.9 (-9.0, 1.2)	-1.1 (-5.0, 2.8)
Fully adjusted[b]	-0.1 (-3.9, 3.7)		0.3 (-3.9, 4.5)	-3.3 (-9.1, 2.5)	-2.3 (-6.2, 1.6)
Fatigue					
Sex and age adjusted	-0.1 (-3.3, 3.0)	REF	-1.5 (-5.1, 2.1)	5.8 (0.4, 11.3)	5.6 (1.4, 9.7)
Fully adjusted[b]	-2.1 (-6.1, 2.0)		-1.6 (-6.0, 2.9)	3.0 (-3.1, 9.2)	7.1 (2.9, 11.2)*

[a] B-vitamin supplement use was defined as the use of dietary supplements containing at least one of the B-vitamins folic acid, vitamin B2, vitamin B6 or vitamin B12.

[b] Fully adjusted model: adjusted for sex, age, cohort (COLON, EnCoRe, ColoCare), neo-adjuvant therapy (yes/no), adjuvant therapy (yes/no), cancer stage (I,II,III), total energy intake (kcal/day), body mass index (kg/m^2), adherence to physical activity guidelines (yes/no), any supplement use (yes/no).

*Statistically significant after adjustment for multiple testing.

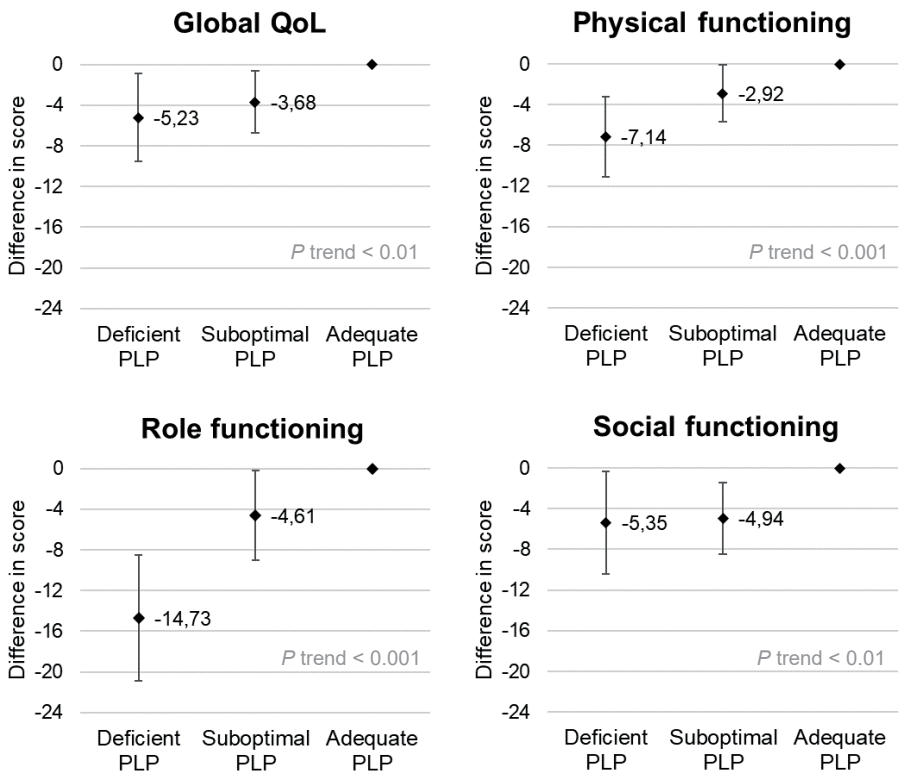


Figure 3. Dose-response relationships between vitamin B6 status, measured as pyridoxal 5'-phosphate (PLP), and quality of life outcomes.

Vitamin B6 deficient, suboptimal, and adequate levels were defined as serum/plasma PLP <20 nmol/L (n=92), 20-30 nmol/L (n=204), and >30 nmol/L (n=791), respectively.

Discussion

The current study aimed to investigate whether B-vitamin biomarkers and B-vitamin supplement use were associated with quality of life in survivors of CRC, both cross-sectionally at 6 months post-diagnosis and through the modeling of changes between diagnosis and 6 months post-diagnosis. Two vitamin B6 markers were associated with quality of life. Specifically, higher circulating concentrations of PLP were cross-sectionally associated with better physical, role, and social functioning, and reduced fatigue at 6 months post-diagnosis. In addition, higher HKr, a novel and inverse marker for vitamin B6 status,[26] was associated with decreased global quality of life and worse physical and role functioning. Dose-response relations were observed when deficient, suboptimal, and adequate levels of vitamin B6 were modeled in relation to global quality of life, physical, role, and social functioning at 6 months post-diagnosis. Only minimal changes were observed in the biomarker concentrations between diagnosis and 6 months post-diagnosis, which may explain the absence of associations between changes in biomarker concentrations and quality of life. In addition, no cross-sectional associations were found between B-vitamin supplement use and quality of life, yet participants who stopped using B-vitamin supplements after diagnosis reported higher fatigue compared to nonusers.

Vitamin B6 status emerged from the current analyses as a potential determinant of quality of life after diagnosis of CRC, while biomarkers of folate, riboflavin and vitamin B12 were unrelated to quality of life. In previous studies, specific focus has been on vitamin B6 in relation to CRC risk[41] and to a lesser extent to survival.[42] Associations of vitamin B6 with quality of life after diagnosis of cancer have previously been investigated in relation to specific conditions, but not in CRC survivors specifically. A systematic review including five intervention studies concluded that vitamin B6 supplementation may have beneficial effects on the prevention of hand-foot syndrome.[43] A mini review on B-vitamins in general showed that it is unclear to what extent B-vitamins play a role in chemotherapy-induced peripheral neuropathy and whether supplementation may be an option for treatment.[44] Further, hyperhomocysteinemia has previously been associated with the development of postoperative cognitive decline in surgical oncology patients.[45] All in all, the results of the current study add to the existing literature on B-vitamins and aspects of quality of life, and provide new insights in the association of vitamin B6 specifically with global quality of life, physical, role, and social functioning, and fatigue in CRC survivors.

The observed associations suggest that elevated vitamin B6 concentrations may be beneficial for quality of life after a CRC diagnosis. Hypothetically, PLP concentrations can be increased relatively easily through the consumption of foods high in vitamin B6 or through the use of vitamin B6 supplements. In our analyses, it was not possible to model vitamin B6 supplement use separately because the vitamin is often part of vitamin complexes and rarely used as single supplement. We modeled the use of B-vitamin containing supplements

altogether, but noted only one association between participants who stopped using B-vitamins and increased fatigue. These analyses provide insufficient evidence for the formulation of an advice on vitamin B6 supplementation or a diet high in vitamin B6 for CRC survivors. Moreover, vitamin B6 status may be a reflection of the general nutritional state of individuals, and the adjustment for total energy intake and BMI may not have been sufficient to account for this. Thus, our findings should be interpreted with caution and no firm conclusion can be drawn based on the observations of this study. Nevertheless, they give important directions for future research on vitamin B6 and quality of life outcomes.

Potential confounding by inflammation was evaluated for the observed statistically significant associations of vitamin B6 with quality of life. As circulating PLP concentrations are known to be low in inflammatory conditions[38,39,46] and because inflammation has been related to quality of life[47], the inflammatory markers CRP and KTR were added to the models to evaluate whether associations could be confounded by inflammation. Although the regression coefficients were slightly attenuated, further research is needed to evaluate the mechanistic role of inflammation in the association between B-vitamins and aspects of quality of life and whether inflammation may influence the association as a possible confounder or mediator.

The ColoCare cohort seemed to differ in comparison to the COLON and EnCoRe cohorts with regard to quality of life, biomarker concentrations, and B-vitamin supplement use. The proportion of ColoCare participants with clinically relevant problems in quality of life and functioning was highest, and participants scored on average lower on quality of life, whereas COLON and EnCoRe participants scored higher, compared to reference data of CRC patients (stage I-IV) with similar age, provided by the EORTC Quality of Life Group.[48] When stratified by tumor location, ColoCare scoring was still significantly lower than COLON and EnCoRe. Other clinical factors, such as the presence of a stoma, and/or cultural factors may be underlying these differences, although ColoCare participants also scored lower on all subscales of quality of life compared to 439 CRC patients from Germany using the same questionnaire.[49] Global quality of life scoring was similar, whereas physical functioning and fatigue scoring was lower, compared to previous analyses from the ColoCare study measured at diagnosis and after 12 months.[50] However, timing may be crucial as patients likely experience the lowest quality of life shortly after therapy. ColoCare participants further had higher total folate concentrations, which may be explained by the faster degradation of folate in plasma (COLON and EnCoRe) compared to serum samples (ColoCare). The higher proportion of rectum cancer patients and/or the lower proportion of supplement users in ColoCare could possibly explain the lower concentrations of PLP of ColoCare participants. All models were adjusted for confounding by cohort and, despite the differences in exposures and outcomes between cohorts, stratified analyses showed comparable associations across cohorts, thereby providing some degree of confidence that the observed

associations were not strongly influenced by these differences.

Important strengths of the current study are its novel and explorative character/ design, as associations between biomarkers of B-vitamins and quality of life have not been investigated in CRC patients before. Furthermore, data were obtained from a consortium of multiple prospective cohorts with comparable participant recruitment and data collection methods and a relatively high number of CRC patients, strengthening the generalizability of our findings. All biochemical analyses were carried out at a centralized laboratory, which delivered a broad panel of targeted and precise profiling of multiple B-vitamins and complementary functional biomarkers.

The study also has limitations. First, the intake of B-vitamins from diet and supplements could not be taken into account due to unavailability of details on dietary B-vitamin intake and exact amount of B-vitamin intake from supplements from all cohorts. Second, biomarker data could have been influenced by several factors, such as the inclusion of both fasting and non-fasting samples, and the small number of COLON participants who may have still been undergoing chemotherapy at the time of the 6 months post-diagnosis measurement. However, these aspects could not be accounted for as details on fasting state were unavailable for all cohorts and therapy end date was unknown for COLON participants. Lastly, the observational nature of the study makes it difficult to differentiate between cause and effect.

In conclusion, the current study provides an important novel lead for one of the multiple possible aspects to improve the health-related quality of life after CRC. Vitamin B6 status was found to be associated with better quality of life, functioning and fatigue in combined analyses of three European prospective studies. However, these associations are based on analyses of two time points with a one-time measurement of the outcome, and minimal associations were observed for B-vitamin supplement use. Vitamin B6 needs to be investigated in future longitudinal studies with more repeated measurements to confirm our findings and to provide a stronger foundation for potential requirement of intervention studies in CRC patients. In addition, future studies should also take the intake of B-vitamins from diet and supplements into account to accurately estimate total B-vitamin intake and further evaluate potential dose-response relationships.

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Supplemental Table 1. Biomarker concentrations (median and interquartile range) stratified by type of B-vitamin supplement user at diagnosis and 6 months.

Biomarker	B-vitamin supplement user types							
	Users *(n=232)*		Starters *(n=91)*		Stoppers *(n=167)*		Nonusers *(n=1173)*	
	Diagnosis	6 months	Diagnosis	6 months	Diagnosis	6 months	Diagnosis	6 months
Total folate[a] (nmol/L)	21.18 [13.21-31.50]	21.02 [14.02-34.33]	12.79 [9.51-17.76]	14.52 [10.11-21.40]	17.15 [11.75-24.39]	12.92 [9.29-18.03]	12.19 [8.69-17.54]	11.84 [8.31-17.48]
Folic acid[b] (nmol/L)	0.00 [0.00-0.88]	0.00 [0.00-0.95]	0.00 [0.00-0.00]	0.00 [0.00-0.00]	0.00 [0.00-0.00]	0.00 [0.00-0.00]	0.00 [0.00-0.00]	0.00 [0.00-0.00]
Riboflavin (nmol/L)	20.60 [11.90-40.60]	21.70 [13.08-39.43]	12.40 [8.43-17.10]	18.90 [11.90-30.00]	15.85 [9.83-23.63]	13.30 [8.05-19.43]	13.10 [8.51-20.13]	13.60 [8.66-21.73]
Pyridoxal 5'-phosphate (nmol/L)	66.80 [46.90-123.00]	70.50 [45.80-112.75]	39.20 [28.70-59.90]	59.60 [32.70-107.50]	53.30 [37.13-80.08]	43.10 [30.75-67.15]	37.13 [26.10-51.93]	38.50 [28.10-53.73]
HKr[c]	0.33 [0.27-0.39]	0.33 [0.27-0.41]	0.36 [0.30-0.46]	0.33 [0.28-0.47]	0.36 [0.28-0.45]	0.35 [0.28-0.45]	0.37 [0.30-0.45]	0.37 [0.30-0.46]
Cobalamin (pmol/L)	451.54 [340.33-551.58]	446.68 [334.65-539.77]	419.49 [333.39-522.67]	443.29 [356.88-564.17]	435.70 [344.83-574.24]	385.80 [329.38-492.29]	379.14 [304.24-469.84]	369.33 [292.57-474.51]
Methylmalonic acid (µmol/L)	0.20 [0.17-0.24]	0.19 [0.16-0.24]	0.19 [0.16-0.24]	0.19 [0.16-0.22]	0.19 [0.15-0.23]	0.20 [0.16-0.25]	0.21 [0.17-0.27]	0.21 [0.17-0.27]

[a] Total folate was analyzed as the sum of 5-methyltetrahydrofolate and 4-α-hydroxy-5-methyltetrahydrofolate.

[b] Folic acid is only detectable after intake of dietary supplements or fortified foods and may end up in the circulation in case of excessive intake of folic acid and dihydrofolate reductase inactivity.

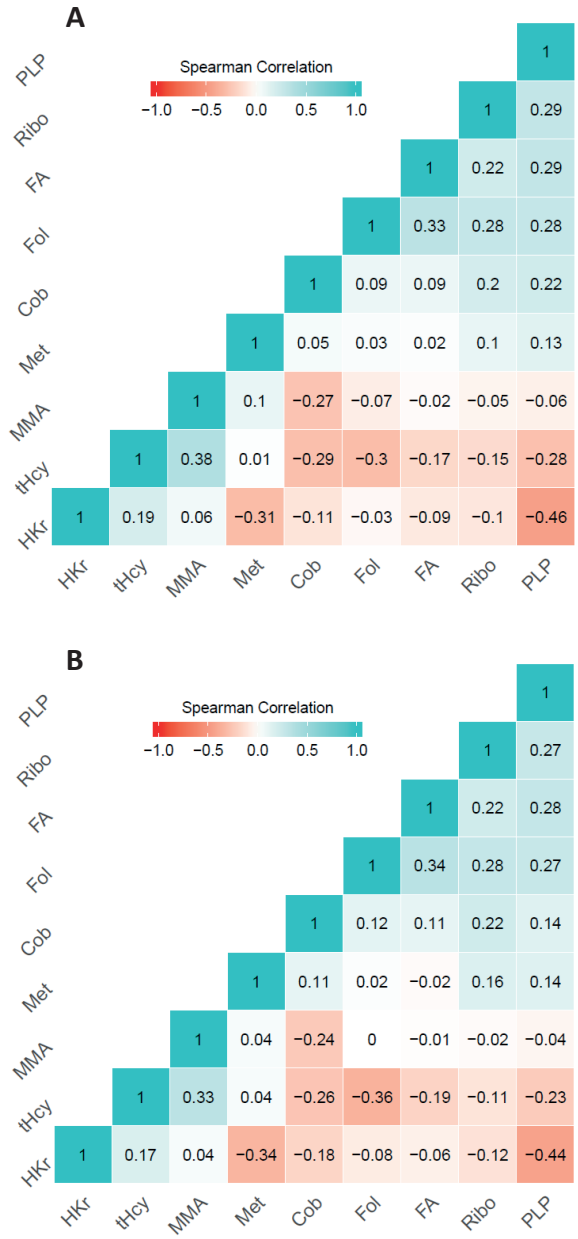
[c] HKr was analyzed as a marker of functional vitamin B6 status and calculated as 3-hydroxykynurenine : (kynurenic acid + xanthurenic acid + 3-hydroxyanthranilic acid + anthranilic acid).

Supplemental Table 2A. Sex and age adjusted results of cross-sectional linear regression analyses of log2-transformed biomarker concentrations with aspects of health-related quality of life 6 months after colorectal cancer diagnosis.

	Global quality of life	Physical functioning	Role functioning	Emotional functioning	Cognitive functioning	Social functioning	Fatigue
	Regression coefficient (95% confidence interval)						
Total folate	-0.75 (-1.94, 0.44)	-0.43 (-1.55, 0.68)	-0.40 (-2.16, 1.35)	-1.05 (-2.22, 0.12)	-0.65 (-1.82, 0.52)	-1.44 (-2.80, -0.07)	1.97 (0.42, 3.51)
Folic acid	-1.56 (-3.65, 0.54)	-0.51 (-2.52, 1.49)	-1.44 (-4.46, 1.58)	0.66 (-1.26, 2.57)	-0.28 (-1.95, 1.40)	-0.33 (-2.48, 1.82)	0.85 (-1.74, 3.45)
Total homocysteine	-0.84 (-3.43, 1.75)	-2.61 (-5.03, -0.19)	-1.64 (-5.45, 2.18)	-1.08 (-3.62, 1.46)	0.90 (-1.64, 3.44)	-0.24 (-3.22, 2.74)	-0.94 (-4.31, 2.42)
Methionine	-0.36 (-3.65, 2.94)	2.82 (-0.26, 5.91)	1.84 (-3.01, 6.69)	-0.55 (-3.79, 2.68)	-0.85 (-4.08, 2.38)	-1.12 (-4.91, 2.67)	3.12 (-1.15, 7.40)
Riboflavin	-0.79 (-1.76, 0.17)	-0.73 (-1.63, 0.17)	-0.74 (-2.15, 0.68)	-0.33 (-1.28, 0.61)	-0.15 (-1.09, 0.80)	-0.27 (-1.38, 0.83)	0.64 (-0.61, 1.89)
Pyridoxal 5'-phosphate	2.19 (0.93, 3.44)	2.76 (1.59, 3.93)	4.71 (2.87, 6.54)	1.84 (0.60, 3.07)	1.00 (-0.23, 2.24)	3.10 (1.67, 4.54)	-3.54 (-5.17, -1.92)
HKr	-5.06 (-7.21, -2.91)	-6.02 (-8.02, -4.03)	-9.12 (-12.26, -5.97)	-2.26 (-4.39, -0.13)	-1.47 (-3.60, 0.66)	-4.76 (-7.24, -2.29)	5.15 (2.34, 7.95)
Cobalamin	-1.62 (-3.72, 0.47)	-0.93 (-2.88, 1.03)	-3.59 (-6.67, -0.51)	0.16 (-1.89, 2.21)	-1.94 (-3.97, 0.09)	-1.62 (-4.02, 0.77)	2.77 (0.06, 5.49)
Methylmalonic acid	0.34 (-1.69, 2.38)	-0.30 (-2.21, 1.60)	1.10 (-1.89, 4.09)	1.31 (-0.68, 3.31)	-0.46 (-2.46, 1.53)	0.75 (-1.58, 3.09)	0.18 (-2.47, 2.82)

Supplemental Table 2B. Sex and age adjusted results of linear regression analyses of standardized changes in biomarker concentrations (concentration at 6 months – concentration at diagnosis) with aspects of health-related quality of life 6 months after colorectal cancer diagnosis.

