Malnutrition and dietary interventions in colorectal cancer patients

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Oslo, February 2018
Hanna Ræder
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*These authors contributed equally

Paper 2


Paper 3


*These authors contributed equally
# Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACS</td>
<td>American Cancer Society</td>
</tr>
<tr>
<td>AICR</td>
<td>American Institute for Cancer Research</td>
</tr>
<tr>
<td>AND</td>
<td>Academy of Nutrition and Dietetics</td>
</tr>
<tr>
<td>ASPEN</td>
<td>American Society for Parenteral and Enteral Nutrition</td>
</tr>
<tr>
<td>BIA</td>
<td>Bioelectrical impedance analysis</td>
</tr>
<tr>
<td>BMI</td>
<td>Body mass index</td>
</tr>
<tr>
<td>CALGB</td>
<td>Cancer and Leukemia Group B</td>
</tr>
<tr>
<td>CBT</td>
<td>Cognitive Behaviour Therapy</td>
</tr>
<tr>
<td>CMS</td>
<td>Consensus Molecular Subtypes</td>
</tr>
<tr>
<td>CPS</td>
<td>Cancer Prevention Study</td>
</tr>
<tr>
<td>CRP</td>
<td>C-reactive protein</td>
</tr>
<tr>
<td>CRC</td>
<td>Colorectal cancer</td>
</tr>
<tr>
<td>CRC-NORDIET</td>
<td>The Norwegian dietary guidelines and colorectal cancer survival</td>
</tr>
<tr>
<td>CT</td>
<td>Computerized tomography</td>
</tr>
<tr>
<td>CVD</td>
<td>Cardiovascular diseases</td>
</tr>
<tr>
<td>DFS</td>
<td>Disease-free survival</td>
</tr>
<tr>
<td>DRM</td>
<td>Disease-related malnutrition</td>
</tr>
<tr>
<td>DXA</td>
<td>Dual energy x-ray absorptiometry</td>
</tr>
<tr>
<td>ESPEN</td>
<td>European Society for Clinical Nutrition and Metabolism</td>
</tr>
<tr>
<td>EWGSOP</td>
<td>European Working Group on Sarcopenia in Older People</td>
</tr>
<tr>
<td>FFM</td>
<td>Fat-free mass</td>
</tr>
<tr>
<td>FFMI</td>
<td>Fat-free mass index</td>
</tr>
<tr>
<td>FM</td>
<td>Fat mass</td>
</tr>
<tr>
<td>GI</td>
<td>Gastrointestinal</td>
</tr>
<tr>
<td>HR</td>
<td>Hazard ratio</td>
</tr>
<tr>
<td>ICD</td>
<td>International classification of diseases and related health problems</td>
</tr>
<tr>
<td>IL</td>
<td>Interleukin</td>
</tr>
<tr>
<td>LBM</td>
<td>Lean body mass</td>
</tr>
<tr>
<td>MD</td>
<td>Mediterranean diet</td>
</tr>
<tr>
<td>MI</td>
<td>Motivational interview</td>
</tr>
<tr>
<td>MNA</td>
<td>Mini Nutrition Assessment</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic resonance imaging</td>
</tr>
<tr>
<td>MST</td>
<td>Malnutrition Screening Tool</td>
</tr>
<tr>
<td>MSTC</td>
<td>Malnutrition Screening Tool for Cancer Patients</td>
</tr>
<tr>
<td>MUST</td>
<td>Malnutrition Universal Screening Tool</td>
</tr>
<tr>
<td>NCP</td>
<td>Nutrition Care Process</td>
</tr>
<tr>
<td>NFBDG</td>
<td>Norwegian food-based dietary guidelines</td>
</tr>
<tr>
<td>NF-κB</td>
<td>Nuclear factor kappa-light-chain-enhancer of activated B cells</td>
</tr>
<tr>
<td>NRS-2002</td>
<td>Nutrition Risk Screening 2002</td>
</tr>
<tr>
<td>NSQ</td>
<td>Nutritional Screening Questionnaire</td>
</tr>
<tr>
<td>OS</td>
<td>Overall survival</td>
</tr>
<tr>
<td>PG-SGA</td>
<td>Patient-generated subjective global assessment</td>
</tr>
<tr>
<td>RCD</td>
<td>Registered clinical dietitian</td>
</tr>
<tr>
<td>RCT</td>
<td>Randomized controlled trial</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>--------------</td>
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</tr>
<tr>
<td>RDN</td>
<td>Registered dietary nutritionist</td>
</tr>
<tr>
<td>RENEW</td>
<td>Reach out to Enhance Wellness</td>
</tr>
<tr>
<td>RT</td>
<td>Radiotherapy</td>
</tr>
<tr>
<td>SGA</td>
<td>Subjective Global Assessment</td>
</tr>
<tr>
<td>SMM</td>
<td>Skeletal muscle mass</td>
</tr>
<tr>
<td>SO</td>
<td>Sarcopenic obesity</td>
</tr>
<tr>
<td>TBW</td>
<td>Total body water</td>
</tr>
<tr>
<td>TNFα</td>
<td>Tumor necrosis factor alpha</td>
</tr>
<tr>
<td>TNM</td>
<td>Tumor node metastasis</td>
</tr>
<tr>
<td>WCRF</td>
<td>World Cancer Research Fund</td>
</tr>
<tr>
<td>WHR</td>
<td>Waist hip-ratio</td>
</tr>
</tbody>
</table>
1 INTRODUCTION