	Global quality of life	Physical functioning	Role functioning	Emotional functioning	Cognitive functioning	Social functioning	Fatigue
	Regression coefficient (95% confidence interval)						
Total folate	-0.85 (-2.28, 0.57)	-1.31 (-2.63, 0.02)	-1.61 (-3.70, 0.48)	0.63 (-0.77, 2.02)	-1.03 (-2.43, 0.36)	-2.33 (-3.95, -0.70)	2.33 (0.49, 4.17)
Folic acid	-0.47 (-1.60, 0.67)	0.06 (-1.01, 1.12)	-0.10 (-1.77, 1.57)	0.10 (-1.01, 1.21)	0.21 (-0.90, 1.33)	-0.09 (-1.39, 1.21)	-0.18 (-1.65, 1.29)
Total homocysteine	0.37 (-1.23, 1.97)	0.28 (-1.22, 1.79)	1.05 (-1.31, 3.41)	0.44 (-1.13, 2.01)	1.44 (-0.13, 3.01)	1.83 (-0.01, 3.67)	-0.65 (-2.73, 1.43)
Methionine	-0.34 (-1.68, 1.00)	0.64 (-0.62, 1.89)	0.36 (-1.61, 2.33)	-0.33 (-1.64, 0.98)	-0.11 (-1.42, 1.20)	-0.55 (-2.09, 0.99)	1.24 (-0.49, 2.98)
Riboflavin	-0.10 (-1.49, 1.28)	0.23 (-1.07, 1.52)	-0.45 (-2.49, 1.59)	-0.16 (-1.51, 1.20)	-0.87 (-2.23, 0.48)	-0.44 (-2.03, 1.15)	-0.14 (-1.94, 1.66)
Pyridoxal 5'-phosphate	0.06 (-1.18, 1.30)	0.46 (-0.71, 1.62)	0.77 (-1.06, 2.59)	0.26 (-0.96, 1.48)	-0.13 (-1.35, 1.09)	0.47 (-0.96, 1.90)	-0.73 (-2.35, 0.88)
HKr	-1.41 (-2.62, -0.20)	-1.29 (-2.42, -0.15)	-2.79 (-4.57, -1.01)	-0.45 (1.65, 0.74)	-0.06 (-1.25, 1.14)	-0.95 (-2.35, 0.45)	1.41 (-0.17, 2.99)
Cobalamin	-1.07 (-2.28, 0.14)	-1.07 (-2.20, 0.07)	-2.37 (-4.16, -0.58)	-0.25 (-1.44, 0.95)	0.53 (-0.66, 1.72)	-0.05 (-1.45, 1.35)	1.56 (-0.02, 3.13)
Methylmalonic acid	1.82 (-0.80, 4.44)	1.04 (-1.42, 3.49)	3.79 (-0.06, 7.65)	1.01 (-1.56, 3.59)	0.74 (-1.83, 3.32)	0.75 (-2.27, 3.76)	-1.43 (-4.84, 1.97)



Supplemental Figure 1. Heatmaps of Spearman correlation coefficients between the nine biomarkers included in the analyses, at diagnosis (A) and 6 months post-diagnosis (B).

CHAPTER 5

Evaluating the validity of a food frequency questionnaire in comparison to a 7-day dietary record for measuring dietary intake in a population of survivors of colorectal cancer

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Abstract

Food frequency questionnaires (FFQs) are a commonly used method to assess dietary intake in epidemiological studies. It is important to evaluate the validity of FFQs in the population of interest. Our aim was to evaluate the validity of a FFQ for measuring dietary intake in survivors of colorectal cancer (CRC), relative to a 7-day dietary record.

Dietary intake was assessed 1 year after the end of CRC treatment. Participants first completed a 7-day dietary record and 2 weeks later a 253-item FFQ that measured intake in the preceding month. Data were used from a subsample of participants (n=100) enrolled in an ongoing prospective study (EnCoRe study) in the Netherlands, from 2015 to 2018. Estimated intakes of total energy, 19 nutrients, and 20 food groups as well as scoring adherence to the dietary recommendations of the World Cancer Research Fund/American Institute for Cancer Research (WCRF/AICR) were compared between both dietary assessment methods. Means and standard deviations, Spearman rank correlations corrected for within-person variation and total energy, and Kappa agreement between quintiles were assessed.

The median Spearman correlation corrected for within-person variation for nutrients and total energy was 0.60. Correlations >0.50 were found for 15 out of 19 nutrients, with highest agreement for vitamin B_{12} (0.74), polysaccharides (0.75), and alcohol (0.91). On average, 73% (range: 60-84%) of participants were classified into the exact same or adjacent nutrient quintile. The median Spearman correlation corrected for within-person variation for food groups was 0.62. Correlations >0.50 were found for 17 out of 20 food groups, with highest agreement for cereals and cereal products (0.96), fish (0.96), and potatoes (0.99). The Spearman correlation between total scores of the WCRF/AICR dietary recommendations was 0.53.

Relative to a 7-day dietary record, the validity of a FFQ for measuring dietary intake among survivors of CRC appeared moderate to good for most nutrients and food groups.

Introduction

The assessment of dietary intake continues to be a challenging and complex practice. Food frequency questionnaires (FFQs) are the most commonly used method to measure habitual dietary intake in epidemiological studies because of their time- and cost-efficiency.[1] However, like all dietary assessment methods, FFQs are not free from measurement error. The predefined list of foods may not comprise the full variety of foods of a person's diet and the retrospective nature of such questionnaires may introduce recall bias, leading to an inaccurate estimation of dietary intake.[2] Moreover, portion sizes are difficult to measure in a standardized way when using a questionnaire. Therefore, FFQs may be considered as a semi-quantitative assessment method generally not intended to assess actual intake but rather to rank individuals according to their habitual intake.[2]

It is important to evaluate the validity of FFQs in the population of interest to assess the credibility and legitimacy of the acquired dietary intake information.[3] Dietary records are considered a suitable comparison method for evaluating the validity of FFQs as the random errors in dietary records, mostly caused by intra-individual day-to-day variation, minimally correlate with bias known to occur in FFQs.[3,4] Dietary records do not rely upon memory and have no limitation to the type or number of recorded food items. Portion sizes are estimated by using standardized methods or by the weighing of foods by participants.[5] Correlation coefficients in previous agreement studies among healthy individuals comparing FFQs to dietary records mostly ranged between 0.3 and 0.7 for nutrients and food groups.[6-8]

To correctly investigate the relation between diet and disease, accurate assessment of dietary intake is of great importance in nutritional epidemiological studies.[5] Aspects of the diet have been identified as potential determinants influencing quality of life and prognosis after colorectal cancer (CRC), but evidence is still limited. A balanced diet with high intakes of fruits and vegetables has previously been associated with higher quality of life outcomes in a cross-sectional study of over 9000 survivors of cancer, including survivors of CRC.[9] In addition, the consumption of red meat, processed meat, low-quality carbohydrates, and sugar-sweetened beverages may play a role in CRC recurrence and mortality, but more research is needed to tailor dietary and lifestyle recommendations for survivors of CRC.[10,11] As specific dietary recommendations for survivors of cancer are lacking, survivors of cancer are currently advised to follow dietary recommendations meant for the prevention of cancer. The 2007 World Cancer Research Fund/American Institute for Cancer Research (WCRF/AICR) lifestyle recommendations have previously been operationalized into a lifestyle score to assess adherence to the recommendations.[12-14] Lifestyle scores can be useful for evaluating diet and lifestyle quality on a population level, also in survivors of CRC.[15,16] The current research group previously found that survivors of CRC with higher adherence to lifestyle recommendations reported

better physical functioning and less fatigue in cross-sectional analyses 2-10 years post-diagnosis using dietary records.[16]

This study aimed to evaluate the validity of a FFQ for measuring dietary intake in a population of survivors of CRC, using a 7-day dietary record as the reference method. Both methods were compared with respect to a selection of nutrients, food groups, and scoring on the WCRF/AICR dietary recommendations.

Methods

Population and study design

The current study was conducted in a subsample of 100 participants enrolled in the Energy for Life after ColoRectal Cancer (EnCoRe) study.[17] This is a multicenter prospective cohort study that is ongoing since 2012 and aims to study how lifestyle factors influence quality of life, health status, functioning and prognosis of survivors of CRC. Patients with stage I, II, or III CRC, including recurrent CRC, are being included upon diagnosis in three Dutch hospitals. Patients are excluded in case of stage IV CRC, comorbidities obstructing participation (e.g. cognitive disorders) or inability to understand the Dutch language. Extensive data is collected at multiple time points from CRC diagnosis until 5 years after the end of initial therapy. Before the start of CRC treatment, a 253-item semi-quantitative FFQ is used to assess habitual dietary intake within the year prior to CRC diagnosis. Subsequently, 7-day dietary records are used to measure intake at follow-up time points 6 weeks, 6 months, and 1, 2, and 5 years after CRC treatment.

For the current study, participants were asked to participate during the regular 1-year post-treatment follow-up visit of the EnCoRe study. Every participant who completed a 7-day dietary record for this visit was eligible to participate in the current study and was asked to fill out an additional FFQ subsequent to the dietary record. The FFQ was sent out by postal mail after the dietary record had been returned and participants were provided with an envelope to also return the FFQ after completion. The FFQ was completed approximately 2 weeks after completion of the 7-day dietary record. Participants of the EnCoRe study were recruited for the current study between August 2015 and January 2018. A total of 100 out of 170 invited persons were willing to participate in the current study (response rate 59%) and filled out and returned the FFQ. Approval for the current study was obtained from the Medical Ethics Committee of the University Hospital Maastricht and Maastricht University, the Netherlands, and all participants provided written informed consent.

Food Frequency Questionnaire

An adapted version of the EnCoRe FFQ that is being used pre-treatment was used for the current study; instead of a reference period of one year, participants were asked to report their intake over the preceding month. In this way, the reference period of the FFQ overlapped with the week in which the 7-day dietary record was completed, to allow for a valid comparison of dietary intakes obtained from both methods. Apart from the reference period, the FFQ to be evaluated was identical to the pre-treatment FFQ. Participants had received extensive verbal instructions on how to fill in the pre-treatment FFQ and written instructions were provided again with the FFQ for the current study. In order to guarantee high-quality data, participants were contacted by telephone after completion of the FFQ to clarify incomplete or contradicting answers. The 101 main questions of the FFQ, covering 253 unique food products, were subdivided into an overarching question on frequency of consumption and a subquestion on quantity of consumption. The question on frequency of consumption ranged from 'not used', '1 day a month', and '2-3 days a month' to '7 days a week' on a 10-point scale, e.g. "How often did you eat bread in the past month?". The question on quantity of consumption ranged from '<1 portion a day' to '>12 portions a day' on a 14-point scale, e.g. "On average, how many slices of bread did you eat on one day?". The answer options for portion/serving size were dependent on the type of product. Intake frequencies of specific types of foods, e.g. types of bread, cheese, red meat, fruits, vegetables, or cooking fats, were included in additional subquestions. Mean nutritional values were calculated based on the 2011 Dutch Food Composition Database which contains energy, macro- and micronutrient values for food products in the Netherlands.[18] One FFQ item could represent multiple food codes in the 2011 Dutch Food Composition Database. A weighted average of the nutritional value of food codes of the 2011 Dutch Food Composition Database was constructed for each FFQ item, based on quantity of consumption according to the Dutch National Food Consumption Survey.[19]

Dietary records

Participants received detailed verbal instructions by a trained research dietitian on how to fill in the 7-day dietary record during the 1-year follow-up home visit. The dietary records also contained detailed written instructions. Participants started recording their diet on the next day and were instructed to return the record by postal mail when 7 consecutive days were completed. The dietary record was pre-structured with a separate page for each mealtime (breakfast, lunch, diner) and for 3 snacking moments in-between. It further contained sufficient open space to note product (brand) names, ingredients, amounts in grams or standardized household measures (e.g. spoon, glass), recipes and preparation methods. Participants were encouraged to be as specific as possible and to write down foods and drinks directly after consumption. After completion, records were checked for incomplete or inconsistent information and, if needed, clarification was requested by telephone by the dietitian who

had visited the participant before. Coding of the dietary records was performed by qualified research dietitians, according to Standard Operating Procedures.[16] In short, the coding of food items was carried out using the 2011 Dutch Food Composition Database[18]. Coding of the quantity of foods was based on a Dutch dietary table providing information on the weight (in grams) of food products and the weight (in grams) of standard portion sizes, often given for small, medium, or large variations of the food product/portion size.[20] After coding, data entry was performed by another dietitian who concurrently performed a quality control check on the coding of the record. Web-based food calculation software was used (Compl-eat), which allows the export of dietary data both on nutrient and food group level.[21] Every participant completed all 7 days of the dietary record.

Nutrients and food groups

The FFQs and dietary records were compared for the intake of total energy, 19 nutrients, and 20 food groups. In the comparison, the macronutrients protein, total fat, as well as saturated fat, monounsaturated fatty acids and polyunsaturated fatty acids, carbohydrates, mono- and disaccharides, polysaccharides, and alcohol were included. Furthermore, a number of nutrients were selected for comparison based on their (potential) relevance in relation to CRC, including dietary fiber, calcium, magnesium, vitamin B_2, vitamin B_6, vitamin B_{12}, vitamin D, folate, folic acid, and dietary folate equivalents. For both methods, food groups were constructed based on the existing food groups in the 2011 Dutch Food Composition Database[18] and included bread; savoury bread spreads; cheese; milk and milk products; eggs; cereals and cereal products; soups; potatoes; vegetables; legumes; meat, meat products and poultry; fish; soy products and vegetarian products; mixed dishes; fats, oils and savoury sauces; fruits; sugar, sweets and sweet sauces; nuts, seeds and snacks; pastry and cookies; and alcoholic and non-alcoholic beverages. The group herbs and spices was not included because information on the consumption of herbs and spices was not collected through the dietary records.

WCRF/AICR dietary recommendations

The WCRF/AICR recommendations consist of components on body composition, physical activity, and the diet. Level of adherence to the dietary recommendations according to both dietary intake assessment methods was compared for the 6 dietary components and the total score; i.e. the recommendation on the energy density of the diet, sugar sweetened drink intake, fruit and vegetable intake, dietary fiber intake, red and processed meat intake, and alcohol intake. Our research group previously operationalized the recommendations according to the 2007 WCRF/AICR guidelines[16], and adapted the operationalization to the updated 2018 guidelines[14] for the current analyses. The update involved adjusted threshold values for dietary fiber (consumption of at least 30 instead of 25 grams per day) and alcohol (preferably no alcohol instead of ≤2 drinks

per day for men and ≤1 drink per day for women). Threshold values for the consumption of sugar sweetened drinks, fruit and vegetables, and red and processed meat did not change. The recommendation on the energy density of the diet was operationalized according to the 2007 recommendations.[13]

Other variables

Clinical information on cancer type and stage was obtained from medical records. Body mass index (kg/m^2) was calculated from body height and weight, measured by dietitians at the pre-CRC treatment (height measurements) and the 1-year post-treatment home visit (only weight measurement). Height and weight were measured without shoes while using the same portable stadiometer and scale (seca 861) for each participant.

Statistical analyses

Extensive data cleaning was performed on nutrient and food product level for both methods. The original questionnaire or record was checked in case of extreme values to evaluate whether values were correctly entered and were plausible based on reported intake. Extreme yet plausible values were not excluded as the study aimed to evaluate how both methods correlated for the entire range of intake, including the extremes.

Descriptive analyses were performed for the included participants (n=100) and compared to the subset of non-responders (n=70) by using independent t-tests for continuous variables and Chi-square tests for categorical variables. Absolute intakes of nutrients and food groups as measured by the FFQ and the 7-day dietary record were compared based on their means and standard deviations. Spearman rank correlations were calculated to assess the agreement in ranking of individuals according to their intake, the main purpose when using FFQs in nutritional epidemiological research. The Spearman correlation coefficients of nutrients and food groups were adjusted for the within-person day-to-day variability that could be measured for the dietary records assessed over seven days. These corrected coefficients were calculated in SAS according to the method as described by Rosner and Glynn.[22] In addition, energy-adjustment was applied by using the residual method, this was done separately for the FFQ and dietary record.[23] In line with previous validation studies on dietary assessment methods[5], correlations >0.50 were considered 'moderate to good'.[24] Sex-specific Bland-Altman plots were constructed for total energy and macronutrients.

The proportion of participants classified into the same quintile, the next (adjacent) quintile and the opposite (extreme) quintile of nutrient intakes based on both methods, were compared. Weighted Cohen's Kappa coefficients were calculated, with linearly decreasing weights for cells further from the diagonal of the 5x5 cross-table; i.e. a weight of 1 was given to the 5 diagonal cells representing exact agreement, weights of 0.75, 0.50 and 0.25 to the 8, 6,

and 4 respective cells in between, and a weight of 0 to the 2 extreme cells on maximal distance from the diagonal. Kappa values (unweighted and weighted) of >0.40 can be interpreted as 'moderate agreement' and were considered acceptable.[24] Furthermore, mean energy-adjusted nutrient intakes from the dietary records were calculated for quintiles based on the FFQ energy-adjusted intakes. Total scoring on the WCRF/AICR dietary recommendations was compared using Spearman correlation coefficients and the scoring of 0, 0.5, or 1 point was compared using weighted Kappa coefficients, with weights of 1, 0.5 and 0 for the consecutive categories.

All statistical analyses were conducted using IBM SPSS Statistics 24[25], except for the analyses of the Spearman crude correlations and correlations corrected for within-person variation that were conducted in SAS 9.4.[26] $P<0.05$ was considered statistically significant.

Results

Study population characteristics

Participants had a mean age of 65 ±8 years and 76% of them were men (Table 1). Thirty-seven percent had been diagnosed with stage III CRC, whereas 23% was diagnosed with stage II CRC, and 35% with stage I CRC. In comparison to the responders, non-responders were significantly older (68.1 vs. 65.4 years, $P=0.047$) and more often women (41% vs. 24%, $P=0.016$). Responders and non-responders were similar in terms of body mass index, cancer stage, cancer type (colon/rectum), and total energy intake as measured by the dietary record method.

Comparing absolute intakes

Energy intake according to the FFQ and dietary record ranged between 1276-4367 and 553-5064 kcal per day, respectively. Mean daily energy intake was on average approximately 100 kcal higher when measured by the FFQ compared to the dietary record (2178 vs. 2083 kcal, Table 2). According to the dietary record, mean total daily energy intake among men was 2193 kcal and among women 1733 kcal, whereas this was 2253 kcal among men and 1941 kcal among women according to the FFQ. Compared to the dietary record, absolute intakes of the macronutrients protein, total fat and carbohydrates were about 6-7% higher in the FFQ, whereas alcohol intake was 22% lower. Mean intakes of all micronutrients except vitamin B_6, folic acid, and dietary folate equivalents were higher when measured by the FFQ. When comparing the intake of the macronutrients protein, total fat, and carbohydrates according to their energy percentages, both methods differed less than 3% (Table 2).

Table 1. Characteristics of responders and non-responders of the validation of a food frequency questionnaire among colorectal cancer survivors, from August 2015 to January 2018 in the South-East of the Netherlands.

	Responders (n=100)	Non-responders (n=70)	*P* value[a]
Sex, n (%)			
Men	76 (76.0)	41 (58.6)	0.016
Women	24 (24.0)	29 (41.4)	
Age in years, mean (SD)	65.4 (7.7)	68.1 (9.2)	0.047
Body mass index in kg/m^2, mean (SD)	28.5 (4.6)	28.5 (4.5)	0.986
Cancer stage[b], n (%)			
I	35 (35.0)	20 (28.6)	0.529
II	23 (23.0)	16 (22.9)	
III	37 (37.0)	32 (45.7)	
Type of cancer, n (%)			
Colon	60 (60.0)	44 (62.9)	0.707
Rectum	40 (40.0)	26 (37.1)	
Total energy intake in kcal/day, mean (SD)	2083 (447)	1963 (450)	0.089
Men	2193 (418)	2110 (407)	0.302
Women	1733 (352)	1754 (432)	0.842

[a] *P* values were obtained using independent t-tests (continuous variables) and Chi-square tests (categorical variables).

[b] Does not add up to 100% because of missing stages.

Table 3 shows the means and standard deviations for the intake of food groups. The intake of bread; cheese; cereals and cereal products; vegetables; meat, meat products and poultry; and fats, oils and savoury sauces differed ≤5% between the FFQ and dietary record. The largest dissimilarities between the two methods were found for savoury bread spreads; soy products and vegetarian products; and mixed dishes, which were all estimated >40% lower by the FFQ. Bland-Altman plots (Figure 1) illustrate that total energy and macronutrient intakes were overestimated by the FFQ compared to the dietary record (mean difference >0 for both men and women). The plots also show that the disagreement between methods appears to increase with higher intakes, especially for men.

Correlations between methods

Spearman correlation coefficients for nutrients and total energy, corrected for within-person variation, ranged between 0.14 and 0.91 (median 0.60) and, adjusted for energy, between 0.26 and 0.79 (median 0.53). Correlations corrected for within-person variation >0.50 were found for 15 out of 19 nutrients, with highest agreement for vitamin B_{12} (0.74), polysaccharides (0.75), and alcohol (0.91) (Table 2). Spearman correlation coefficients for food groups, corrected for within-person variation, ranged between 0.31 and 0.99 (median 0.62). Correlations corrected for within-person variation >0.50 were found for 17 out of 20 food groups, with highest agreement for fish (0.96), cereals and cereal products (0.96), and potatoes (0.99) (Table 3).

Table 2. Daily nutrient intakes and Spearman correlation coefficients comparing intakes based on a food frequency questionnaire (FFQ) to intakes based on a 7-day dietary record in colorectal cancer survivors, from August 2015 to January 2018 in the South-East of the Netherlands.

Nutrients	Intake from dietary record		Intake from FFQ		% difference[a]	Spearman correlation coefficients		
	Mean	SD	Mean	SD		Crude	Energy-adjusted	Corrected for within-person variation[b]
Energy (kcal)	2083	447	2178	545	4.6	0.55	-	0.62
Protein (g)	77.9	15.6	83.3	19.7	6.9	0.45	0.53	0.55
Total fat (g)	81.0	20.2	85.7	30.7	5.8	0.49	0.49	0.58
Saturated fat (g)	29.7	9.0	30.9	12.3	4.0	0.54	0.56	0.62
Monounsaturated fatty acids (g)	28.0	7.4	29.9	11.1	6.8	0.43	0.38	0.53
Polyunsaturated fatty acids (g)	16.3	4.8	17.7	8.2	8.6	0.42	0.29	0.53
Carbohydrates (g)	214.6	53.7	227.5	62.9	6.0	0.64	0.65	0.70
Mono- and disaccharides (g)	82.4	31.5	92.4	39.1	12.1	0.60	0.64	0.65
Polysaccharides (g)	132.1	33.8	135.0	37.9	2.2	0.66	0.55	0.75
Dietary fiber (g)	21.8	6.5	26.5	8.8	21.6	0.54	0.56	0.58
Alcohol (g)	18.9	25.8	14.7	18.3	-22.2	0.86	0.79	0.91
Calcium (mg)	793.8	260.0	920.0	358.8	15.9	0.41	0.50	0.46
Magnesium (mg)	332.9	90.3	375.6	104.5	12.8	0.57	0.57	0.61
Vitamin B_2 (mg)	1.3	0.4	1.4	0.4	7.7	0.38	0.39	0.42
Vitamin B_6 (mg)	1.8	0.6	1.7	0.5	-5.6	0.50	0.54	0.61
Vitamin B_{12} (µg)	4.6	2.7	4.7	2.3	2.2	0.53	0.56	0.74
Vitamin D (µg)	4.0	1.9	4.0	2.0	0.0	0.45	0.40	0.67
Folate (µg)	231.4	62.9	251.4	75.0	8.6	0.49	0.47	0.57

Table 2 continued.

Nutrients	Intake from dietary record		Intake from FFQ		% difference[a]	Spearman correlation coefficients		
	Mean	SD	Mean	SD		Crude	Energy-adjusted	Corrected for within-person variation[b]
Folic acid (µg)	24.2	44.0	7.2	16.5	-70.2	0.13	0.26	0.14
Dietary folate equivalents (µg)	272.4	97.7	263.5	7.9	-3.3	0.43	0.39	0.47
Protein (% energy)	15.9	2.3	16.3	2.6	2.5	0.60	-	0.74
Total fat (% energy)	35.2	5.4	34.9	6.1	-0.9	0.47	-	0.56
Carbohydrates (% energy)	43.3	5.7	44.1	6.5	1.8	0.64	-	0.75

[a] Formula for % difference: ((intake FFQ–intake dietary record)/intake dietary record)*100.
[b] Corrected for within-person day-to-day variation of the 7-day dietary records.

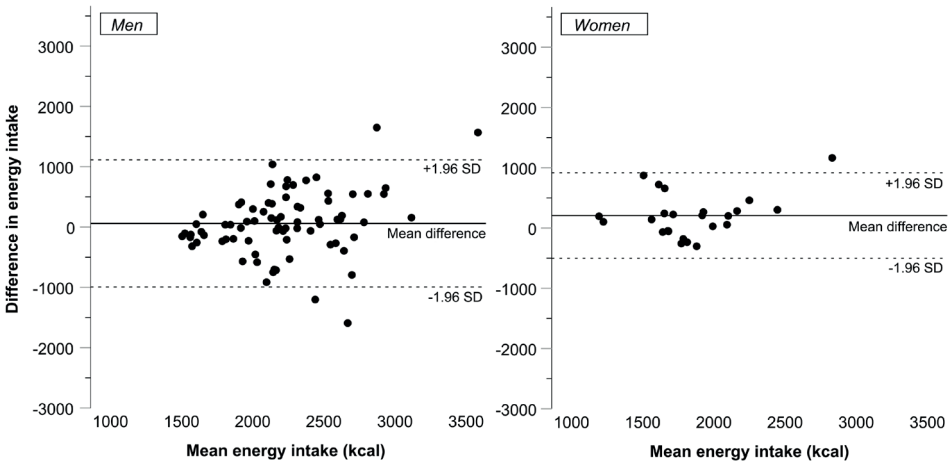
Table 3. Daily food group intakes and Spearman correlation coefficients comparing intakes based on a food frequency questionnaire (FFQ) to intakes based on a 7-day dietary record in colorectal cancer survivors, from August 2015 to January 2018 in the South-East of the Netherlands.

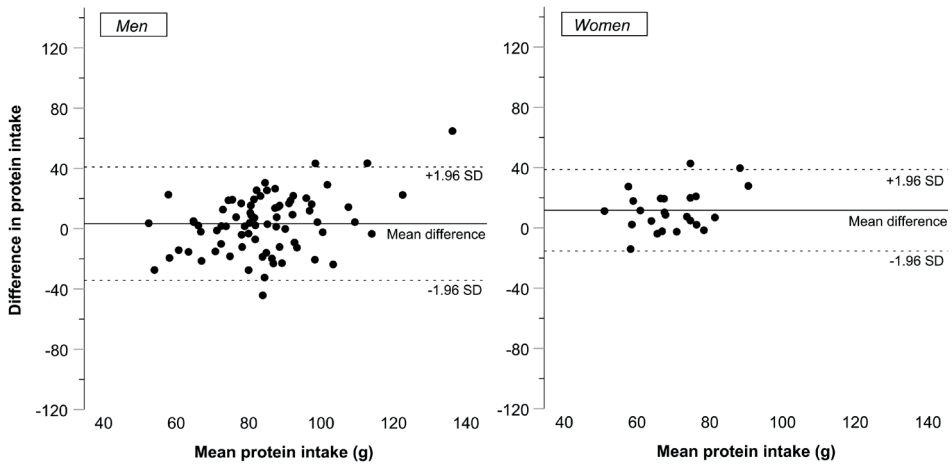
Food groups[a]	Intake from dietary record		Intake from FFQ		% difference[b]	Spearman correlation coefficients	
	Mean	SD	Mean	SD		Crude	Corrected for within-person variation[c]
Bread (g)	151.0	56.6	153.2	66.2	1.5	0.70	0.77
Savoury bread spreads (g)	5.9	4.2	3.2	6.4	-45.8	0.51	0.61
Cheese (g)	31.2	18.4	31.1	27.5	-0.3	0.53	0.63
Milk and milk products (g)	175.8	125.7	223.3	141.7	27.0	0.57	0.63
Eggs (g)	21.2	16.4	18.8	14.7	-11.3	0.41	0.58
Cereals and cereal products (g)	51.9	42.8	49.7	38.8	-4.2	0.65	0.96
Soups (g)	85.0	66.2	77.4	92.5	-8.9	0.42	0.53
Potatoes (g)	90.1	50.1	120.2	65.1	33.4	0.54	0.99
Vegetables (g)	139.1	74.3	141.0	82.5	1.4	0.46	0.57
Legumes (g)	23.9	18.1	18.0	19.7	-24.7	0.21	0.31
Meat, meat products and poultry (g)	105.1	49.6	100.5	50.7	-4.4	0.68	0.87
Fish (g)	32.9	30.5	24.9	22.3	-24.3	0.59	0.96
Soy products and vegetarian products (g)	23.4	65.0	11.6	49.2	-50.4	0.77	0.87
Mixed dishes (g)	59.2	44.0	29.9	29.9	-49.5	0.43	0.79
Fats, oils and savoury sauces (g)	47.2	18.6	45.6	28.9	-3.4	0.29	0.43
Fruits (g)	136.5	90.9	178.4	130.8	30.7	0.54	0.61
Sugar, sweets and sweet sauces (g)	28.5	23.7	30.4	26.0	6.7	0.62	0.68
Nuts, seeds and snacks (g)	27.8	18.2	25.3	16.8	-9.0	0.28	0.39

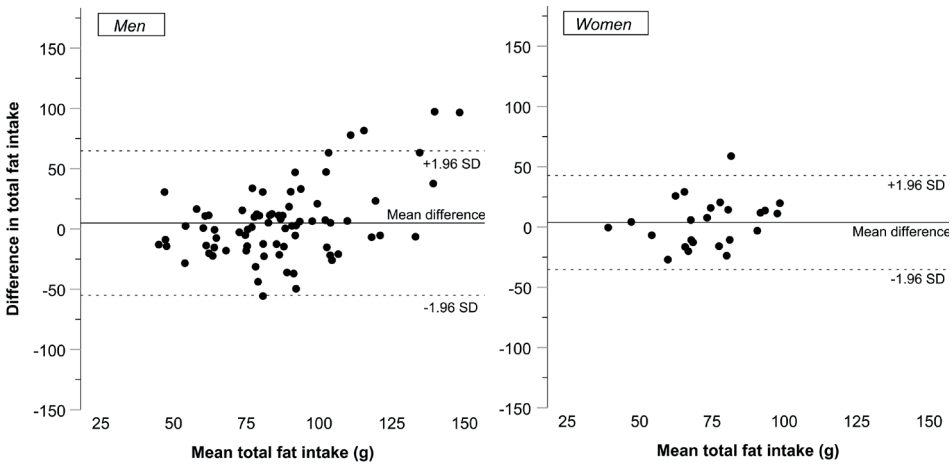
Table 3 continued.

Food groups[a]	Intake from dietary record		Intake from FFQ		% difference[b]	Spearman correlation coefficients	
	Mean	SD	Mean	SD		Crude	Corrected for within-person variation[c]
Pastry and cookies (g)	40.6	35.8	28.2	22.4	-30.5	0.48	0.59
Alcoholic and non-alcoholic beverages (g)	1554.0	580.4	1452.9	542.3	-6.5	0.56	0.60

[a] Food groups were adopted from the Dutch Food Composition Database.[18]
[b] Formula for % difference: ((intake FFQ–intake dietary record)/intake dietary record)*100.
[c] Corrected for within-person day-to-day variation of the 7-day dietary records.


Men
Women
Difference in energy intake
3000
2000
1000
0
-1000
-2000
-3000
+1.96 SD
Mean difference
-1.96 SD
1000
1500
2000
2500
3000
3500
Mean energy intake (kcal)


Men
Women
Difference in protein intake
120
80
40
0
-40
-80
-120
+1.96 SD
Mean difference
-1.96 SD
40
60
80
100
120
140
Mean protein intake (g)


Men
Women
Difference in total fat intake
150
100
50
0
-50
-100
-150
+1.96 SD
Mean difference
-1.96 SD
25
50
75
100
125
150
Mean total fat intake (g)

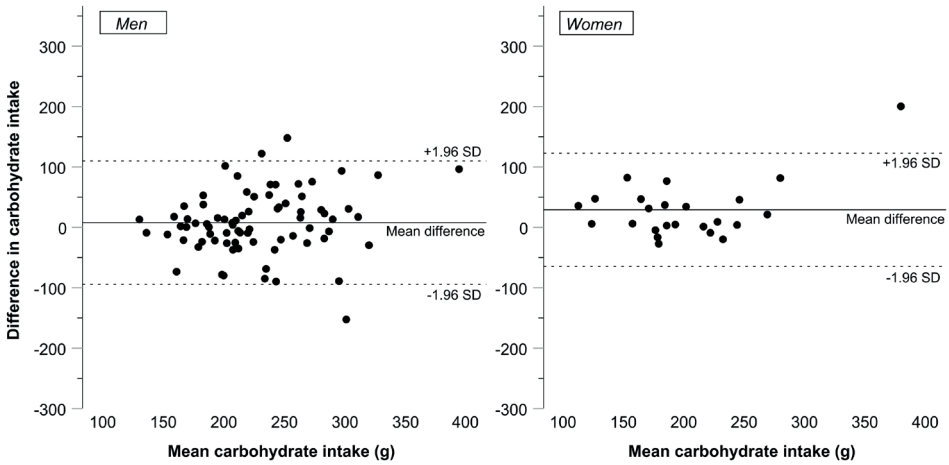


Figure 1. Bland-Altman plots for total energy and macro-nutrients showing level of agreement of daily intakes based on a food frequency questionnaire (FFQ) and a 7-day dietary record, respectively, in colorectal cancer survivors, from August 2015 to January 2018 in the South-East of the Netherlands. y-axis: (FFQ intake - dietary record intake), x-axis: (FFQ intake + dietary record intake) / 2.

Agreement between quintile distributions

Table 4 shows the mean nutrient intakes from the dietary record in quintiles of intake based on the FFQ. Intake of most nutrients steadily increased with increasing quintiles, except for folic acid, which showed no clear pattern. When quintiles based on the FFQ were compared with quintiles based on the dietary record, on average 35% (range 26-51%) of participants were classified into the exact same quintile, 38% (range 33-49%) into the adjacent quintile, and only 3% (range 0-6%) into the opposite quintile (Q1 vs. Q5). Cohen's linearly weighted Kappa's ranged between 0.18 (folic acid) and 0.58 (alcohol), with a median of 0.37.

WCRF/AICR dietary recommendations

Figure 2 illustrates the distribution of scores on the WCRF/AICR dietary recommendations according to each of the methods for the total population, and for men and women separately. The Spearman correlation between the total scores was 0.53. A median score of 2.5 (range: 0.5-4.0) was obtained by the FFQ and a median score of 2.0 (range: 0.5-5.5) by the dietary record. In addition, women scored a median of 3.0 points based on both methods, and men scored a median of 2.5 points based on the FFQ and a median of 2.0 points on the dietary record. Linearly weighted Kappa's ranged from 0.23 (dietary fiber intake) to 0.59 (alcohol intake) (Table 5). The recommendation to not consume alcohol showed the highest Spearman correlation (0.88) between methods, whereas the lowest correlation (0.50) was observed for the recommendation to eat a diet high in plant foods.

Table 4. Comparison of quintile classifications by a food frequency questionnaire (FFQ) and a 7-day dietary record in colorectal cancer survivors, from August 2015 to January 2018 in the South-East of the Netherlands.

Nutrient intake/day from dietary record	FFQ-based quintiles					Quintile classifications[a]			Linearly weighted Kappa
	Q 1 *(n=20)*	Q 2 *(n=20)*	Q 3 *(n=20)*	Q 4 *(n=20)*	Q 5 *(n=20)*	Exact (n)	Adjacent (n)	Opposite (n)	
Energy (kcal)	1756	1989	2090	2155	2424	41	38	2	0.44
Protein (g)	69.5	75.2	78.4	79.7	87.2	38	34	3	0.36
Total fat (g)	70.8	76.4	78.4	86.9	88.0	26	43	1	0.29
Saturated fat (g)	25.0	29.2	30.5	30.7	36.8	38	36	1	0.39
Monounsaturated fatty acids (g)	25.4	28.1	27.4	31.5	31.1	29	34	2	0.24
Polyunsaturated fatty acids (g)	14.0	15.5	18.1	15.2	17.4	31	33	5	0.21
Carbohydrate (g)	183.5	206.0	214.4	232.4	235.7	33	45	1	0.41
Mono- and disaccharides (g)	59.2	71.9	90.3	91.7	100.6	39	43	1	0.46
Polysaccharides (g)	115.4	120.3	133.9	143.0	146.5	38	35	3	0.38
Dietary fiber (g)	16.9	22.8	23.0	24.2	26.8	39	41	2	0.41
Alcohol (g)	0.0	9.8	13.9	20.3	47.7	51	33	0	0.58
Calcium (mg)	621.3	744.1	775.6	917.2	915.2	27	49	3	0.33
Magnesium (mg)	279.5	314.8	339.8	338.3	390.5	37	38	2	0.39
Vitamin B_2 (mg)	1.84	1.98	2.11	2.14	2.22	29	41	4	0.28
Vitamin B_6 (mg)	1.95	1.98	2.21	2.50	2.55	36	41	4	0.38
Vitamin B_{12} (µg)	4.30	4.00	4.84	5.14	7.66	41	34	3	0.43
Vitamin D (µg)	3.32	3.56	4.07	4.55	5.31	32	33	2	0.26
Folate (µg)	195.4	217.6	239.9	247.1	262.2	35	37	3	0.33
Folic acid (µg)	22.9	9.7	38.7	14.3	30.4	26	34	6	0.18
Dietary folate equivalents (µg)	233.7	264.9	259.2	291.7	311.1	31	41	6	0.28

Footnote Table 4.