1.1 Dietary aspects of cancer

There is strong evidence for the link between lifestyle and the risk of developing cancer [1]. World Cancer Research Fund (WCRF) recently stated that 1/3 of the most common cancers could be prevented through optimal diet, weight regulation and physical activity [2]. A diet based on plant foods and a limited intake of salt, high-calorie foods, sugary drinks and alcohol has shown a protective effect in several common cancers. Based on the extensive evidence for the role of diet in cancer prevention, comprehensive and detailed dietary recommendations for cancer prevention are currently available [1].

On the other hand, the role of diet for cancer patients is far less investigated. What is the optimal diet for cancer patients? Should there be similar dietary recommendations for all cancer patients, independently of cancer type and disease extent? May diet after diagnosis have impact on recurrence from the primary cancer, new morbidity including secondary cancer and survival?

The cancer prevalence continues to grow due to increase in incidence and improved survival. The first is mainly due to an increase in population and a change in age distribution towards older patients at higher risk for developing cancer. Despite this strong increase in cancer prevalence and the known impact of diet on cancer development, few studies have addressed the impact of diet on survival and the risk of developing a second serious chronic disease in cancer patients after cancer diagnosis.

The nutritional challenges following a cancer diagnosis vary according to several factors such as type of cancer, extent of disease, age, presence of comorbidities and the general health status. Moreover, nutritional problems often change during the course of survivorship. At the time of diagnosis, cancer patients are particularly vulnerable to unintentional weight loss and malnutrition due to loss of appetite and decreased food intake caused by the tumor and/or cancer treatment. After treatment is completed, some patients recover within weeks or months, whereas others, even patients in complete remission, experience persistent side effects from cancer treatment and difficulties in regaining weight and lean body mass.
Prolonged depletion of lean body mass (LBM) may lead to progressive functional impairment and decreased quality of life. Since cancer survivors have a life-long increased risk of developing second primary cancers [3], as well as increased risk of chronic diseases such as cardiovascular disease, obesity, chronic pulmonary disease and diabetes [4], they may benefit from guidance on a healthy diet targeting prevention of these diseases. However, documentation of the impact of diet is so far inadequate and data from randomized controlled trials are limited.

### 1.2 Colorectal cancer

Colorectal cancer (CRC) is cancer with origin in the colon or rectum. Tumors in colon are most commonly localized in the right-sided colon and sigmoideum (Figure 1). More than 90% of the colorectal cancers are adenocarcinomas originating from epithelial cells of the mucosa layer of the intestine wall. Less common types of colorectal carcinomas are neuroendocrine, squamous cell, adenosquamous, spindle cell and undifferentiated carcinomas [5].

![An anatomic overview of colon and rectum](https://via.placeholder.com/150)

**Figure 1.** An anatomic overview of colon and rectum. ©2017, WebMD, LCC. All rights reserved.
Colorectal carcinomas develop from dysplastic adenomatous polyps, the most common form of premalignant precursor lesions, through a multistep process known as the “adenoma-carcinoma sequence”, where inactivation of a variety of tumor suppressor genes and DNA-repair genes and activation of oncogenes occurs in a sequential manner [6]. These genetic alterations may be inherited or acquired, and the colorectal carcinogenesis may take more than 10 years [7].

1.2.1 Diagnosis and staging

Asymptomatic CRC can be diagnosed by different screening procedures including fetal blood examination or colonoscopy and a national screening programme for Norway is planned to start in 2019. Most CRC cases are, however, currently diagnosed after the onset of symptoms such as rectal bleeding, anemia, abdominal pain, diarrhea, constipation, and weight loss [8, 9]. The clinical presentation is depending on the localization of the cancer. Change in bowel habits and abdominal pain due to obstruction are more common in patients with left-sided tumours than patients with right-sided tumours [9]. Hematochezia is typical for tumours located in the rectum. Anemia is most frequent in patients with tumours in coecum and ascendens affecting 75% of these patients [10]. About 15-25 % of the CRC cases are presented with acute ileus, severe bleedings or bowel perforation [11]. Approximately 20 % have metastases to intraabdominal lymph nodes, peritoneum, liver or lungs at the time of diagnosis [12].

Staging of the cancer, i.e. grading of the cancer with regard to size and extent, performed at the time of diagnosis is important to initiate appropriate treatment as well as to determine prognosis. The Tumor Node Metastasis (TNM) staging system developed by The Union for International Cancer Control and the American Joint Comitee on Cancer [13] has been used for more than 50 years and describes the magnitude of the tumor (T), the extent of spread to lymph nodes (N) and the presence of metastasis (M). Based on the combination of T, N and M, i.e. the TNM status, an overall disease stage is determined (Table 1). Stage 0 corresponds to carcinoma in situ, stage I and II correspond to localized cancer, stage III spread to regional lymph nodes and stage IV is metastatic cancer (Figure 2).
**Table 1. Pathological staging according to the TNM classification system**

<table>
<thead>
<tr>
<th>Stage</th>
<th>T</th>
<th>N</th>
<th>M</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Tis</td>
<td>N0</td>
<td>N0</td>
</tr>
<tr>
<td>I</td>
<td>T1</td>
<td>N0</td>
<td>M0</td>
</tr>
<tr>
<td></td>
<td>T2</td>
<td>N0</td>
<td>M0</td>
</tr>
<tr>
<td>IIA</td>
<td>T3</td>
<td>N0</td>
<td>M0</td>
</tr>
<tr>
<td>IIIB</td>
<td>T4a</td>
<td>N0</td>
<td>M0</td>
</tr>
<tr>
<td>IIIC</td>
<td>T4b</td>
<td>N0</td>
<td>M0</td>
</tr>
<tr>
<td>IIIA</td>
<td>T1-T2</td>
<td>N1/N1c</td>
<td>M0</td>
</tr>
<tr>
<td></td>
<td>T1</td>
<td>N2a</td>
<td>M0</td>
</tr>
<tr>
<td>IIIB</td>
<td>T3-T4a</td>
<td>N1/N1c</td>
<td>M0</td>
</tr>
<tr>
<td></td>
<td>T2-T3</td>
<td>N2a</td>
<td>M0</td>
</tr>
<tr>
<td></td>
<td>T1-T2</td>
<td>N2b</td>
<td>M0</td>
</tr>
<tr>
<td>IIIC</td>
<td>T4a</td>
<td>N2a</td>
<td>M0</td>
</tr>
<tr>
<td></td>
<td>T3-T4a</td>
<td>N2b</td>
<td>M0</td>
</tr>
<tr>
<td></td>
<td>T4b</td>
<td>N1-N2</td>
<td>M0</td>
</tr>
<tr>
<td>IVA</td>
<td>Any T</td>
<td>Any N</td>
<td>M1a</td>
</tr>
<tr>
<td>IVB</td>
<td>Any T</td>
<td>Any N</td>
<td>M1b</td>
</tr>
</tbody>
</table>

TNM: Tumor Node Metastasis.
The table is a modified version of a table included in a free poster by American Joint Committee on Cancer; Colon and Rectum Cancer Staging 7th edition.

Disease stage (I-IV) is determined based on the combination of Tumor (T), nodes (N) and metastasis (M). Stage 0 corresponds to carcinoma in situ, stage I, and II corresponds to localized cancer, stage III spread to regional lymph nodes and stage IV is metastatic cancer. M1a and M1b denote metastases to one or more than one organs, respectively.
Figure 2. The colorectal cancer stages. Colorectal carcinomas develop from adenomatous polyps to adenocarcinomas through four stages. In stage 0, carcinoma in situ, the cancer has not grown beyond mucosa, i.e. the inner layer of the colon/rectum wall. In stage 1, the cancer has grown through mucosa. It has not spread to nearby lymph nodes or distant sites. In stage 2, the cancer has grown further into the mucosa layers. It has not yet spread to lymph nodes or distant sites. In stage 3, the tumor has grown through the mucosa into submucosa and spread to lymph nodes. It has not yet spread to distant sites. In stage 4, the cancer has spread to 1 or more distant organs. Liver and peritoneum are the most affected organs. ©2017, WebMD, LCC. All rights reserved.

Recently, an international effort based on sharing of large-scale data and coordinated analytics, has resulted in the definition of four consensus molecular subtypes (CMS) [14, 15]. The four CMS represent the best current description of CRC heterogeneity, and form the basis for the future clinical stratification and subtype-based interventions.

1.2.2 Incidence, mortality and survival

CRC is the third most common cancer in the world, with approximately 1.4 million new cases in 2012 [16]. Norway is one of the top ten countries with highest incidence in the world [16] and according to the annual report from the Cancer Registry of Norway, 4268 new cases of CRC were reported in 2015 [17]. About 80 % of these cases are stage I-III whereas about 20 % are metastatic disease.
There has been a significant increase in incidence (rates per 100,000) of CRC for both genders from 1965 to today (Figure 3). For the last decade there has been a slight, but steady increase in incidence of colon cancer, whereas the rates for rectum cancer have stabilized.