[a] 'Exact' defined as classification into the exact same quintile by both methods; 'Adjacent' defined as classification into one of the adjacent quintiles (e.g. 2nd quintile for FFQ and 3rd quintile for dietary record); 'Opposite' defined as classification into the extreme quintiles (i.e. 1st quintile for FFQ and 5th quintile for dietary record, and vice versa).

Table 5. Adherence to the World Cancer Research Fund/American Institute for Cancer Research (WCRF/AICR) dietary recommendations according to a food frequency questionnaire (FFQ) and a 7-day dietary record in colorectal cancer survivors, from August 2015 to January 2018 in the South-East of the Netherlands.

WCRF/AICR dietary recommendations 2018[a]	Operationalization[b]	Scoring	Dietary record			FFQ			Linearly Weighted Kappa[c]	Spearman correlation coefficient[d]
			n	Mean intake	SD	n	Mean intake	SD		
Limit consumption of 'fast foods' and other processed foods high in fat, starches or sugars	Energy density of diet								0.29	0.54
	≤ 125 kcal/100 g/day	1	5	121.0	2.8	8	105.6	9.4		
	> 125 to < 175 kcal/100 g/day	0.5	49	158.7	11.9	58	151.2	15.6		
	≥ 175 kcal/100 g/day	0	46	196.8	17.1	34	195.0	20.2		
Do not consume sugar sweetened drinks	Sugar sweetened drink intake								0.41	0.52
	0 g/day	1	25	0.0	0.0	11	0.0	0.0		
	> 0 to ≤ 250 g/day	0.5	62	81.8	65.3	74	76.1	61.3		
	> 250 g/day	0	13	356.9	95.5	15	446.1	203.5		
Eat a diet high in all types of plant foods including at least five portions (at least 400 grams in total) of a variety of non-starchy vegetables and fruit every day	Fruit and vegetable intake								0.32	0.50
	≥ 400 g/day	1	18	456.6	47.9	23	538.4	147.4		
	≥ 200 to < 400 g/day	0.5	45	283.4	60.9	53	277.9	53.9		
	< 200 g/day	0	37	122.2	54.3	24	114.7	44.3		
Consume a diet that provides at least 30 grams per day of fiber from food sources	Dietary fiber intake								0.23	0.52
	≥ 30 g/day	1	10	34.0	3.6	25	36.9	9.9		
	≥ 15 to < 30 g/day	0.5	74	22.1	4.1	70	23.8	3.7		
	< 15 g/day	0	16	13.0	2.0	5	11.7	3.2		

Table 5 continued.

WCRF/AICR dietary recommendations 2018[a]	Operationalization[b]	Scoring	Dietary record			FFQ			Linearly Weighted Kappa[c]	Spearman correlation coefficient[d]
			n	Mean intake	SD	n	Mean intake	SD		
If you eat red meat, limit consumption to no more than about three portions per week. Three portions is equivalent to about 350 to 500 grams cooked weight of red meat. Consume very little, if any, processed meat.	Red and processed meat intake								0.34	0.55 (red) 0.62 (processed)
	< 500 g red meat/week and < 3 g processed meat/day	1	6	16.7 0.5	30.3 0.8	2	322.8 1.3	93.0 1.7		
	< 500 g red meat/week and ≥ 3 to < 50 g processed meat/day	0.5	34	257.6 26.3	139.3 13.3	53	245.8 26.6	143.4 13.3		
	≥ 500 g red meat/week or ≥ 50 g processed meat/day	0	60	403.6 66.3	239.7 31.6	45	524.2 61.2	224.3 31.1		
For cancer prevention, it's best not to drink alcohol	Alcohol intake[e]								0.59	0.88
	0 g/day	1	19	0.0	0.0	3	0.0	0.0		
	> 0 to ≤ 10 g/day	0.5	28	4.0	2.8	49	3.3	3.1		
	> 10 g/day	0	53	33.6	28.1	48	27.4	19.6		

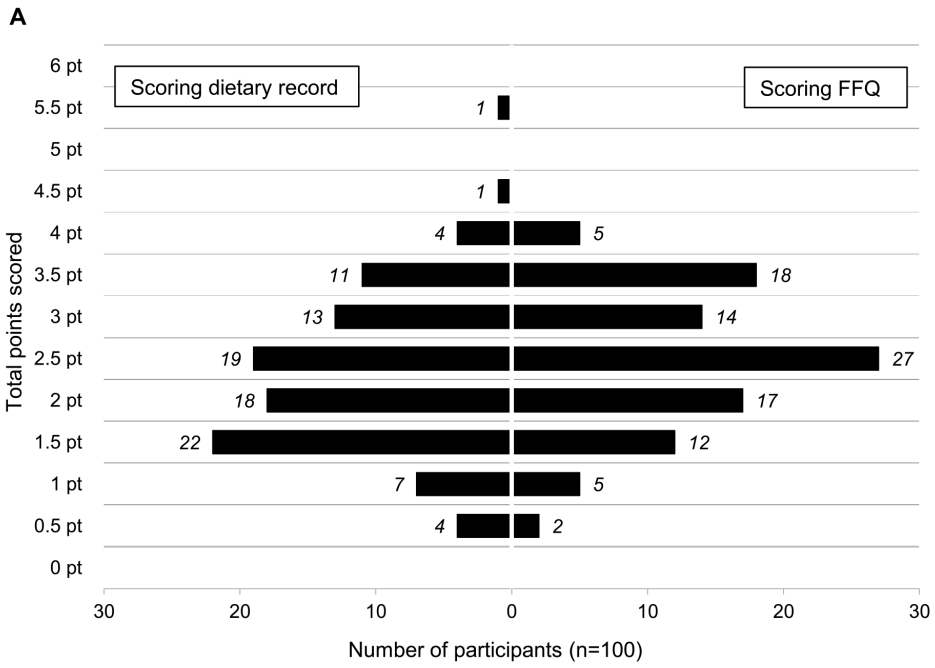
[a] Spearman correlation between total scores FFQ and dietary record: 0.53. Median score dietary record: 2.0. Median score FFQ: 2.5.

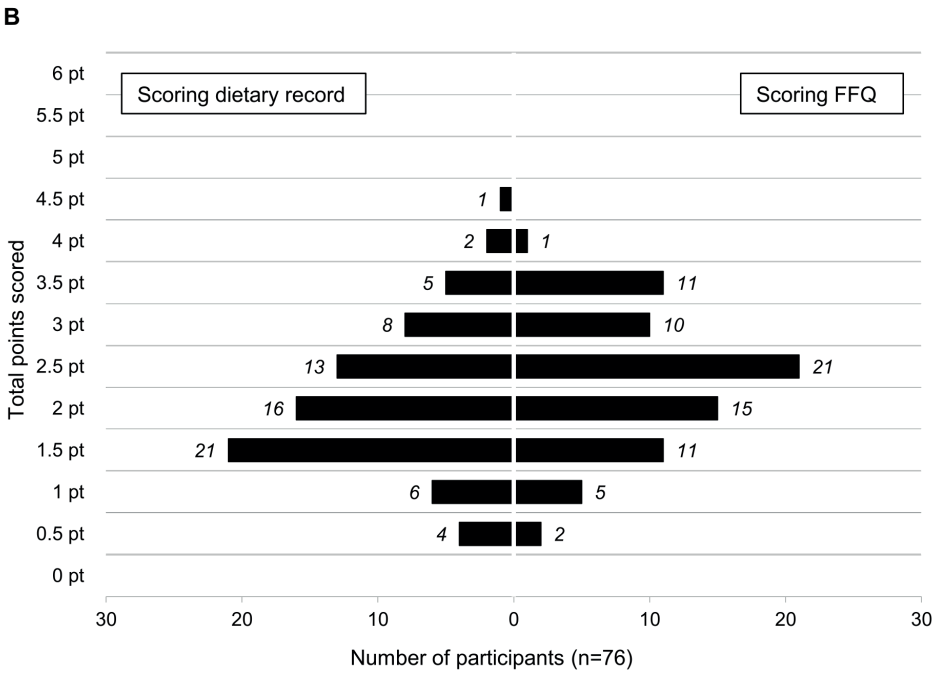
[b] Operationalization based on the 2018 and 2007 recommendations.[13,12]

[c] Linearly weighted Kappa calculated for scoring 0, 0.5 or 1 point on the WCRF/AICR score compared for both methods.

[d] Crude Spearman correlations calculated between absolute intakes of both methods for each recommendation.

[e] Calculated as the nutrient alcohol. For the FFQ, the calculation of alcohol intake also comprised food products containing alcohol (e.g. tiramisu, chocolate pralines), while this was not the case for the dietary records. As a result, 12 out of 15 participants in total who reported to not have consumed alcoholic drinks in the past month were misclassified in the middle category because of the consumption of foods containing alcohol.


A
Scoring dietary record
Scoring FFQ
6 pt
5.5 pt
5 pt
4.5 pt
4 pt
3.5 pt
3 pt
2.5 pt
2 pt
1.5 pt
1 pt
0.5 pt
0 pt
1
1
4
5
11
18
13
14
19
27
18
17
22
12
7
5
4
2
Total points scored
30
20
10
0
10
20
30
Number of participants (n=100)


B
Scoring dietary record
Scoring FFQ
6 pt
5.5 pt
5 pt
4.5 pt
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3.5 pt
3 pt
2.5 pt
2 pt
1.5 pt
1 pt
0.5 pt
0 pt
1
2
1
5
11
8
10
13
21
16
15
21
11
6
5
4
2
Total points scored
30
20
10
0
10
20
30
Number of participants (n=76)

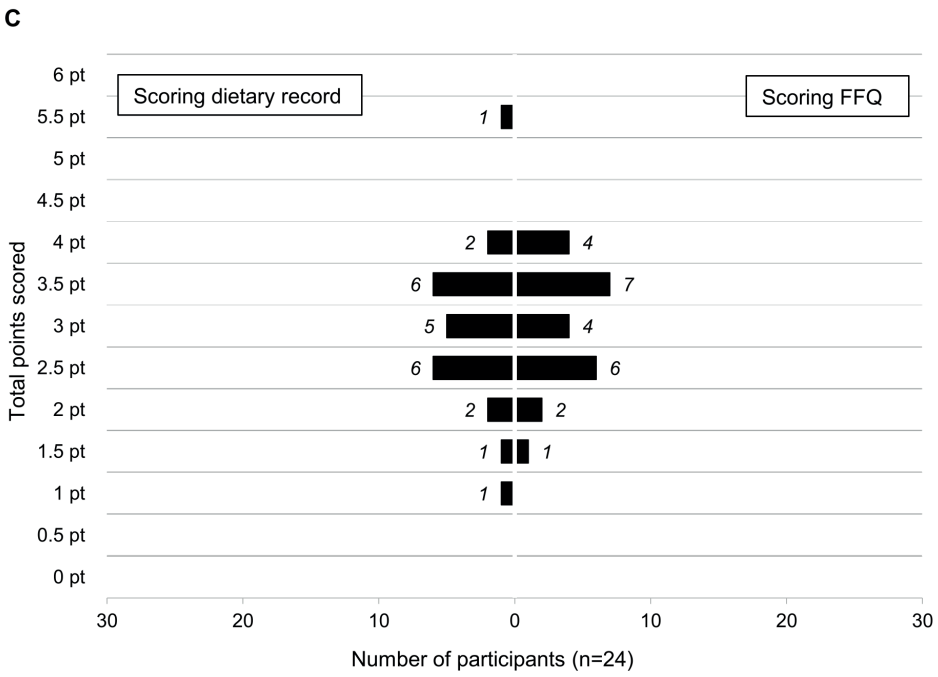

Figure 2. Distribution of scoring on the World Cancer Research Fund/American Institute for Cancer Research (WCRF/AICR) dietary recommendations according to a dietary record (left side) and a FFQ (right side) in a FFQ validation study among colorectal cancer survivors, in total (A), men (B), and women (C), from August 2015 to January 2018 in the South-East of the Netherlands.

Discussion

This study assessed the validity of a FFQ against a 7-day dietary record in a population of survivors of CRC. The FFQ performed moderate to good when evaluating the Spearman correlation coefficients for nutrients and food groups. Correlations >0.50 were found for total energy, all macronutrients, most micronutrients and food groups. The FFQ performed less well (correlations <0.50) for the micronutrients folic acid, vitamin B_2, calcium, and dietary folate equivalents, and the food groups legumes; nuts, seeds and snacks; and fats, oils and savoury sauces. Although Kappa coefficients were relatively low (<0.40) for protein, total fat, minerals such as calcium and magnesium, and vitamins B_2 and B_6, for all these nutrients approximately 70-80% of participants were classified in the exact or adjacent quintile and very few (≤4%) in the opposite quintile.

Correlation coefficients observed in the current study are comparable to previous findings. Results could not be compared with other FFQ validation

studies among adult survivors of cancer as this was the first validation study in this population. The FFQ used in the current study was based on a FFQ designed for a population-based study on type 2 diabetes, where total energy and macronutrient correlations of 0.38-0.56 and food group correlations of 0.30-0.69 were observed when evaluated against 1 to 5 24h dietary recalls and biomarkers.[27] The European Prospective Investigation into Cancer and Nutrition (EPIC) observed crude total energy and macronutrient correlations of 0.51-0.71 for men and 0.51-0.66 for women, and food group correlations of 0.21-0.78 for men and 0.31-0.87 for women when evaluating the Dutch EPIC FFQ against 12 monthly 24-hour recalls.[28,29]

FFQs and dietary records are prone to several types of measurement bias that could have influenced the results of the current study. FFQs are known to have relatively high systematic bias because dietary intake tends to be overestimated when a large number of fixed food items is presented and needs to be recalled over a long period of time.[30] Dietary records are more prone to random measurement error caused by within-person day-to-day variation.[5] The bias of social desirability affects both methods. Individuals are, to a greater or lesser extent, influenced by social norms and values when reporting their dietary intake.[31] Furthermore, participants may simply forget or find it too bothersome to report certain items which may lead to underreporting in both a FFQ and dietary record.[31] Another type of bias, applicable to dietary records, is the altering of the diet during the period of recording.[31] Inevitably, participants become increasingly aware of their dietary behaviors when recording their diet, and this can, consciously or unconsciously, lead to changes in 'normal' eating behaviors. All aforementioned types of bias are difficult to avoid and estimating dietary intake therefore remains complex.[32] Moreover, when a true 'gold standard' is lacking it is specifically challenging to identify the exact source (FFQ or dietary record), magnitude, and the direction of bias. The designs of both methods differ in their purpose and perfect correlation would never be obtained. Although the within-person variation in the dietary record was accounted for by calculating the adjusted Spearman coefficients, person-related propensity to over- or underestimate the intake of specific foods cannot be completely ruled out.[5] However, the correlated errors of FFQs and dietary records are believed to be relatively low[4] and the observed agreement between methods is likely to be true agreement instead of an agreement of errors.

For folic acid in particular, correlations were low and the agreement between quintiles was weak. This was likely due to differences between the methods regarding the selection of underlying food items from the Dutch Food Composition Database. Folic acid is only found in a limited number of foods, such as folic acid fortified (low fat) margarines and breakfast cereals, but there is large variety in folic acid content between those foods. The selection of a different food between the methods could easily have led to low agreement as the selected answer option in the FFQ could be low in folic acid, whereas the reported/coded food item in the dietary record could be high in folic acid. In

addition, the estimated FFQ intake of the food group mixed dishes was 49.5% lower than the intake according to the dietary record. This could have been caused by a difference in the assessment of mixed dishes by both methods. Mixed dishes may have been recorded and coded as mixed dishes or as the separate ingredients in the dietary records whereas they were likely reported as mixed dishes in the FFQ as seven different types of mixed dishes were included. The food groups with low agreement indicate where the FFQ could potentially be improved for future use.

Regarding the WCRF/AICR dietary recommendations, the correlation of 0.53 between the total scores of both methods was considered moderate to good. Higher scores were obtained by the FFQ compared to the dietary record, and Kappa coefficients for the distribution of scoring 0, 0.5, or 1 point were below 0.40 for the individual recommendations on energy density, fruit and vegetables, dietary fiber, and red and processed meat. In contrast, correlation coefficients between individual recommendations were all ≥0.50. Thus, when ranking individuals according to their adherence scores, both methods showed moderate to good agreement, whereas the exact number of points scored was less comparable between methods.

The Bland-Altman plots implied that there was increased disagreement in the higher ranges of intake, especially for men. Extreme intakes may more likely be exposed when recording actual intake in the dietary record[33] whereas FFQs represent a longer period of reference with individuals summarizing usual intake. As the Bland-Altman plots showed unadjusted intakes, this divergence between methods could have led to the greater disagreement for higher intakes.

Our study had several strengths and also limitations. An important strength of the current study involves the comparison of the FFQ with a 7-day dietary record, which is a suitable method to evaluate the validity of a FFQ.[3] Another strength involves the high-quality and completeness of the data, which was achieved by the thorough instructions provided by trained research dietitians during the home visits. Additionally, all required details on dietary intake were obtained by checking the instruments directly after completion, and contacting participants by telephone if additional clarification was needed. Additional quality control checks were performed during data entry and data cleaning. Furthermore, different aspects of validity of the FFQ were investigated by comparing absolute intakes as well as Spearman rank correlations, and agreement based on kappa coefficients and Bland-Altman plots.

A limitation of the current study may be that the validation population might consist of the more motivated and healthy participants as they voluntarily consented to participate in both the EnCoRe and the validation study. It is unknown whether these participants were indeed more likely to report with less measurement error than the average survivor of CRC. Differences between responders and non-responders were small but when interpreting the results,

it must be considered that participants were more often men and had a lower mean age than the overall population. Another potential limitation is that the dietary record was completed approximately 2 weeks before the FFQ, while for evaluation purposes it is often preferred to administer the instrument to be evaluated before the reference instrument.[3] In the current study, however, reference periods of both methods would not have overlapped when the FFQ had been administered first. Keeping a dietary record could have made participants more aware of their dietary intake. On the one hand, this may have influenced the response to the FFQ, causing correlation coefficients to be somewhat increased. One the other hand, the increased awareness of intake could have led to a more accurate completion of the FFQ.[5]

To our knowledge, the current study was the first FFQ validation study among adult survivors of cancer. The results contribute to the existing literature on the evaluation of FFQs and are important for the general understanding of the agreement between FFQs and 7-day dietary records in populations of survivors of cancer. Moreover, the results are relevant in meta-analyses to pool data with other cohorts of survivors of CRC using FFQs.