Figure 3. Age-standardized incidence, mortality and 5-year survival curves for CRC. A) Colon cancer (ICD-10 C18) and B) Rectum and rectosigmoid cancer (ICD-10 C19-20). Tables from the report Cancer in Norway 2015 [17].
The main reason for the increase in incidence is the aging population, as the disease is strongly associated with increased age. Median age at diagnosis for colon and rectum cancer is 73 and 69 years, respectively. According to the 2016 Cancer in Norway report [18], more than 2/3 of the patients operated for localized colon cancer were 75 years old or older. Figures 4 A and 4 B show the age distribution in patients treated for localized colon and rectum cancer, respectively. The mortality rates for colon cancer have been stable from the mid 1960ies in contrast to a significant mortality reduction for rectum cancer the last 25 years (Figure 3).

Figure 4 A) Gender and age distribution in patients treated by surgery for primary colon cancer stage I-III. Printed with permission from The Norwegian Colorectal Cancer Registry report (2015) [18]. B) Gender and age distribution in patients treated by surgery for primary rectum cancer stage I-III. Printed with permission from The Norwegian Colorectal Cancer Registry report (2015) [18].
Similar to other high-income countries, there has been a significant improvement in survival from CRC in Norway. Since the late 1970s, 5-year relative survival has increased from 40 to 60% and from 37 to 66% for colon and rectum cancer, respectively. The improvement in survival from colon cancer is explained by earlier diagnosis and the introduction of adjuvant chemotherapy for stage III patients in the late 1990s [19]. For rectum cancer, the implementation of total mesorectal excision in 1993, advancements in radiology (introduction of magnetic resonance imaging (MRI)) and increased use of chemotherapy and radiotherapy have contributed to the increase in survival rates.

Survival is however highly dependent on the TNM stage at diagnosis. In Norway during 2010-2014, the estimated 5-year relative survival in localized CRC was 77-78% compared to 12-18% for metastatic CRC [20].

1.2.3 Etiology

Both genetic and environmental factors play roles in the etiology of CRC, however, only 3-5% of the CRC cases are attributed to hereditary syndromes, where Familial adenomatous polyposis and Hereditary non-polyposis colorectal cancer (Lynch syndrome) are the two major ones [7]. Family history of CRC, i.e. having one or more first-degree relatives with CRC is present in about 25% of the patients [21].

A majority of the CRC cases are attributed to unhealthy lifestyle. Smoking, overweight, abdominal fat distribution, diabetes type 2, physical inactivity and an unhealthy diet are established risk factors [1, 22]. According to the last update from WCRF and American Institute for Cancer Research (AICR), i.e. the colorectal cancer continuous update report released in 2017, there is strong evidence for the protective effects of whole-grains, foods containing dietary fiber and physical activity, and moreover, for the increased risk by consuming red and processed meat and alcoholic drinks [23]. It is concluded by the WCRF/AICR that about 45% of CRC cases could be prevented by improved lifestyle [1].
1.2.4 Treatment of localized CRC

The major treatment modalities for CRC are surgery and chemotherapy and for rectal cancer also radiotherapy. The majority of the CRC patients undergo surgery, which is the only curative treatment. In Norway, treatment and follow-up of CRC is practiced in accordance with the standardized national guidelines from the Norwegian directorate for health [12].

For colon cancer, surgery includes total removal of the tumor-bearing segment and the corresponding lymph nodes. In most patients undergoing uncomplicated colectomy it is possible to reestablish intestinal continuity by the use of primary anastomosis [12]. In some cases, a temporary colostomy or ileostomy is necessary. The standard surgical procedure for curative resection of rectum cancer is total mesorectal excision, a technique that removes the tumor-bearing part of the rectum with the surrounding mesorectal fat, mesorectal lymph nodes and its border, the mesorectal fascia. According to the national guidelines, pre-operative radiochemotherapy is standard of care for patients with locally advanced rectum cancer [12]. For rectum cancer, patients aged < 75 years with stage III colon cancer are offered adjuvant chemotherapy whilst adjuvant chemotherapy is not standard of care for patients with rectal cancer.

1.2.5 Follow-up

After surgery, patients with localised CRC are systematically followed until 5 years with regard to local recurrence (i.e. reoccurrence of cancer in the same site as the primary tumor) or distant metastases (i.e. spread of the disease to other organs, including distant lymph nodes) [12]. According to The Norwegian Colorectal Cancer Registry, 16 % of the patients with localised colon cancer disease-free after surgery, develop distant metastases during the first 5 years. Liver and peritoneum are the most commonly affected organs for colon cancer metastases. Twenty-two % of the patients with localized rectum cancer develop metastases [18]. Metastases are most commonly localized to the liver or lungs. Estimated proportion affected by local recurrence is 5 % and less than before introduction of the total mesorectal excision procedure.
1.3 Colorectal cancer-associated malnutrition and sarcopenia

1.3.1 Definitions and diagnostic criteria of malnutrition

Malnutrition is often observed in CRC patients. There is however currently no universally accepted definition of malnutrition. ESPEN (The European Society of Clinical Nutrition and Metabolism) recently published guidelines on definitions and terminology of clinical nutrition to be used in clinical practice and research [24]. According to these guidelines, malnutrition is defined as “a state resulting from lack of intake or uptake of nutrition that leads to altered body composition (decreased fat-free mass (FFM)) and body cell mass leading to diminished physical and mental function and impaired clinical outcome from disease” [24]. To diagnose malnutrition, ESPEN has published a set of diagnostic criteria [25] (Table 2). These criteria are different from the criteria proposed by the Academy of Nutrition and Dietetics (Academy) and American Society for Parenteral and Enteral Nutrition (ASPEN) (Table 3) [26]. Whereas low body mass index (BMI) is a central part of the ESPEN criteria, it is not included in the ASPEN criteria. Moreover, the ASPEN criteria encompass energy intake, loss of body fat, fluid accumulation and hand grip strength which are not included in the ESPEN criteria. Weight loss and low muscle mass/FFM are however present in both set of criteria.

Table 2. The ESPEN diagnostic criteria for malnutrition

| 1) | BMI < 18.5 |
| 2) | Weight loss (unintentional) > 10 % indefinite of time, or > 5 % over the last 3 months combined with either BMI < 20 kg/m² if < 70 years of age, or < 22 if > 70 years of age or FFMI < 15 and 17 kg/m² in women and men, respectively |

BMI; body mass index, FFMI; fat-free mass index.

Two alternative ways to diagnose malnutrition according the ESPEN Consensus Statement [25]. Prior to diagnosis, it is mandatory to be considered “at risk” by a validated risk screening tool.
Table 3. The clinical characteristics for the diagnosis of malnutrition in the Consensus Statement by the Academy/ASPEN.

<table>
<thead>
<tr>
<th>Clinical characteristic</th>
<th>Malnutrition in the context of chronic illness</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nonsevere (moderate) malnutrition</td>
</tr>
<tr>
<td>Energy intake</td>
<td>&lt;75% of estimated energy requirement for ≥1 month</td>
</tr>
<tr>
<td>Weight loss</td>
<td>5% in 1 month 7.5% in 3 months 10% in 6 months 20% in 1 year</td>
</tr>
<tr>
<td>Body fat</td>
<td>Mild</td>
</tr>
<tr>
<td>Muscle mass</td>
<td>Mild</td>
</tr>
<tr>
<td>Fluid accumulation</td>
<td>Mild</td>
</tr>
<tr>
<td>Reduced grip strength</td>
<td>NA</td>
</tr>
</tbody>
</table>

NA, not applicable

The table is a modified version from the table published in The Consensus statement: Academy of nutrition and dietetics and American Society for Parenteral and Enteral Nutrition: Characteristics Recommended for the identification and documentation of adult malnutrition (undernutrition) [26]. A minimum of 2 of the 6 characteristics in the table must be fulfilled for the diagnosis of malnutrition. Chronic is defined as a disease/condition lasting 3 months or longer. Weight loss must be evaluated in light of under or overhydration. Body fat (loss of subcutaneous fat), muscle mass and fluid accumulation is evaluated by a physical exam. Hand grip strength is evaluated in accordance with normative standards presented by the manufacturer of the measurement device.
The national guidelines for prevention and treatment of malnutrition [27] have based the malnutrition codes in the International Classification of Diseases (ICD-10) [28] on low BMI, weight loss and reduced food intake (Table 4).

**Table 4. The ICD-10 classification of malnutrition**

<table>
<thead>
<tr>
<th>Malnutrition code</th>
<th>Criteria</th>
</tr>
</thead>
</table>
| E46: Unspecified protein-energy malnutrition | NRS 2002 ≥ Score 3  
MUST score > 1  
MNA score < 11  
SGA B |
| E44: Moderate protein-energy malnutrition | Weight loss > 10 % the preceding 3-6 months  
or > 5 % the last 2 months  
BMI < 18.5 kg/m\(^2\) (> 70 years old: BMI < 20)  
BMI < 20 kg/m\(^2\) (>70 years: BMI < 22) and weight loss > 5 % the last 6 months  
Food intake <1/2 of calculated requirements the last week |
| E43: Severe protein-energy malnutrition | Weight loss > 15 % the preceding 3-6 months  
or > 5 % the last month  
BMI < 16 kg/m\(^2\) (> 70 years old: BMI < 18.5)  
BMI < 18.5 kg/m\(^2\) (>70 years: BMI < 20) and weight loss > 5 % the last 3 months  
Food intake <1/4 of calculated requirements the last week |

BMI: Body mass index, MNA; Mini Nutrition Assessment, MUST; Malnutrition Universal Screening Tool, NRS; Nutritional Risk Screening.