Conclusions

The current study investigated the validity of a FFQ and showed that ranking of individuals according to their nutrient and food group intakes was moderate to good (most Spearman correlations >0.50) in comparison with a 7-day dietary record. Direct comparison of absolute intakes between both methods is not justified as potential differences are more likely a result of the difference in assessment method. Instead, the results could be used to rank individuals according to their intake in the current study and in the pooling of dietary data with other cohorts of survivors of CRC.

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CHAPTER 6

General Discussion

This thesis investigates the roles of vitamins and dietary supplements in relation to fatigue and quality of life after a colorectal cancer diagnosis. More specifically, this thesis aims to gain deeper insight in the potential benefit of the use of supplements in general and of supplements containing vitamin D and B-vitamins, for reducing fatigue and increasing the quality of life of colorectal cancer survivors up to 2 years after the end of treatment. Furthermore, this thesis investigates associations of blood biomarkers of vitamin D and B-vitamins with fatigue and quality of life, and evaluates the relative validity of a food frequency questionnaire in comparison to a 7-day dietary record.

The three aims of this thesis, as formulated in chapter 1, were:

I. To examine the association of supplement use in general, and the use of supplements containing vitamin D and B-vitamins, with quality of life and fatigue in colorectal cancer survivors (chapters 2, 3, and 4).
II. To investigate whether blood biomarkers of vitamin D and B-vitamins are associated with quality of life and fatigue in colorectal cancer survivors (chapters 3 and 4).
III. To evaluate the validity of a food frequency questionnaire in comparison to a 7-day dietary record to measure dietary intake in colorectal cancer survivors (chapter 5).

Part A of this chapter starts with a summary and discussion of the main findings of this thesis according to each of the three aims. Figure 1 provides a summary of the observed statistically significant associations amongst all those investigated. The use of any supplements was associated with more fatigue in inter-individual analyses. In addition, biomarker concentrations of 25-hydroxyvitamin D_3 ($25OHD_3$) and vitamin B6, measured as high pyridoxal 5'-phosphate (PLP) or low 3'-hydroxykynurenine ratio (HKr), were associated with better quality of life outcomes. The current chapter further reflects on a number of methodological aspects (part B), addresses the practical implications of this work, and gives directions for future research (part C).

Ch. 2: Supplement use and Fatigue
6 weeks to 2 years post-treatment[a] (EnCoRe)

Supplement use	Fatigue[b] Overall	Fatigue[b] Inter-individual	Fatigue[b] Intra-individual
Any supplement use			

Ch. 3: Vitamin D and Quality of Life 6 weeks to 2 years post-treatment[a] (EnCoRe)

Vitamin D	Fatigue[b] Overall	Fatigue[b] Inter-individual	Fatigue[b] Intra-individual	Global quality of life Overall	Global quality of life Inter-individual	Global quality of life Intra-individual	Cognitive functioning Overall	Cognitive functioning Inter-individual	Cognitive functioning Intra-individual	Depression/ anxiety Overall	Depression/ anxiety Inter-individual	Depression/ anxiety Intra-individual
25-hydroxyvitamin D_3												
Vitamin D sufficiency												
Vitamin D supplement use							n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
Intake from diet + supplements							n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
Intake from supplements							n.i.	n.i.	n.i.	n.i.	n.i.	n.i.

Ch. 4: B-vitamins and Quality of Life at diagnosis and 6 months post-diagnosis[c,d] (FOCUS)

B-vitamin biomarkers	Fatigue[b] At 6 months	Fatigue[b] Changes	Global quality of life At 6 months	Global quality of life Changes	Cognitive functioning At 6 months	Cognitive functioning Changes	Social functioning At 6 months	Social functioning Changes	Role functioning At 6 months	Role functioning Changes	Emotional functioning At 6 months	Emotional functioning Changes	Physical functioning At 6 months	Physical functioning Changes
Total folate														
Folic acid														
Homocysteine														
Methionine														
Riboflavin (vit B2)														
Pyridoxal 5'-phosphate (vit B6)														
HKr[e]														
Cobalamin (vit B12)														
Methylmalonic acid														
B-vitamin supplement use[f]														
B-vitamin supplement use[g]														

Legend:
- positive association as hypothesized
- positive association, not as hypothesized
- inverse association, as hypothesized
- inverse association, not as hypothesized
- no association

Figure 1. *Title and footnote on next page.*

Figure 1. Overview of observed statistically significant associations in this thesis, adjusted for relevant confounders.

Abbreviations: not investigated (n.i), 3'-hydroxykynurenine ratio (HKr).
[a] Overall associations include both inter- and intra-individual associations over time. Inter-individual associations indicate the difference between participants' mean value in the exposure variable over time. Intra-individual associations indicate the changes made in the exposure variable within a participant over time.
[b] Higher scoring on fatigue indicates more fatigue whereas higher scoring on the other outcomes indicates better quality of life.
[c] Analyses on B-vitamins were additionally adjusted for multiple testing.
[d] Changes were calculated by subtracting biomarker concentrations at diagnosis from concentrations at 6 months.
[e] HKr (3'-hydroxykynurenine ratio) was analyzed as an inverse marker of functional vitamin B6 status.
[f] B-vitamin supplement use was defined as the use of dietary supplements containing at least one of the B-vitamins folic acid, vitamin B2, vitamin B6, or vitamin B12.
[g] Participants who stopped using B-vitamin supplements after diagnosis reported higher fatigue as compared to nonusers of B-vitamin supplements.

A. Summary and interpretation of main findings

Aim I – Supplement use

Main findings on supplement use

At the time of colorectal cancer diagnosis, 39% of participants used any type of dietary supplement. Of participants with at least two available measurements in the period from diagnosis to 2 years post-treatment, 28% were consistent users of supplements, 45% were nonusers, and 27% reported both use and nonuse. Between 6% and 16% of participants started the use of a new supplement or stopped the use of a supplement at the time points 6 weeks, 6 months, 1 year, and 2 years post-treatment. The most commonly reported motivation for supplement use throughout the study included complaints not related to the bowel, such as peripheral neuropathy. Other motivations were: to complement the diet, to improve bone health, or because use was recommended by a physician or relative. No overall longitudinal association was observed between the use of supplements and fatigue from 6 weeks to 2 years post-treatment (β 4.0, 95% confidence interval -0.7; 8.7). However, in contrast to what was hypothesized, inter-individual associations showed that users of supplements had higher fatigue scores compared to nonusers over time (β 7.0, 95% confidence interval 0.3; 13.7).

Despite the fairly high prevalence of overall supplement use, the prevalence of vitamin D use was relatively low considering the national recommendations on supplement use that applied to about half of the study population; 24% of

participants aged >70 years and 33% of women aged 50-70 years used vitamin D supplements at diagnosis. No longitudinal associations were observed between vitamin D supplement use and quality of life in the period from 6 weeks to 2 years post-diagnosis. Furthermore, B-vitamin supplements were used by 24% of participants at diagnosis and by 19% of participants at 6 months post-diagnosis in the analyses using data of the FOCUS consortium. B-vitamin supplement use was not associated with quality of life outcomes in cross-sectional analyses at 6 months post-diagnosis. However, participants who stopped using B-vitamin supplements between diagnosis and 6 months post-diagnosis reported higher fatigue compared to nonusers.

Supplement use and fatigue

The observed inter-individual associations between the use of any supplements and fatigue in chapter 2 are likely explained by the possibility that survivors use supplements as a result of their fatigue. Individuals with higher levels of fatigue may be inclined to use supplements as an attempt to reduce their complaints. Likewise, individuals who feel fit and healthy may not feel the need to use supplements. An inverse association between perceived health status and supplement use has previously been observed by Miller et al.[1] Cancer survivors with underlying chronic conditions, such as high blood pressure or asthma, were more likely to use supplements than survivors without chronic conditions.[1] This corresponds to our findings as the motivations for use that were reported by study participants were frequently related to specific health problems and physical complaints. Besides the biological effects of supplement use, such as elevated vitamin levels, findings from chapter 2 suggest another aspect of supplement use, namely that supplements may be used by cancer survivors as a health management strategy.[2] This aspect could interfere with the biological associations of blood biomarkers and single vitamin D and B supplement use with quality of life, and was therefore taken into account in chapters 3 and 4 by additionally adjusting the analyses for the use of any supplements.

The relation of supplement use with higher levels of fatigue further implies that colorectal cancer survivors are searching for ways to alleviate their complaints. Survivors supposedly take supplements with the presumption that it cannot do any harm. In the past decade or so, supplements have become a major profitable industry with large-scale marketing strategies,[3] making individuals believe that supplements are able or even necessary to alleviate certain physical or psychological complaints. Moreover, it remains unclear whether the use of supplements, e.g. anti-oxidants during chemo- and radiotherapy, is safe.[4] It is therefore of importance for colorectal cancer survivors to have access to reliable information and options on how to effectively deal with chronic conditions and complaints such as fatigue that frequently occur after a cancer diagnosis and anti-cancer therapy.

Use of vitamin D and B-vitamin supplements

The results on vitamin D and B-vitamin supplement use in chapters 3 and 4 implied no benefit of supplement use for quality of life. However, the possibility that survivors use supplements because of their complaints, as described above, may have interfered with the associations – despite the adjustment for the use of any supplements – and explain some of the findings. For instance, it may also explain the observed (non-statistically significant) inter-individual associations between vitamin D supplement use and increased levels of fatigue while the intra-individual associations suggested decreased levels of fatigue for individuals who started using vitamin D supplements during follow-up, as discussed in chapter 3.

Altogether, based on these findings, it is not justified to encourage the use of vitamin D or B-containing supplements for colorectal cancer survivors for the specific purpose of decreasing fatigue complaints and improving quality of life after treatment. When higher circulating blood concentrations of these vitamins would be proven beneficial for better quality of life, it remains uncertain whether supplements are necessary to reach adequately high concentrations or whether concentrations can be reached by conventional ways such as sun exposure and dietary intake. Regarding vitamin D, it would be favorable if colorectal cancer survivors aged >50 years (women) and >70 years (women and men) would have knowledge on and be actively encouraged to adhere to the national recommendations on vitamin D supplementation and sun exposure. In the Netherlands, 15 to 30 minutes of daily sunlight exposure between 12:00h and 15:00h, from March to November, is generally sufficient for adequate vitamin D synthesis.[5] Intervention studies are needed to demonstrate whether vitamin D supplementation would be beneficial for increasing vitamin D levels, reducing fatigue and improving the quality of life of colorectal cancer survivors.

Aim II – Biomarkers

Main findings on vitamin D

Longitudinal associations were investigated between $25OHD_3$ concentrations and global quality of life, fatigue, cognition, depression, and anxiety from 6 weeks to 2 years after colorectal cancer treatment. Higher $25OHD_3$ concentrations were associated with better global quality of life and less fatigue over time, both inter- and intra-individually. A high proportion of participants (45%) were vitamin D deficient at diagnosis, and having a vitamin D deficiency was longitudinally associated with decreased quality of life and more fatigue. Intra-individual associations demonstrated that participants who changed from having deficient to sufficient levels over time, reported better quality of life and lower levels of fatigue. Dose-response relations were observed for participants with severely deficient, deficient, and adequate concentrations of $25OHD_3$ in relation to quality of life and fatigue.

Biological mechanisms of vitamin D

Immune responses and inflammatory processes play a potentially mediating role in the association between vitamin D and fatigue. $25OHD_3$ is converted to its biologically active form, 1,25-dihydroxyvitamin D_3 ($1,25OH_2D_3$), by the enzyme CYP27B1.[6] Immune cells, such as monocytes, T cells, and B cells, are able to synthesize the active metabolite $1,25OH_2D_3$ and express the vitamin D receptor.[7,8] Consequently, $1,25OH_2D_3$ is involved in the inhibition of pro-inflammatory cytokines, including IL-6, IL-8, and TNF-alpha, and the production of anti-inflammatory cytokines such as IL-10.[7-9] Through these mechanisms, vitamin D contributes to an effective immune response, and a deficiency in $25OHD_3$ may lead to a dysregulation of immunoreactivity.[6] Moreover, inflammation has been recognized as a key mechanism in the development of cancer-related fatigue.[10] When pro-inflammatory cytokines are released by immune cells, for instance as a result of anti-cancer therapy, signals are conveyed to the central nervous system that may induce feelings of fatigue.[10] It could be hypothesized that when vitamin D is capable of diminishing inflammatory processes, this may also result in less fatigue. For that reason, inflammation can be a mediator in the association between vitamin D and fatigue.

Inflammation could, however, also confound the associations between $25OHD_3$ and quality of life since inflammatory processes may be a cause instead of a consequence of a reduction in circulating $25OHD_3$ concentrations. Therefore, when inflammation impacts $25OHD_3$ concentrations and simultaneously has a negative impact on fatigue, it confounds the association between $25OHD_3$ and fatigue. This was tested by evaluating the addition of an inflammatory z-score to the regression model, but no effect on the regression coefficients was observed. Nonetheless, it must be noted that the z-score may not have given the most accurate reflection of an inflammatory state and the potential role of inflammation as a confounder or mediator needs to be further evaluated in future studies.

Main findings on B-vitamins

The B-vitamins and related biomarkers were investigated in relation to global quality of life, functioning scales, and fatigue. Increased circulating PLP (vitamin B6) concentrations were cross-sectionally associated with better physical, role, and social functioning, and reduced fatigue at 6 months post-diagnosis. In addition, higher HKr was associated with decreased global quality of life and lower physical and role functioning. Biomarkers of folate, vitamin B2, and vitamin B12 were not associated with quality of life. Dose-response relations were observed for deficient, suboptimal, and adequate concentrations of PLP in relation to global quality of life, physical, role, and social functioning at 6 months post-diagnosis. No associations were observed for changes in biomarker concentrations between diagnosis and 6 months post-diagnosis in relation to quality of life outcomes, potentially because only minimal changes in biomarker concentrations were observed between the two time points.

Biological mechanisms of vitamin B6

As for vitamin D, underlying pathways related to inflammation may also cover part of the biological explanation for the association between vitamin B6 and quality of life. During inflammatory conditions, plasma PLP concentrations are often low and PLP seems to be redistributed in tissues.[11] PLP has been found to be inversely associated with inflammatory markers such as C-reactive protein (CRP), neopterin, and the kynurenine/tryptophan ratio (KTR).[11,12] However, the direction of the associations between PLP and inflammation needs further study as it is unclear whether a vitamin B6 deficiency induces inflammatory responses, or whether inflammation contributes to lower vitamin B6 concentrations.[11] Two pathways that shed further light on the relation between vitamin B6 and inflammation are the kynurenine pathway and metabolism of the amino acids serine and glycine, which are also involved in folate-mediated one-carbon metabolism. In short, PLP is an important co-factor in kynurenine metabolism, thereby promoting the anti-inflammatory function of some kynurenines.[11] In addition, the conversion of serine to glycine is catalyzed by the PLP-dependent enzyme serine hydroxymethyltransferase (SHMT), thereby influencing the anti-inflammatory activities of serine and glycine metabolism.[11] Inflammation may subsequently lead to increased fatigue and lower quality of life, making inflammation a mediator in the association between vitamin B6 and quality of life. However, as noted above, the direction of the association between vitamin B6 and inflammation may be bi-directional and inflammation may therefore also be considered a confounder in these observations. The mechanistic role of vitamin B6 in relation to quality of life and fatigue needs to be further unraveled before clear conclusions can be drawn on the exact role of vitamin B6. Nevertheless, these results call for further study as vitamin B6 may be a possible lead in enhancing the quality of life of colorectal cancer survivors.

Aim III – Food frequency questionnaire validation study

The validity of the food frequency questionnaire that is used within the EnCoRe study was evaluated in comparison to a 7-day dietary record to obtain insight in the performance of the questionnaire in a population of colorectal cancer survivors. To the best of our knowledge, this was the first time that the validity of a food frequency questionnaire was evaluated in an adult population of cancer survivors. The study therefore adds to the general understanding of the performance of food frequency questionnaires to measure dietary intake in this particular population.

The Spearman correlation coefficient for total energy intake, adjusted for within-person variation, was 0.62. Highest correlations were observed for the nutrients alcohol (0.91), polysaccharides (0.75), and vitamin B12 (0.74). For food groups, the highest correlations were observed for potatoes (0.99), cereals and cereal products (0.96), and fish (0.96). The intake of total energy and most nutrients were overestimated by the food frequency questionnaire, except for

the intake of alcohol, vitamin B6, folic acid, and dietary folate equivalents, which were underestimated as compared to the dietary record. The Spearman correlation adjusted for within-person variation for vitamin D was 0.67, for vitamin B2 0.42, for vitamin B6 0.61, for folic acid from fortified foods 0.14, for folate 0.57, for dietary folate equivalents 0.47, and for vitamin B12 0.74. All in all, the ranking of subjects according to their nutrient and food group intakes by the food frequency questionnaire was moderate to good in comparison with a 7-day dietary record. Further considerations concerning the food frequency questionnaire validation study are discussed in part B of this chapter.

B. Methodological considerations

In part B of this chapter, a number of methodological aspects regarding the research conducted in this thesis are discussed. First, the measurement of the main variables is evaluated along with the possible influence of information bias. Second, the external validity of the results and the issue of selection bias are discussed. Third, the potential relevance of confounding is evaluated, and fourth, a reflection is given on causality and what we can conclude about the direction of the observed associations.