The World Health Organization ICD-10 classification (version 2016) [28] of malnutrition and the criteria as defined by the National guidelines for prevention and treatment of malnutrition [27].
According to the definitions and terminology by ESPEN, malnutrition can be further categorized into sub categories according to etiology (Figure 5). Malnutrition may be caused by disease, i.e. disease-related malnutrition (DRM), or other factors such as hunger or psychological factors. DRM may occur with or without inflammation. When inflammation is present production of inflammatory mediators induces anorexia and affects the metabolic pathways leading to increased tissue break down (muscle mass and/or adipose tissue). DRM with inflammation is also known as cancer cachexia [24].

**Figure 5. Malnutrition sub categories.** Printed with permission from *ESPEN guidelines on definitions and terminology of clinical nutrition (2017)* [24].
1.3.2 Fat-free mass depletion

According to the definition of malnutrition as described in the ESPEN guidelines, malnutrition is characterized by decreased FFM [24]. Low FFM is according to the ESPEN consensus diagnostic criteria [25] defined as FFM index (FFMI), i.e. FFM adjusted for height (FFM (kg)/height(m²)), below 15 kg/m² and 17 kg/m², in females and males, respectively.

The two-compartment model simply divides the body into fat mass (FM) and FFM. FFM consists of all that are not fat; minerals, glycogen, proteins and water (Figure 6). On tissue and organ level, FFM is comprised of skeletal muscle, bone, organs and total body water. In the literature, FFM is often used synonymously with LBM. However, this is not correct since LBM does not include bone mineral compartment. FFM represents the sum of LBM and bone [29].

![Figure 6. The two-compartment model of body composition.](image)

The body is simply divided into fat and fat-free mass. Body fat consists of storage fat and essential fat. Storage fat is localized subcutaneously and surrounds the internal organs whereas essential fat is utilized by different organs. Fat-free mass consists of water, protein, minerals and glycogen. The amount of water in the adult human body is in the range 50-65 %. Printed with permission from Glucocorticoid therapy and body composition, Nature Reviews Rheumatology [30].

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The two-compartment model is shown to contribute with important information regarding nutritional status, since the amount of FFM reflects the amount of skeletal muscle and moreover, that changes in FFM mainly reflect changes in skeletal muscle mass, since the other components of FFM are considered to be stable. Nevertheless, in advanced cancer changes in FFM may be attributed to changes in organs and tumor as well as depletion of skeletal muscle. Lieffers and colleges observed increases in liver and tumor mass in CRC patients with unresectable liver metastases, quantified by the use of computer tomography (CT) images [31].

Since the cut-offs for FFMI were published in 2015 [25], validation studies have confirmed the prognostic impact of the malnutrition criteria on clinical outcomes [32] and survival [33]. FFM can be estimated by the use of different modalities, including air displacement plethysmography, labeled water-isotope dilution techniques, dual energy x-ray absorptiometry (DXA) and bioelectrical impedance analysis (BIA). DXA and BIA were used in the current work and will be further discussed in the chapter General discussion.

1.3.3 Sarcopenia and sarcopenic obesity

Sarcopenia and frailty may be considered nutritional related disorders in addition to malnutrition/undernutrition, overweight, obesity, micro-nutrient abnormalities and re-feeding syndrome (Figure 7). This conceptual categorization of nutrition disorders is however schematic and the conditions may in practice be overlapping.

**Figure 7.** Nutrition disorders and nutrition related conditions. Printed with permission from ESPEN guidelines on definitions and terminology of clinical nutrition (2017) [24].
Sarcopenia is defined as “a syndrome characterized by progressive and generalized loss of skeletal muscle mass and strength with increased risk of adverse outcomes such as physical disability, poor quality of life and death” [34]. Frailty is a geriatric syndrome characterized by unintentional weight loss, exhaustion, weakness, slow walking speed and low physical activity, associated with increased risk of adverse health outcomes such as falls, hospitalization, institutionalization and mortality [35]. Sarcopenia and frailty are both associated with advanced age and share several characteristics, such as weight loss, weakness and reduced physical performance. Frailty is however also characterized by other factors related to age such as cognitive status and social status (e.g. loneliness and depression).