Measurement of the outcome

Health-related quality of life

In this thesis, quality of life, functioning domains, and fatigue were measured by validated questionnaires, using both generic and condition-specific instruments. Quality of life is known to be a challenging construct to measure given its multidimensional and subjective nature.[13] Quality of life has become a widely used outcome measure in oncological care to understand and identify the impact of problems during treatment as well as of late effects after treatment (such as fatigue).[13,14] Given the subjectivity of the concept, self-report is considered the best method to assess quality of life and its related aspects.[14] The use of generic instruments, such as the Checklist Individual Strength to measure fatigue, allows comparisons between various populations, including non-cancer populations. By using condition-specific questionnaires, such as the EORTC QLQ-C30, multiple health problems and dimensions of quality of life and functioning are assessed that are of particular relevance in relation to cancer and its treatment, thereby increasing the clinical relevance of the outcome.[13]

Two phenomena that are worth discussing in relation to the measurement and interpretation of quality of life over time are ‘response shift’ and ‘benefit finding’. A response shift is described as the adjustment of patients’ internal standards and values regarding the perception of their quality of life during the process of a cancer diagnosis, its treatment, and thereafter, in which patients have to deal with their diagnosis, adapt to new situations, and live with the lasting

consequences of cancer.[15] Due to evolving controllability and coping abilities, the relative importance of the domains associated with quality of life may change over time, and patients' responses to questions may consequently alter, even though their health status may remain unchanged.[15] Furthermore, benefit finding refers to the sense of progress or personal growth that cancer survivors may experience as a consequence of the cancer.[16] In spite of the health and functioning problems that commonly accompany a cancer diagnosis, survivors may experience an increased appreciation of life due to closer relations with relatives and the increased acceptance of life's imperfections. Benefit finding is generally higher closer to diagnosis and was found to be positively associated with quality of life.[16]

Response shift and benefit finding are not considered types of bias as they are natural responses after a cancer diagnosis. Cancer survivors might report better overall quality of life due these phenomena, thereby providing a possible explanation for the overall good quality of life that has previously been reported in cancer survivors.[17] However, results of this thesis also suggested that elevated and severe levels of fatigue persisted in more than one third of the study population up until 2 years post-treatment. Therefore, fatigue itself may be less influenced by response shift or benefit finding yet the impact of fatigue on reported quality of life may be reduced.[18]

Measurement of the exposures

Supplement use

Within the EnCoRe study, supplement use was assessed during home visits. There were low chances for measurement error to be introduced since participants were asked to show the original packaging of the supplement and received detailed questions about use, after which a dietitian wrote all details on standardized registration forms. Recall bias might have been an issue when participants were asked to report use over a longer period of time, such as at diagnosis or 2 years post-diagnosis (both a 1 year recall), in case they had stopped using the supplement some time before the home visit and had discarded the package. In addition, there are no strong norms or standards around the use or nonuse of supplements so participants were likely honest about their use, causing the influence of socially desirable answers to be relatively low compared to for instance dietary intake assessment. Furthermore, it could have been challenging for participants to report and for dietitians to register irregular supplement use. However, an advantage of the performance of home visits in comparison to self-reported measures is the possibility of the dietitian to encourage the participant to recall use and ask for clarification, in order to report use in the most correct possible way. As supplement use was defined as use at least once a week for a period of one month or more, occasional and irregular use were not taken into account in the final analyses. Potential errors in the registration of irregular use might therefore not have influenced

the analyses. Vitamins or minerals that were used less than once a week, but contained a high dose to cover the intake for a longer period of time (e.g. vitamin D) were also taken into account. Although supplements were defined according to the broadly used definition of the European Food Safety Authority (see chapter 2), disparities may appear between studies regarding the in- and exclusion of certain types of 'supplements', e.g. vitamin injections (considered as medication and not as a supplement in this thesis) or food items such as chia seeds or apple vinegar (considered part of the diet and not as a supplement in this thesis).

Within the analyses using data of the FOCUS consortium (chapter 4), the COLON and ColoCare study used self-administered questionnaires to assess supplement use. These methods were previously found adequate to assess supplement use in comparison to in-person interviews and biological markers.[19,20] Potential sources of measurement error that were reported in these studies were the estimation of the micronutrient composition of supplements and the distinction between multivitamins and single supplements, in particular regarding calcium.[19] Since the analyses in chapter 4 only included frequency of B-vitamin use (yes/no), there was reduced possibility for measurement error with regard to these two sources of error.

In sum, the methods used in this thesis to measure the use of supplements were valid and reliable, and the performed analyses with quality of life as outcome were not believed to be largely influenced by potential errors in the measurement of supplement use.

Blood biomarkers of vitamin D and B-vitamins

Biomarker measurements were obtained for vitamin D, the B-vitamins, and related biomarkers such as total homocysteine. The analyses of all markers were performed using high quality and state-of-the-art techniques.[21-26] Liquid chromatography coupled to tandem-mass spectrometry (LC-MS/MS) was used to measure circulating $25OHD_3$ concentrations, which is the superior and preferred method compared to the traditional and more widely used automated immunoassay.[21,27] In addition, the measurement of folate as the sum of 5-methyltetrahydrofolate and 4-α-hydroxy-5-methyltetrahydrofolate is a favored method because it takes the loss of folate due to its rapid degradation during storage into account, whereas this is not done in other methods such as a microbiological assay based on the growth of *Lactobacillus rhamnosus*, which is therefore a less reliable method.[28] Within the FOCUS consortium, blood samples were shipped by each cohort to a renowned laboratory in Bergen, Norway, where all analyses were performed. Therefore, the analysis techniques were identical between the cohorts, facilitating the harmonization of data. In comparison to questionnaire data, biomarker data are more objective and subject to other types of bias. Bias may for instance be introduced in the phase of collecting and storing the blood samples, such as the use of fasting and

non-fasting samples as was the case in the FOCUS consortium. In addition, genetic factors could have influenced the measurements, such as a variation in the methylenetetrahydrofolate reductase (MTHFR) gene, responsible for the conversion of 5,10-methylene tetrahydrofolate to 5-methyl tetrahydrofolate, causing homocysteine levels to increase.[29] Data on these factors (prandial status and genetics) were not available, and could therefore account for some of the variation between the samples.

Measuring dietary intake

A perfect instrument to measure dietary intake is lacking and studies often evaluate the relative validity of a food frequency questionnaire in comparison to a second dietary assessment method.[30] Since the errors of food frequency questionnaires and dietary records are largely independent given their difference in purpose (retrospective measurement of habitual intake versus measurement of actual intake), dietary records are considered a suitable comparison method to evaluate the validity of a food frequency questionnaire, even though they do not provide unbiased estimations of intake.[30,31] An alternative method to evaluate the validity of a food frequency questionnaire is the use of biomarkers.[32] Recovery biomarkers, a category of biomarkers directly related to intake, are preferred as they provide largely unbiased reference measurements.[33,34] However, markers are currently only available for energy intake using doubly labeled water, for protein intake using urinary nitrogen excretion, and for sodium and potassium intake using urinary sodium and potassium excretion.[33,34] Other categories of biomarkers are concentration biomarkers and predictive biomarkers, which are available for some dietary compounds, such as blood concentrations of certain vitamins and the urinary excretion of sucrose and fructose, yet those are not preferred to evaluate the validity of dietary assessment methods because they may be highly influenced by person-related factors like metabolism and lifestyle, or give an incomplete reflection of dietary intake.[34] It is not always feasible to make use of dietary biomarkers to evaluate the validity of food frequency questionnaires given their limited availability, the high costs involved, and the possible high respondent burden.[35] Duplicate portions are another potential reference method, characterized by participants keeping an identical portion of all foods and drinks consumed, and the concurrent completion of a weighed dietary record.[36] Biochemical analyses are carried out to measure food composition. The duplicate portions technique is, however, unsuitable for large scale studies because they are expensive, burdensome, and require extreme motivation of the participants. Consequently, validation studies opt for reference methods that are less-biased and considered superior to a food frequency questionnaire, such as dietary records.[30] This approach is believed to give a good impression of the performance of the food frequency questionnaire in ranking subjects according to their intake.[30] Nevertheless, it needs to be kept in mind that the validity of the food frequency questionnaire was evaluated against a method that is also not free from measurement error, potentially causing the correlation coefficients to be somewhat inflated.[35]

In this thesis, the food frequency questionnaire was administered approximately 2 weeks after participants had completed the dietary record. Preferably, however, the instrument to be validated is administered before the reference instrument, in order to prevent the reference instrument from influencing the instrument to be validated, as discussed in chapter 5. In this study, the main reason to administer the dietary record first, was the subsequent overlap between the reference periods of both methods; the one month reference period of the food frequency questionnaire included the one week that the dietary record was completed. Regardless of the sequence, the second instrument will always be influenced by the first instrument.[30] It is important to note that perfect correlation was not expected, and would never be obtained, because both methods differ in their purpose and reference periods only partially overlapped. Regarding the measurement of vitamin D intake, the food frequency questionnaire performed moderate to good in comparison to a 7-day dietary record. However, the measurement of dietary vitamin D intake is of limited relevance because exposure to sunlight is the main source of this vitamin. Regarding the measurement of B-vitamin intake, Spearman coefficients of <0.50 were observed for vitamin B2 and folic acid, and linearly weighted kappa coefficients of <0.40 were observed for vitamin B2, vitamin B6, folate, and folic acid in comparison to the dietary record. The food frequency questionnaire data may therefore not adequately reflect the complete dietary intake of an individual over a certain period of time, especially concerning a number of micronutrients, such as the B-vitamins. Nonetheless, the food frequency questionnaire is useful in the estimation of the majority of estimated nutrients and food groups, and results are valuable for potential regression calibration when performing future analyses between the diet and certain health outcomes using the data as measured by this food frequency questionnaire.[35]

External validity

A question with important consequences for the implications of this thesis is: to what extent are the study participants a representation of the general population of colorectal cancer survivors? The answer tells us whether the results of this thesis are generalizable to the general population of colorectal cancer survivors seen in everyday clinical practice. Within the EnCoRe study, participant recruitment took place by asking newly diagnosed colorectal cancer patients in three hospitals to participate in the study. Several in- and exclusion criteria were applied; see Table 1. Importantly, patients with stage I to III disease were included, but stage IV patients were excluded, because their quality of life is likely not primarily determined by lifestyle but by the poor prognosis associated with stage IV disease. Apart from the criteria in Table 1, the selection of participants was independent of specific characteristics that would limit the generalizability of the population.

Upon study inclusion, selection bias could have been introduced if participants with unhealthier lifestyle behaviour and (increased risk of) worse quality of life

were less likely to participate. In addition, a higher proportion of participants included in the EnCoRe study were men (69%), compared to data of incident colorectal cancer cases in the Netherlands in 2015 (57%).[38] The age of EnCoRe participants was comparable to data on age at colorectal cancer diagnosis of the national cancer registry, on average 67 and 69 years, respectively.[38] Despite the potential differences, it remains uncertain whether this could have led to selection bias and could have distorted the observed associations.

Table 1. In- and exclusion criteria of the EnCoRe prospective cohort study.[37]

Inclusion criteria
• Men and women, aged >18 years; • Established diagnosis of stage I, II, or III colorectal cancer, including recurrent colorectal cancer, at Maastricht University Medical Center+, VieCuri Medical Center, and Zuyderland Medical Center.
Exclusion criteria
• Diagnosis of stage IV colorectal cancer; • Current home address not in the Netherlands; • Inability to understand the Dutch language in speech as well as in writing (e.g. reading disorders or illiteracy); • Presence of comorbidities that may obstruct successful participation, including cognitive disorders such as Alzheimer disease, and severe visibility or hearing disorders such as complete blindness and/or deafness.

A major strength of the EnCoRe study is the high response rate (>90%) for follow-up time points as a result of the high level of commitment of participants, most likely due to the performance of home visits and the close contact that is kept with the participants during study follow-up. However, when participants with worse health outcomes, and worse quality of life, more often died or dropped out during post-treatment follow-up, a more healthy study population would remain and explain part of the observed improvements in quality of life over time. However, given the high response rates and the relatively low number of deaths — 17 participants (5%), see flow diagram in chapter 2, the observed improvements in quality of life were likely due to actual improvement rather than a result of a selection of healthy participants staying in the study. The decreasing numbers of participants over time who were included in the longitudinal analyses are a result of the method of data processing that was used. Data collected until November 1st 2016, were processed, cleaned, and used in the analyses in this thesis. This led to a lower number of included participants for the follow-up time points, simply because these time points were not yet reached by participants at November 1st 2016. These participants were therefore not lost to follow-up because they remained in the study and received follow-up measurements after November 1st 2016, which are included in future analyses of the EnCoRe study. In the analyses, data were used of participants with at least one follow-up visit with available data on both the exposure and the outcome.

To come back to the central question at the start of this paragraph, the results of thesis can be generalized to the general population of stage I-III colorectal cancer survivors. It must be noted that it is unknown whether the observed associations were altered as a consequence of a healthier study population with a better quality of life and higher proportion of men, as compared to the general population of colorectal cancer survivors. The additional use of data from the FOCUS consortium in chapter 4 led to a more diverse study population because of the use of data from multiple studies, thereby improving the generalizability of the results. Whether the results of this thesis are generalizable to a broader population of cancer survivors needs to be evaluated in future studies. Other populations of cancer survivors, e.g. breast cancer survivors, may encounter different health problems compared to colorectal cancer survivors because of the exposure to different types of treatment that target other parts of the body. Moreover, populations of cancer survivors differ with regard to demographic and lifestyle characteristics, such as sex, age, and the prevalence of supplement use and vitamin deficiencies. Nonetheless, when future intervention studies would, for instance, show that vitamin D supplement use is beneficial for reducing fatigue in colorectal cancer survivors with low vitamin D levels, this would give a strong motive for evaluating this effect in other populations of cancer survivors.

Confounding

For all analyses of this thesis, potential confounding variables were carefully selected based on their relevance in relation to both the exposure and the outcome under investigation by making use of the literature and causal diagrams. The sets of confounding variables that were used always covered a broad selection of variables from multiple domains, i.e. demographic variables such as sex and age, clinical variables such as therapy, and lifestyle variables such as physical activity and body mass index. Thus, efforts were made to reduce the chance of residual confounding to the lowest possible level. The presence of comorbidities was another variable that was considered an important confounder in the analyses with data of the EnCoRe study. Due to the unavailability of a harmonized variable on the presence of comorbidities in the FOCUS consortium, the analyses on B-vitamin biomarkers and supplement use were not adjusted for the presence of comorbidities. Although the analyses were thoroughly adjusted for other confounding variables, the inability to adjust for the presence of comorbidities was a limitation of these analyses, potentially causing an overestimation of the regression coefficients. The adjustments that were made in the analyses with data of the EnCoRe study were very comprehensive and likely left little room for residual confounding. The influence of inflammation as a potential confounder needs to be investigated in future studies, as explained in part A of this discussion. All in all, the observed associations in this study were believed to be minimally influenced by residual confounding given the thorough adjustment that was performed in all analyses.

Causality

The analyses in this thesis were performed using data from prospective cohort studies. A major advantage of such studies is the possibility to study the exposure and outcome variables over time, as well as their joint development.[39] The observational nature of the data, however, makes it challenging to draw firm conclusions on the direction of observed associations. The associations between supplement use and increased fatigue (chapter 2), for instance, were presumably caused by an association in the reversed direction. This could be explained by the possibility that participants with more fatigue use supplements in response to their complaints, as was recognized in previous literature[1] and has been discussed in part A of this chapter. In addition, the associations between higher vitamin D concentrations and reduced quality of life could have been directed by individuals with a better quality of life spending more time outdoors, causing an increase in vitamin D status, rather than a high vitamin D status being responsible for an improved quality of life. Even though intra-individual associations, which represent the changes made within an individual over time, can be disentangled with longitudinal data, cause and effect cannot be discriminated in these analyses.

The criteria for causality by Bradford Hill are the most commonly used to evaluate whether an observed association is causal or not.[40] One criterion is temporality, which can only be assessed when the exposure variable precedes the outcome. In chapter 2, time-lag analyses were performed by modeling the variables in such a way that the exposure variable (supplement use) was associated with the outcome variable (fatigue) on the next time point.[39] Although not statistically significant, the altered beta-coefficients of overall, inter- and intra-individual associations suggested that the initially observed associations were directed the other way, i.e. higher levels of fatigue causing participants to use supplements. The results of time-lag analyses should be interpreted with caution because power is decreased due to the elimination of the first and last time point from the analyses. In addition, the lag-model is less useful when the time between measurements is long and does not match biologically plausible duration for associations.[39] Moreover, a certain amount of time lag can already be present in the measurement of the exposure variable, which was the case with the retrospective report on supplement use in this thesis (use since the previous time point). Another important point to consider with regard to causality and time-lag analyses in this thesis is the continued presence of the outcome variable over time. The associations at one time point could therefore be confounded by quality of life at the preceding time point.

Two other criteria for causality are the presence of a biological gradient and a plausible biological mechanism underlying the association. Both for the associations of vitamin D and vitamin B6 with quality of life, dose-response associations were observed, indicating that an increase in the exposure was associated with an increase in the outcome. In addition, the analyses were based

on *a priori* defined hypothesis, and observed associations could be biologically explained as both vitamin D and vitamin B6 are involved in pathways that could work towards the alleviation of health problems, consequently leading to better quality of life. These findings therefore strengthen the argument for a relationship that is potentially biologically relevant.

Another criterion refers to the strength of the observed associations, which can be evaluated by comparing the observed effect sizes against the minimally important differences for clinical relevance, as have been previously defined for the different outcomes.[41-43] The effect sizes from the analyses in this thesis mostly did not reach the minimally important differences. Relevant contrasts in the exposure variable were used when possible, such as the one standard deviation (20 nmol/L) increase in $25OHD_3$ concentration, while a doubling in biomarker concentrations was used for the analyses on B-vitamins, which might not represent a realistic contrast. The contrasts between deficient and adequate concentrations likely represent relevant contrasts, which also more often reached the level of minimally important difference, as shown in the results of dose-response relations. Although effect sizes can be small, they can still be causal and relevant.[44] Associations were also in line with the literature and therefore seemed to show consistency in different populations and circumstances; another criterion of causality. Related to this, associations of vitamin D and B6 concentrations with the different outcomes consistently pointed in the same direction, i.e. better quality of life, improved functioning, and reduced fatigue.

To conclude this paragraph, caution is warranted in drawing conclusions about cause and effect based on the analyses in this thesis. When evaluating the criteria for causation, a number of criteria are met that could point at causal relationships, such as biological plausibility and consistency, but there are also some criteria that are not or only partially met, e.g. temporality and the strength of associations. The results of this thesis give a strong foundation for forthcoming studies on vitamin D and vitamin B6 in relation to aspects of quality of life in colorectal cancer survivors, and results need to be interpreted with caution when formulating implications, e.g. regarding the use of supplements.

C. Implications for practice and future research directions

The results of this thesis add to the body of evidence that is needed to formulate clear-cut and evidence-based lifestyle recommendations for colorectal cancer survivors, in order to provide them with practical guidance in how they can personally work towards an improved quality of life after the end of cancer treatment. The current absence of such recommendations, specifically developed for cancer survivors and targeting quality of life, creates a gap between the patient's need for support and the options available for practitioners to provide tailored care. The final part of this chapter describes important implications that arise from this thesis and which are relevant to current colorectal cancer survivorship practice. In addition, research directions are formulated for future studies.

Implications for practice

Supplement use

A central theme in this thesis is the use of supplements by colorectal cancer survivors over time. Since evidence supporting the potential beneficial effects of supplements is lacking, current guidelines discourage use.[45-47] In spite of this advice, it was observed that supplement use is still common among this population in the first 2 years after colorectal cancer treatment. Guidelines may therefore not always reach patients and practitioners, may be misinterpreted or may possibly be disregarded. This thesis observed inter-individual associations between supplement use and increased fatigue, thereby indicating that fatigue may be a reason for supplement use. Supplement use may therefore be perceived as a self-managing strategy of colorectal cancer survivors to reduce their complaints, as also implied by the reported motivations for supplement use in chapter 2, with the reduction of specific complaints being the most frequently reported reason for use.