Sarcopenia was originally described by Irwin Rosenberg in 1997 as “age-related loss of muscle mass” [36]. It is now recognized that sarcopenia is not only restricted to the elderly, and the syndrome may be caused by secondary factors to age such as physical inactivity, inadequate dietary intake, malabsorption, advanced organ failure and cancer [34]. The pathogenesis of sarcopenia is complex and multifactorial, encompassing atrophy of muscle fibers, increased muscle fat infiltration, increased proteolysis and decreased protein synthesis [37]. In patients with cancer, the process and onset of sarcopenia may be accelerated due to impaired dietary intake, malabsorption, physical inactivity and the impact of inflammation.

During the last 20 years, the concept of sarcopenia has been widely discussed and various definitions have been proposed. In 2010, experts from the European Union Geriatric Medicine society, European Society for Clinical Nutrition and Metabolism, the International Academy of Nutrition and Aging, and the International Association of Gerontology and Geriatrics – Europe Region, gathered to create a study group, the European Working Group on Sarcopenia in Older People (EWGSOP) in order to propose diagnostic criteria for use in both clinical practice and research [34]. The experts agreed that the diagnosis should be based on the following criteria; 1) low muscle mass 2) low muscle strength 3) Low physical performance, where criterion 1 and either criterion 2 or 3 must be present to fulfill the diagnosis. Skeletal muscle depletion in combination with excessive adipose tissue, recognized as “sarcopenic obesity” (SO), is considered to be particularly detrimental since
the health risks from obesity and sarcopenia are combined. Moreover, excessive adipose tissue may lead to increased fat infiltration in skeletal muscle, a condition termed myosteatosis, leading to decreased muscle density and impaired physical function [38]. SO is demonstrated to be a significant predictor for functional status and lower survival in patients with gastrointestinal cancers [39, 40].

1.3.4 Malnutrition and sarcopenia in CRC patients

The prevalence of malnutrition in CRC patients are reported to be in the range of 23-84 % [41-47] depending on methodology used to assess nutritional status and disease stage. Most data on prevalence and outcomes of malnutrition in CRC patients are collected at the time of diagnosis or during CRC treatment. Prior to treatment, the tumor may cause symptoms such as constipation, abdominal pain and bleedings, subsequently affecting dietary intake and absorption of nutrients. It is documented that significant weight loss prior to chemotherapy is prevalent in more than one third of the patients with CRC [48]. Losing weight prior to chemotherapy increases the risk of chemotherapy-induced toxicity such as stomatitis, plantar-palmar syndrome, diarrhea, nausea, neuropathy, fever or fatigue, which subsequently may lead to dose reductions, delays or discontinuation of chemotherapy, and consequently the effect of treatment. Several studies have shown the impact of weight loss and malnutrition on toxicity and mortality in CRC patients receiving adjuvant or palliative chemotherapy [48-50].

CRC patients on radiotherapy (RT) may experience symptoms such as anorexia, nausea, diarrhea and vomiting. These symptoms may be persistent and affect long-term outcomes. In a randomized controlled trial in CRC patients referred to RT, patients not receiving individualized dietary counseling targeting RT induced symptoms had poorer food intake, more symptoms, poorer nutritional status and lower scores on quality of life (QoL) than patients who received intensive counseling during RT at the end of RT [51]. Furthermore, the long-term (median 6.5 years) follow-up of this study showed that the patients with a poorer dietary intake, worse nutritional status and lower QoL scores at the end of radiotherapy had a significantly shorter median survival and increased incidence of late effects of toxicity from radiotherapy [45].
The prevalence of sarcopenia in CRC patients is high. Based on various definitions and cut-offs used, prevalence of sarcopenia is reported to be in the range 39-71% [50, 52, 53]. The majority of the studies have used CT to measure low skeletal muscle mass and defined sarcopenia by the use of cut-offs for sarcopenia published by Prado and coworkers in 2008 [40], which were derived in a mixed sample of patients with tumors in the gastrointestinal and respiratory tracts. Studies have reported that sarcopenia in CRC patients is associated with postoperative complications [39, 52-55], increased chemotherapy toxicity [50, 56] and higher mortality [39, 57]. The impact of sarcopenia on survival has also been shown in studies with mixed populations of patients were gastrointestinal (GI) cancers were included [40, 58].

Taken together, the literature shows that malnutrition and sarcopenia assessed at the time of diagnosis are associated to adverse short-term outcome measurements and survival in CRC patients. The prevalence of malnutrition and sarcopenia assessed later in the course of the disease, as well as the impact on quality of life and survival is however not well investigated. Persistent nutritional problems may lead to continued loss of body weight and poor nutritional status. In addition, body weight increase in terms of fat instead of FFM may lead to increased fat infiltration in skeletal muscle, which may accelerate functional decline.