An important implication that can be extracted from these findings is the need for specific attention regarding advice on supplement use during the post-treatment survivorship trajectory. Cancer survivors would benefit from awareness and alertness among practitioners about the frequency of supplement use over time among this population. Since supplement use is generally conceived as innocent and harmless, it is particularly important that survivors are reminded of the fact that supplement use is unnecessary when consuming a varied and healthy diet, as well as of the potential detrimental consequences of supplement use, such as interactions with therapy and intakes exceeding the tolerable upper intake level.[4,48] The frequent use of supplements by cancer survivors over time underlines the importance of an open dialogue between survivors, their families, and practitioners about supplements, both

before and after treatment. Remarkably, Dutch oncology nurses often (43%) perceive themselves as incompetent for providing tailored advice on nutrition because of insufficient knowledge on the topic.[49] Moreover, nurses with insufficient knowledge more often recommended the use of supplements.[49] Dutch dietitians follow national guidelines from the Netherlands Comprehensive Cancer Organisation (Oncoline) and advise against high doses of antioxidants, fish oil supplements, and the consumption of fatty fish during chemo and/or radiotherapy.[50] In general, supplement use is not encouraged by dietitians, unless dietary intake is inadequate.[50] As part of current colorectal cancer follow-up care, referral to specialized health care professionals, such as dietitians or practitioners specialized in integrative medicine, may be necessary more often, to assist survivors with questions about nutrition and supplement use.

Colorectal cancer follow-up

In follow-up practice, colorectal cancer survivors would further benefit from evidence-based advice and information about effective options on how to deal with problems like fatigue and diminished functioning. Fatigue in particular requires increased awareness given its long-lasting impact and high prevalence up to 10 years after treatment.[51,52] A patient-centered approach is warranted since the underlying cause(s) and contributing factors of fatigue may differ per individual.[53] A number of national and international guidelines are currently available for the treatment of cancer-related fatigue, including the Dutch guideline for cancer-related fatigue in palliative care (Oncoline) and the standards of care for cancer-related fatigue developed by the National Comprehensive Cancer Network.[54,55] Both guidelines emphasize the initial focus on cause-specific management, regular assessment and evaluation, and appropriate referral to other health care professionals.[54,55] Non-specific treatment of fatigue is aimed at physical activity, behavioural and psychosocial programs, and pharmacologic interventions such as corticosteroids.[54,55] The guidelines also emphasize the increased need for awareness of fatigue, both in research and in practice, because the symptom is currently insufficiently recognized and acknowledged.[54,55] Furthermore, survivors themselves should be encouraged to report symptoms of fatigue to their practitioners so that screening of fatigue can take place and treatment can be initiated.

In the Netherlands, colorectal cancer follow-up is primarily provided by surgeons (71%) and to a lesser extent by nurse practitioners (10%).[56] The majority of survivors have four outpatient clinic visits during the first year and two visits during the second year after treatment, mainly comprising surveillance for metastases and recurrences.[56] The level and type of involvement of general practitioners (GPs) during follow-up cancer care varies.[57] A Dutch qualitative study reported that GPs played a significant role in providing support regarding psychosocial issues, lifestyle-related advice, and management of symptoms during cancer follow-up, even though GPs formally have no responsibilities in follow-up care.[58] A greater role of GPs is envisioned since alternative follow-

up strategies, including GP-led, nurse-led, and remote follow-up, are being evaluated in light of the increasing number of cancer survivors.[57,59]

Surgeons and GPs are currently the principal health care professionals to provide colorectal cancer survivors with guidance concerning the health problems that are encountered. There is a tendency towards supporting survivors and their families in the self-management of symptoms as a core strategy in improving the quality of life of cancer survivors.[60,61] Engagement in their own care, by using self-acquired knowledge and skills, may empower survivors to make sustainable lifestyle changes, such as adaptations in nutritional habits, to manage health-related problems, and live a healthy life after cancer. Surgeons and GPs would be the primary persons to support survivors, refer them to specialized (para)medic care, mental health professionals, or available websites and online tools, such as *Voeding & Kankerinfo*[62] and *Kanker Nazorg Wijzer.*[63] As a result, colorectal cancer survivors would be prevented to search for solutions themselves, potentially ending up with strategies, such as the use of supplements, that appear harmless but could be unsafe.

Vitamin D

The results of this thesis demonstrated low compliance to the prevailing vitamin D supplementation recommendations in women aged >50 years and women and men aged >70 years, comprising about half of the population under study. Compliance to the recommendations, that have been set up for the prevention of osteoporosis, is also known to be low among the general Dutch population;[64,65] 18-26% of the elderly used vitamin D supplements according to the Dutch National Food Consumption Survey Older Adults 2010-2012.[66] Previous research showed that 65-70% of Dutch geriatricians and GPs were aware of the vitamin D supplementation recommendations, and about half of them prescribed vitamin D for the elderly aged >70 years.[65] There is uncertainty among GPs and geriatricians on whether they should actively prescribe vitamin D or not,[64] and this could partially explain the low compliance to the recommendations. The susceptibility of colorectal cancer survivors for low vitamin D status[67-69] and the potential negative consequences of a deficiency, call for more active information provision to survivors about the guidelines on vitamin D supplementation and sun exposure – 15 to 30 minutes of daily exposure from March to November.[5] Survivors who are not part of the age groups of the supplementation guideline, and who encounter symptoms as fatigue, would likely benefit from a check of their vitamin D status, after which necessary measures can be taken. Moreover, when time outdoors is spent actively, e.g. going for a walk, a short run, or a bicycle ride, both the recommendations on sun exposure and decreasing sedentary behaviour could rather easily be met. Awareness of vitamin D deficiencies among GPs, surgeons and other health care professionals would contribute to a more active and integrated approach towards reducing the prevalence of vitamin D deficiencies in colorectal cancer survivors.

Next to vitamin D, the results of this thesis showed that vitamin B6 status was positively associated with quality of life, and 27% of participants had suboptimal or deficient circulating PLP concentrations. Vitamin B6 is therefore another vitamin of interest to colorectal cancer survivors, and its possible contribution to a better quality of life needs further study.

Future research directions

This thesis provides important leads for future research. A strong foundation is given for further investigation into circulating vitamin concentrations in relation to quality of life and fatigue in colorectal cancer survivors. Consistent longitudinal associations, both inter- and intra-individually, and dose-response relations were observed between $25OHD_3$ and quality of life, functioning, and fatigue. Future experimental studies should target survivors with low vitamin D concentrations to assess whether an increase in vitamin D towards adequate concentrations has beneficial effects on complaints of fatigue and the quality of life of colorectal cancer survivors. A placebo-controlled trial is currently ongoing at the German Cancer Research Center (DKFZ).[70] Colorectal cancer survivors within 6 months after diagnosis, with a vitamin D deficiency and mild to severe fatigue, receive vitamin D supplements for a period of 12 weeks, after which fatigue and quality of life will be evaluated. The trial will give insight in whether associations between $25OHD_3$ and quality of life are causal or not.

Two markers of vitamin B6 were cross-sectionally associated with quality of life outcomes at 6 months post-diagnosis. Additional dose-response relations were observed for PLP concentrations with quality of life. Prospective studies are required to investigate whether markers of vitamin B6 are also associated with quality of life in longitudinal analyses, preferably with separation of inter- and intra-individual associations. In addition, longitudinal associations of the intake of vitamin B6 through the diet with quality of life needs to be further examined as the diet is an important source of vitamin B6 intake; e.g. meat, eggs, fish, bread, potatoes, vegetables, and dairy products contain vitamin B6. Such longitudinal associations between vitamin B6 intake, through the diet and supplements, and quality of life would give stronger indication for conducting intervention studies on whether vitamin B6 intake could be effective for improving quality of life.

For identifying colorectal cancer survivors who may be in need of lifestyle advice for the benefit of their quality of life, prediction models can be useful in predicting which individuals are at risk of low quality of life.[71] When these individuals are identified in an early stage, targeted interventions could be implemented to prevent deterioration of quality of life. Interventions could, for instance, comprise the introduction of lifestyle changes, such as optimization of vitamin D status. But first, conclusive evidence from experimental studies must be available to support the positive impact of such interventions on the quality of life of a specific population at risk. Prediction models may facilitate

selection of individuals eligible for intervention studies.

As discussed in part A of this chapter, inflammation could work as a possible mediator in the associations of vitamin D and vitamin B6 with fatigue and quality of life. It would be insightful to conduct mediation analyses to evaluate whether the associations of these vitamins with fatigue and quality of life are mediated by inflammatory processes. When inflammation turns out to be mediating these associations, further studies could evaluate whether approaches to reduce inflammation may be of benefit for decreasing fatigue and improving quality of life. In addition, the potential role of inflammation as a confounding variable should be investigated more comprehensively in the associations between vitamin D and quality of life. Moreover, other specific inflammatory markers, such as prostaglandins, growth factors, and cytokines could additionally be used to obtain a more comprehensive reflection of the inflammatory processes in the tumor micro-environment.[72,73]

In addition to inflammation, other themes for future research are the underlying mechanisms that contribute to cancer-related fatigue. Those remain largely unclear and would, if better understood, specify areas for therapies to intervene in order to alleviate the symptom. Furthermore, the harmful consequences of supplement use during and after treatment, i.e. interference with therapy and consequences of high doses, need to be clarified.

To conclude, the research in this thesis has demonstrated longitudinal associations between supplement use and increased fatigue, potentially because colorectal cancer survivors use supplements in order to decrease symptoms of fatigue. Further, higher biomarker concentrations of vitamin D and B6 were associated with better quality of life. Although the direction of these associations could not be demonstrated, vitamin D and B6 are promising leads for future studies in this field. Important implications that were drawn from this thesis are the need for attention and alertness among health care professionals about the use of supplements by colorectal cancer survivors, the need for increased recognition and dialogue about the symptom of fatigue, and more awareness on the prevalence and consequences of vitamin deficiencies in this population.

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SUMMARY

A growing number of patients survive colorectal cancer. This is attributable to detection in an earlier stage of the cancer and to enhancing treatment options. The so-called survivors of colorectal cancer are individuals living with a current or past diagnosis of colorectal cancer. Colorectal cancer survivors often continue to experience health problems due to the tumour and anti-cancer therapy, that negatively impact their quality of life in the years after treatment. Fatigue is one of the most common and distressing symptoms of colorectal cancer survivors. There is a need for effective and tailored strategies on how to improve the quality of life and reduce fatigue in colorectal cancer survivors.

As introduced in **chapter 1** of this thesis, we aimed to obtain insight in the role of supplements, vitamin D, and B-vitamins (status and intake) in relation to quality of life and fatigue in the growing population of colorectal cancer survivors. Previous studies already showed that colorectal cancer survivors are frequent users of supplements. Current recommendations, such as from the World Cancer Research Fund, advise against the use of supplements and recommend to obtain an adequate intake by the diet alone. Furthermore, the use of validated methods to estimate dietary intake in research is essential to correctly estimate the relation between dietary intake, including the intake of vitamins, and a certain health outcome, such as quality of life.

Three main aims were addressed:

I. To examine the association of supplement use in general, and the use of supplements containing vitamin D and B-vitamins, with quality of life and fatigue in colorectal cancer survivors (chapters 2, 3, and 4).
II. To investigate whether blood biomarkers of vitamin D and B-vitamins are associated with quality of life and fatigue in colorectal cancer survivors (chapters 3 and 4).
III. To evaluate the validity of a food frequency questionnaire in comparison to a 7-day dietary record to measure dietary intake in colorectal cancer survivors (chapter 5).

Data from the EnCoRe study (Energy for Life after ColoRectal Cancer) and the FOCUS consortium (Biomarkers related to Folate-dependent One-carbon metabolism in colorectal Cancer recUrrence and Survival) were used. The EnCoRe study is an ongoing prospective cohort study, initiated in 2012, recruiting stage I-III colorectal cancer patients upon diagnosis from three hospitals in the southeast of the Netherlands. The objective of the EnCoRe study is to investigate longitudinal associations between lifestyle factors and health-related quality of life, functioning, and prognostic outcomes in colorectal cancer survivors up to two years after the end of treatment. The international FOCUS consortium comprises four cohort studies and has the objective to analyze associations of circulating folate and related biomarkers with survival, recurrence, and quality of life in colorectal cancer survivors.

Chapter 2 of this thesis described the use of supplements in colorectal cancer survivors, and investigated longitudinal associations between supplement use and fatigue. About 40% of the participants used supplements over the period from diagnosis to two years post-treatment. Of participants with at least two available measurements, 28% were consistent users and 45% were consistent nonusers. The remaining 27% of participants were inconsistent users, reporting both use and nonuse over time. Overall longitudinal analyses showed no statistically significant associations between supplement use and fatigue. In inter-individual analyses, supplement users reported higher fatigue compared to nonusers of supplements over time. These findings suggest that increased levels of fatigue may be a reason instead of a consequence for supplement use among colorectal cancer survivors.

In **chapter 3**, longitudinal associations between serum vitamin D concentrations and vitamin D supplements and intake with quality of life, cognitive functioning, fatigue, depression, and anxiety were investigated. Circulating 25-hydroxyvitamin D_3 ($25OHD_3$) concentrations decreased after diagnosis and subsequently increased during follow-up. Vitamin D deficiencies were present in 45% of colorectal cancer patients upon diagnosis. Longitudinal associations were observed between circulating $25OHD_3$ concentrations with better quality of life and reduced fatigue, both inter- and intra-individually. No associations were found between vitamin D supplement use and quality of life. In addition, $25OHD_3$ concentrations were not longitudinally associated with cognitive functioning, depression, and anxiety.

Chapter 4 investigated associations of B-vitamin related biomarkers and B-vitamin supplement use with quality of life using data of the FOCUS consortium. Two markers of vitamin B6 status were statistically significantly associated with quality of life in cross-sectional analyses at six months post-diagnosis. First, higher pyridoxal 5'-phosphate (PLP) concentrations were associated with better physical, role, and social functioning, and reduced fatigue. Second, increased 3'-hydroxykynurenine ratio (HKr), an inverse marker of vitamin B6 status, was associated with reduced global quality of life, and lower levels of physical and role functioning. Biomarkers of folate, vitamin B2, and vitamin B12 were not associated with quality of life. No associations were observed for changes in biomarker concentrations between diagnosis and six months. Notably, participants who stopped using B-vitamin supplements after diagnosis reported higher fatigue compared to nonusers of supplements.

The validity of a 253-item food frequency questionnaire was evaluated in a subsample of 100 participants of the EnCoRe study at one year post-colorectal cancer treatment, as reported in **chapter 5**. Dietary intake over the preceding month as measured by a food frequency questionnaire was compared to intake as measured by a 7-day dietary record. Spearman correlations >0.50 were found for 15 of 19 nutrients, with highest agreement for vitamin B12, polysaccharides, and alcohol. Correlations >0.50 were found for 17 of 20 food groups, with

highest agreement for cereals and cereal products, fish, and potatoes. The FFQ performed less well (correlations <0.50) for the micronutrients folic acid, riboflavin, calcium, and dietary folate equivalents, and the food groups legumes; nuts, seeds, and snacks; and fats, oils, and savory sauces. The Spearman correlation between total scores of the World Cancer Research Fund/ American Institute for Cancer Research dietary recommendations was 0.53. All in all, the food frequency questionnaire performed moderately well to good for most nutrients and food groups in comparison to the dietary record.

Chapter 6 gave a summary and interpretation of the main results, reflected on methodological aspects such as external validity and causality, gave important implications that could be drawn from the results, and formulated directions for future research. There is a need for specific attention regarding the current general recommendations to obtain an adequate intake of nutrients through the diet, and not to use supplements, during the post-treatment survivorship trajectory, both among health care professionals and survivors themselves. In addition, it is of importance for colorectal cancer survivors to have access to reliable information and options on how to effectively deal with health problems such as fatigue that frequently occur after a cancer diagnosis. Further, this thesis pointed out that vitamin D deficiencies are common among this population. Awareness of vitamin D deficiencies among GPs, surgeons and other health care professionals would contribute to a more active approach in terms of monitoring and guidance in working towards reducing the prevalence of vitamin D deficiencies in colorectal cancer survivors.

Targeting vitamin D and B6 status are promising potential approaches for reducing fatigue and improving the quality of life of colorectal cancer survivors. First, however, more longitudinal and experimental studies are needed to further unravel the nature of the associations, to draw conclusions about cause and effect, and to develop and evaluate effective approaches to improve the quality of life of colorectal cancer survivors.

SAMENVATTING

Steeds meer patiënten overleven colorectaalkanker. Dit komt doordat de diagnose tegenwoordig vaker in een vroeger stadium wordt gesteld en doordat behandelingsmogelijkheden blijven verbeteren. De zogeheten overlevers van colorectaalkanker zijn mensen die colorectaalkanker hebben of in het verleden hebben gehad. Overlevers van colorectaalkanker blijven vaak gezondheidsproblemen ervaren, als gevolg van de tumor en de behandeling daarvan, welke hun kwaliteit van leven tot jaren na de behandeling negatief kunnen beïnvloeden. Vermoeidheid is één van de meest voorkomende en ingrijpende symptomen onder overlevers van colorectaalkanker. Er zijn nog onvoldoende effectieve en op maat gemaakte methoden om de kwaliteit van leven in deze specifieke populatie te verbeteren en vermoeidheidsklachten te verminderen.

Zoals ingeleid in **hoofdstuk 1** onderzoeken we in dit proefschrift wat de rol is van supplementgebruik en vitamine D en B-vitamines (zowel status als inname) in relatie tot de kwaliteit van leven en vermoeidheid in de groeiende populatie van overlevers van colorectaalkanker. Eerdere onderzoeken hebben laten zien dat overlevers van colorectaalkanker veelvuldig supplementen gebruiken. De huidige adviezen, onder andere van het Wereld Kanker Onderzoek Fonds, raden supplementgebruik echter af en adviseren om benodigde voedingsstoffen binnen te krijgen door middel van de voeding.

De drie doelstellingen die aan de orde komen in dit proefschrift zijn:

I. Het bestuderen van associaties van supplementgebruik in het algemeen en het gebruik van supplementen die vitamine D of B-vitamines bevatten, met kwaliteit van leven en vermoeidheid bij overlevers van colorectaalkanker (hoofdstuk 2, 3 en 4).
II. Het onderzoeken van associaties tussen biomarkers van vitamine D en B-vitamines in het bloed en de kwaliteit van leven en vermoeidheid bij overlevers van colorectaalkanker (hoofdstuk 3 en 4).
III. Het evalueren van de validiteit van een voedselfrequentievragenlijst in vergelijking met een 7-daags voedingsdagboek om de voedingsinname van overlevers van colorectaalkanker te schatten (hoofdstuk 5).

Er is gebruik gemaakt van gegevens van de EnCoRe-studie (Energie voor het leven na ColoRectaalkanker) en het internationale FOCUS-consortium (Biomarkers related to Folate-dependent One-carbon metabolism in colorectal Cancer recUrrence and Survival). De EnCoRe-studie is een prospectieve cohortstudie en includeert sinds 2012 colorectaalkankerpatiënten vanuit drie ziekenhuizen in de provincie Limburg: het Maastricht Universitair Medisch Centrum+, het Zuyderland Medisch Centrum in Sittard-Geleen en het VieCuri Medisch Centrum in Venlo/Venray. Er vinden vanaf de diagnose tot twee jaar na afloop van de behandeling vijf huisbezoeken plaats om longitudinale associaties te onderzoeken tussen leefstijlfactoren en kwaliteit van leven, functioneren, en prognostische uitkomsten. Het internationale FOCUS-consortium bestaat uit vier cohortstudies, waaronder de EnCoRe-studie, en heeft als doel associaties te

onderzoeken tussen folaat en gerelateerde biomarkers en overleving, terugkeer van kanker en de kwaliteit van leven van overlevers van colorectaalkanker.

In **hoofdstuk 2** van dit proefschrift is een beschrijving gegeven van het gebruik van supplementen door overlevers van colorectaalkanker, onder andere de frequentie, het type supplementen en de redenen voor gebruik zijn beschreven. Daarnaast is onderzocht of supplementgebruik over de tijd geassocieerd is met vermoeidheid. Uit de analyses bleek dat 40% van de studiedeelnemers supplementen gebruikt tussen diagnose en twee jaar na afloop van de behandeling. Van de mensen waarvan ten minste twee herhaalde metingen beschikbaar waren, was 28% een consistente gebruiker van supplementen en 45% een consistente niet-gebruiker. De overige 27% was een inconsistente gebruiker; zij rapporteerden zowel gebruik als niet-gebruik. In de longitudinale analyses waarin tussen- en binnenpersoonsassociaties werden gecombineerd, werden geen associaties geobserveerd met vermoeidheid. De tussenpersoonsassociaties lieten zien dat supplementgebruikers meer vermoeid waren in vergelijking met de niet-gebruikers. Deze bevindingen suggereren dat vermoeidheid een reden zou kunnen zijn voor het gebruik van supplementen door overlevers van colorectaalkanker.

In **hoofdstuk 3** hebben we associaties onderzocht van serum vitamine D concentraties, vitamine D supplementgebruik en inname door de voeding, met kwaliteit van leven, cognitief functioneren, depressie en angst. De 25-hydroxyvitamin D_3 ($25OHD_3$) concentratie in het bloed nam na diagnose eerst af en nam vervolgens vanaf zes weken na het einde van de behandeling weer toe. Bij diagnose had 45% van de colorectaalkankerpatiënten een vitamine D deficiëntie. Er werd een longitudinale dosis-respons associatie geobserveerd tussen hogere $25OHD_3$ concentraties en een betere kwaliteit van leven en minder vermoeidheid, zowel binnen als tussen personen. $25OHD_3$ concentraties waren niet longitudinaal geassocieerd met cognitief functioneren, depressie en angst. Er werden ook geen associaties gevonden tussen vitamine D supplementgebruik en kwaliteit van leven.

In **hoofdstuk 4** is gebruik gemaakt van de gegevens van het FOCUS-consortium om te onderzoeken of B-vitamine biomarkers en het gebruik van supplementen die B-vitamines bevatten geassocieerd zijn met kwaliteit van leven. Twee markers van vitamine B6 waren statistisch significant geassocieerd met kwaliteit van leven in cross-sectionele analyses op het tijdspunt zes maanden na diagnose. Hogere concentraties van pyridoxaal-5-fosfaat waren geassocieerd met beter fysiek-, rol-, en sociaal functioneren, en minder vermoeidheid. Daarnaast was een hogere 3'-hydroxykynurenine ratio, een inverse marker van vitamine B6 status, geassocieerd met een verminderde globale kwaliteit van leven, en met lager fysiek- en rol functioneren. De overige onderzochte B-vitamines – biomarkers voor folaat, vitamine B2 en B12 – waren niet geassocieerd met kwaliteit van leven. Verder werden er geen associaties gevonden voor veranderingen in biomarkerconcentraties tussen diagnose en zes maanden en kwaliteit van leven.

Wel werd gezien dat deelnemers die stopten met het gebruik van B-vitamine supplementen na diagnose meer vermoeidheid rapporteerden in vergelijking met niet-gebruikers.

In een subgroep van 100 deelnemers van de EnCoRe-studie is de relatieve validiteit van een voedselfrequentievragenlijst onderzocht op het meetmoment één jaar na afloop van de behandeling, zoals beschreven in **hoofdstuk 5**. De voedingsinname over de voorafgaande maand zoals gemeten door een voedselfrequentievragenlijst werd vergeleken met de inname gemeten door een 7-daags voedingsdagboekje. Spearman correlatiecoefficiënten van boven de 0.50 werden gevonden voor 15 van de 19 onderzochte nutriënten, met de hoogste overeenkomsten voor vitamine B12, polysachariden en alcohol. Verder werden correlatiecoefficiënten van boven de 0.50 gevonden voor 17 van de 20 onderzochte productgroepen, met de hoogste overeenkomst voor graanproducten en bindmiddelen, vis en aardappelen. De voedselfrequentievragenlijst toonde minder goede resultaten (correlatiecoëfficiënten <0.50) voor de micronutriënten foliumzuur, riboflavine (vitamine B2), calcium en folaatequivalenten, en de productgroepen peulvruchten; noten, zaden en snacks; en vetten, oliën en hartige sauzen. De Spearman correlaties tussen de totale scores van de World Cancer Research Fund/American Institute for Cancer Research aanbevelingen op het gebied van voeding was 0.53. Over het algemeen kunnen we concluderen dat de voedselfrequentievragenlijst zoals gebruikt binnen de EnCoRe-studie voldoende tot goed scoort in vergelijking met een 7-daags voedingsdagboekje.

Hoofdstuk 6 gaf, als laatste hoofdstuk, een samenvatting en interpretatie van de belangrijkste resultaten uit het proefschrift, evenals een reflectie op de gebruikte methodologie. Tot slot werden er enkele belangrijke implicaties van de resultaten en richtingen voor toekomstig onderzoek geformuleerd. De resultaten uit dit proefschrift lieten zien dat er in de periode na de behandeling van colorectaalkanker wellicht meer aandacht nodig is, zowel onder gezondheidsprofessionals als onder overlevers van colorectaalkanker, voor het geldende advies om geen supplementen te gebruiken en te streven naar een adequate inname via de voeding voor het binnenkrijgen van benodigde voedingsstoffen. Daarnaast is het van belang dat overlevers van colorectaalkanker toegang hebben tot betrouwbare informatie in het omgaan met gezondheidsproblemen, zoals vermoeidheidsklachten, en vanuit de zorgverlener handvatten krijgen aangereikt om klachten die zij na afloop van de behandeling kunnen ervaren te verminderen. Uit dit proefschrift bleek verder dat vitamine D deficiënties vaak voorkomen onder deze populatie. Er is daarom aandacht nodig van huisartsen, chirurgen en andere gezondheidsprofessionals voor de lage vitamine D concentraties in deze populatie zodat er een meer actieve benadering komt met betrekking tot het monitoren van de vitamine D status en het verlagen van deficiënties ten behoeve van het voorkomen en verminderen van mogelijke gezondheidsklachten.

Vitamine D en B6 zijn veelbelovende vitamines die mogelijk een rol kunnen

spelen bij het verminderen van vermoeidheid en het verbeteren van de kwaliteit van leven van overlevers van colorectaalkanker. Er moet echter eerst meer longitudinaal en experimenteel onderzoek gedaan worden om oorzaak en gevolg uit elkaar te kunnen halen, om verder uit te diepen wat aan de gevonden associaties ten grondslag ligt, en om passende methoden te ontwikkelen en te evalueren voor het optimaliseren van de kwaliteit van leven van overlevers van colorectaalkanker.

KNOWLEDGE VALORISATION

The results of this thesis shed light on the role of supplement use and blood biomarkers of vitamin D and B-vitamins in relation to fatigue and quality of life of colorectal cancer survivors. As stated in this thesis, findings contribute to the body of scientific evidence necessary for the development of clear-cut recommendations and personalized advice regarding the use of supplements and the vitamin status of colorectal cancer survivors. This paragraph will take a broader perspective and elaborate on the value of the findings for areas outside the scientific field, such as clinical practice and society in general.

Growing population of colorectal cancer survivors

The number of people diagnosed with colorectal cancer continues to rise. Colorectal cancer is the third most commonly diagnosed cancer worldwide, after lung- and breast cancer, and accounted for 10% of the total number of new cancer cases in 2018.[1] In the Netherlands in 2019, 13,000 new cases of colorectal cancer were identified and the 5-year prevalence was 55,000.[2] The growing population of colorectal cancer survivors results in an increasing demand for health care services. In addition, the increase in the population of colorectal cancer survivors has economic consequences due to the increased number of individuals who are (temporarily) removed from the workforce and other social roles such as volunteer work, resulting in a loss of societal- and work-related productivity.[3,4]

Following successful anti-cancer treatment, follow-up care of colorectal cancer survivors comprises the surveillance for recurrences and metastases, and also involves guidance in the management of lasting health problems after cancer, such as fatigue, that may contribute to a decreased quality of life.[5] This guidance, however, currently differs in content and intensity between care providers and may be improved in terms of structure and consonance between health care providers involved in follow-up care of colorectal cancer survivors.[6] The development of effective lifestyle recommendations for colorectal cancer survivors may aid in the prevention and/or reduction of health problems in this population, thereby reducing the demand for continued follow-up care. Moreover, self-management strategies, e.g. lifestyle changes that can be made by colorectal survivors themselves, empower colorectal cancer survivors to take control over their own health, resulting in shared involvement of the patients, their social environment, and the health care providers. Consequently, the pressure on the health care provider may be alleviated. In addition, the sooner colorectal cancer survivors fully recover from the consequences of cancer and its treatment, the sooner they will feel fit enough to return to their jobs, resume personal tasks, and participate in society.

Main findings of this thesis

Considering the observational nature of the research, the results of this thesis cannot be translated directly into implementable recommendations or strategies for improving quality of life of colorectal cancer survivors. This thesis

showed that supplements were used by 40% of colorectal cancer survivors from diagnosis to 2 years post-treatment. Associations were observed between the use of supplements and increased fatigue, which implies that colorectal cancer survivors may initiate the use of supplements as a consequence of fatigue. It was also observed that higher circulating $25OHD_3$ concentrations were longitudinally associated with better quality of life and reduced fatigue from 6 weeks to 2 years post-treatment. In addition, two biomarkers of vitamin B6 status were cross-sectionally associated with better quality of life outcomes at 6 months post-diagnosis. Participants who stopped using B-vitamin supplements after diagnosis reported higher fatigue compared to nonusers of supplements. The evaluation of a food frequency questionnaire to measure dietary intake in this population was moderate to good for most nutrients and food groups in comparison to a 7-day dietary record. Findings contribute to the body of evidence that is needed as a scientific foundation for guidelines and advice for this population. Further, as also observed in previous studies, this thesis showed that fatigue is a common problem among colorectal cancer survivors up to 2 years after treatment. This thesis may therefore direct attention towards the problem of fatigue and contribute to the recognition and acknowledgement of this problem among patients, their social environment, and health care providers. It also gives some important directions for further research regarding the use of supplements and vitamin status, as described in chapter 6. For instance, additional prospective studies are needed to further unravel the potential role of vitamin B6 in relation to the quality of life of colorectal cancer survivors.

Supplement use

This thesis discloses several points of interest that are relevant to current colorectal survivorship follow-up practice. An important observation was the frequent use of supplements by colorectal cancer survivors over time, in particular by survivors experiencing fatigue. These findings could be an impetus for colorectal cancer survivorship care providers to discuss the use of supplements with survivors and/or to refer survivors to specialized health professionals for additional guidance on the subject. Health care professionals involved in the follow-up trajectory of colorectal cancer survivorship are generally surgeons, general practitioners, and nurse practitioners. Referral to dietitians and other specialized professionals may be necessary more often to tailor advice on supplement use and better meet the needs of survivors. Colorectal cancer survivors themselves, being the principal subject of this research, would likely benefit from increased information provision, such as evidence-based knowledge about the use of supplements and its needlessness when consuming a sufficiently varied diet, as well as the potential harmful consequences of high doses. Furthermore, the common use of supplements, despite the recommendation to not use supplements (only in case of deficiencies), reveals low guideline adherence and demonstrates that there may be room for improvement in guideline communication and implementation.

Vitamin D

This thesis could additionally serve as a scientific foundation for potential actions that may need to be taken by health care professionals regarding the frequently low vitamin D status of colorectal cancer survivors. Individuals with vitamin D deficiencies would benefit from knowledge regarding the improvement of vitamin D levels, such as spending time outdoors in order to expose the skin to sunlight. Consequently, potential health problems that result from vitamin D deficiencies, possibly including fatigue and low quality of life, could be prevented or alleviated. Checks of vitamin D status may be necessary more often to evaluate whether vitamin deficiencies are at hand and whether a deficiency is a potentially underlying cause for complaints. The Dutch vitamin D supplementation recommendation, although implemented for the prevention of osteoporosis, might be brought under the attention of older colorectal cancer survivors more. Given the association between low $25OHD_3$ concentrations and increased colorectal cancer risk, there may be need for increased attention for vitamin D deficiencies among colorectal cancer survivors, especially among them experiencing fatigue or decreased quality of life. The advice to spend more time outdoors could effectively be integrated with the recommendation to reduce sedentary time by, for instance, encouraging survivors to go for a walk outside. Hence, two important lifestyle messages can be communicated at once, which can favor the motivation of survivors. However, it must be noted that the message on vitamin D and sun exposure can be complex when considering the increased risk of skin cancer, such as melanoma, also related to sunlight exposure.

Communication of information

In chapter 6, the practical implications of this thesis were discussed. Important implications were the need for increased attention and alertness among health care professionals about the use of supplements by colorectal cancer survivors, the need for increased recognition and dialogue about the symptom of fatigue, and awareness on the prevalence and possible consequences of vitamin deficiencies in this population. There are a number of channels for the communication of new insights regarding oncological survivorship care, both concerning the communication of research outcomes to care providers and communication from care providers to the patient. Oncoline is an important platform for guidelines in oncological care, including follow-up care in the Netherlands. Besides clinical practice, Dutch platforms to communicate lifestyle recommendations to cancer survivors are the website voedingenkankerinfo.nl and the online tool the Kanker Nazorg Wijzer.[7,8] The integration of these tools into standard follow-up care may aid in providing a more structured, univocal, and personalized approach towards improving the quality of life of colorectal cancer survivors.

Dissemination of results

This PhD project was funded by Kankeronderzoekfonds Limburg (KOFL).

During the trajectory, the results of this thesis have been presented at several meetings organized by KOFL, including meetings of “Business Vrienden Kankeronderzoekfonds Limburg”, aimed at raising funds for cancer research. The results of chapter 2 have been published on the website and in the newsletter of the Wereld Kanker Onderzoek Fonds (WKOF).[9] The results of chapter 3 have been published on the website and in the newsletter of the World Cancer Research Fund (WCRF) international.[10] These organisations reach both scientists and professionals involved in colorectal cancer survivorship care. Lastly, results were disseminated within the FOCUS consortium, were published in scientific articles, and presented at several conferences and meetings that attract both scientists and professionals working in clinical practice.

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Joke, je gezellige Belgische accent zorgde vaak voor een glimlach op mijn gezicht. We delen onze liefde voor de natuur en ik vond het erg leuk om samen in het Green Impact team te zitten om te proberen de afdeling wat 'groener' te maken. We hebben in ieder geval de plastic vlaaibordjes en -vorkjes weten te verbannen. Verre reizen zijn nu even niet aan de orde, maar dankzij mijn 'magazine' weet ik alle leuke plekjes dichtbij huis te vinden!

José, ik denk dat wij samen nog de meeste discussies gehad hebben over alles wat maar met het meten van voeding te maken heeft. Het onderscheid tussen supplementen, medicatie en voeding is nog niet zo makkelijk te maken als het lijkt, en dan heb ik het nog niet eens over rood en bewerkt vlees. We wisten dat we uit moesten kijken niet te verzanden in de details, zeker tijdens de codeeroverleggen, maar uiteindelijk kwamen we er altijd wel uit en hadden we er ook veel lol in. Met een beetje 377 erbij is alles op te lossen!

Jolanda, je hebt veel taken van me overgenomen toen ik me meer ging richten op het analyseren en schrijven van artikelen, en ik wist dat je het nog zorgvuldiger ging doen dan ik. Ik was blij dat jij die laatste maanden ook liever op DEB werkte dan thuis, je was er praktisch elke dag en daardoor kreeg ik weer wat meer (hoognodige) motivatie om ook aan de slag te gaan en die laatste loodjes af te maken. Dankjewel daarvoor!

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ABOUT THE AUTHOR

Annaleen Koole was born on July 6th 1989, in Hengelo (ov), the Netherlands.

She graduated from secondary school in 2007 at the Greijdanus College and moved to Nijmegen to study Biomedical Sciences at the Radboud University. Given her interest in nutrition, she switched after one year to Nutrition and Dietetics at the HAN University of Applied Sciences in Nijmegen. During this programme, she completed a specialization in Quantitative Research from Wageningen University & Research. She wrote her bachelor thesis at the same university on vitamin B12 and folate status in elderly people using data of a randomized controlled trial (*B-PROOF study)*. This thesis was awarded the second prize in the Nestlé prize for Dietetics 2011.

After graduating in 2013, she moved to the south of the Netherlands and started the master programme Global Health at Maastricht University. She studied at Thammasat University in Bangkok, Thailand for three months to learn about health care on a global scale. Subsequent to her stay in Thailand, she attended the symposium “Bridging Different Worlds” at Manipal University, India, and wrote her master thesis about the nutrition transition in India.

In September 2014, Annaleen started working as a Research Dietitian for the EnCoRe prospective study at the department of Epidemiology at Maastricht University. She performed home visits and measurements, and was involved in the coordination of activities, data processing and analyses. In 2016, she started her PhD project on supplement use and quality of life outcomes with data from the EnCoRe study, funded by Kankeronderzoekfonds Limburg (KOFL). During her PhD, Annaleen became an Academy Fellow of WCRF International (World Cancer Research Fund). Annaleen aspires to continue her career in the field of global nutrition.

LIST OF PUBLICATIONS

This thesis

Koole JL, Bours MJL, Breedveld-Peters JJL, van Roekel EH, Breukink SO, Janssen-Heijnen MLG, Vogelaar FJ, Aquarius M, Keulen E, Stoot J, Weijenberg MP. **Is dietary supplement use longitudinally associated with fatigue in stage I-III colorectal cancer survivors?** Clin Nutr. 2020 Jan;39(1):234-241.

Koole JL, Bours MJL, Breedveld-Peters JJL, van Roekel EH, van Duijnhoven FJB, van den Ouweland J, Breukink SO, Janssen-Heijnen MLG, Keulen E, Weijenberg MP. **Higher serum vitamin D concentrations are longitudinally associated with better global quality of life and less fatigue in colorectal cancer survivors up to 2 years after treatment.** Cancer Epidemiol Biomarkers Prev. 2020 Jun;29(6):1135-1144.

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