Moreover, most of the studies investigating associations between malnutrition and sarcopenia and to clinical outcome measurements and survival in CRC patients have included high proportions of patients with locally advanced or metastatic disease. Only one study [57] has investigated the impact of sarcopenia in patients with non-metastatic CRC and this study showed that sarcopenia was found to be associated with reduced survival in this population. Due to the limited number of studies, data on prevalence and clinical impact of malnutrition and sarcopenia in non-metastatic patients is currently scarce.
1.4 Nutritional risk screening and assessment tools in oncology

The primary nutritional evaluation is screening, which aims to systematically identify individuals who are malnourished or are at risk of malnutrition. The initial screening may be undertaken by a nurse and allows for a rapid decision of whether the patient should be further evaluated by a nutritional professional with specialized expertise, for instance a registered clinical dietitian. An in-depth evaluation should include assessment of symptoms affecting food intake and an evaluation of food intake, appetite, weight status, muscle mass, subcutaneous fat stores, fluid status, comorbidities and laboratory status [59].

According to the Oncology Evidence-Based Nutrition Practice Guideline for Adults from the Academy of Nutrition and Dietetics, “adult oncology patients should be screened using a malnutrition tool validated in the setting in which the tool is intended for use” [60]. More specifically, Malnutrition Screening Tool (MST) [61], Malnutrition Screening Tool for Cancer patients (MSTC) [62] and Malnutrition Universal Screening Tool (MUST) [63] are the tools recommended for hospitalized patients, whereas MST is recommended for ambulatory/outpatient settings. For nutritional assessment, the Academy recommends that “registered dietary nutritionists (RDN) should use an assessment tool validated in the setting in which the tool is intended for use as part of the complete nutrition assessment”. These include the Patient-generated subjective global assessment (PG-SGA) [64] and the Subjective Global Assessment [65].

According to ESPEN guidelines on nutrition in cancer patients [66], the recommendation concerning screening is as follows; “To detect nutritional disturbances at an early stage, we recommend to regularly evaluate nutritional intake, weight change and BMI, beginning with cancer diagnosis and repeated depending on the stability of the situation” [66]. Next, “In patients with abnormal screening, we recommend objective and qualitative assessment of nutritional intake, nutritional impact symptoms, muscle mass, physical performance and the degree of systemic inflammation” [66]. The ESPEN guidelines include various methods for the purpose of screening, such as BMI, evaluation of weight loss, evaluation of food intake, or screening tools such as Nutritional Risk Screening 2002 (NRS-2002)[67], MUST, MST or Mini Nutritional Assessment Short Form Revised [68]. Regarding nutritional assessment,
ESPEN recommends that patients identified as “at risk” should be assessed with attention to factors such as dietary intake, body composition, physical activity and predominant metabolic pattern (i.e. metabolic derangements caused by systemic inflammation) without recommending any specific nutritional assessment tool [66].

In Norway, NRS 2002, MUST, MNA [68] and SGA are the available recommended tools to use for identification of patients with increased risk of malnutrition [27]. An overview of the most commonly used screening and assessment tools in oncology is given in Table 5.

Table 5. An overview of screening and assessment tools in oncology

<table>
<thead>
<tr>
<th>Screening tools</th>
<th>Dietary intake</th>
<th>Nutritional impact symptoms</th>
<th>Appetite</th>
<th>Weight loss</th>
<th>BMI</th>
<th>Body composition</th>
<th>Anthropometry</th>
<th>Physical function/mobility</th>
<th>Psychological stress</th>
<th>Disease</th>
<th>Neuropsychological problems</th>
<th>Metabolic stress</th>
</tr>
</thead>
<tbody>
<tr>
<td>NRS 2002</td>
<td>X</td>
<td>X</td>
<td>X</td>
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<td>MST</td>
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<tr>
<td>MUST</td>
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<tr>
<td>MSTC</td>
<td>X</td>
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<tr>
<td>PG-SGA SF</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
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<td></td>
<td></td>
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<td>X</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Assessment tools</th>
<th>Dietary intake</th>
<th>Nutritional impact symptoms</th>
<th>Appetite</th>
<th>Weight loss</th>
<th>BMI</th>
<th>Body composition</th>
<th>Anthropometry</th>
<th>Physical function/mobility</th>
<th>Psychological stress</th>
<th>Disease</th>
<th>Neuropsychological problems</th>
<th>Metabolic stress</th>
</tr>
</thead>
<tbody>
<tr>
<td>SGA</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
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</tr>
<tr>
<td>PG-SGA</td>
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<td>X</td>
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<td></td>
<td></td>
<td>X</td>
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<tr>
<td>MNA</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
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<td>X</td>
</tr>
</tbody>
</table>

Mini Nutrition Assessment (MNA); MST: Malnutrition Screening Tool; MSTC: Malnutrition Screening Tool for Cancer Patients; MUST: The Malnutrition Universal Screening Tool; NRS: Nutritional Risk Screening; PG-SGA: Patient-Generated Subjective Global Assessment; PG-SGA SF: Patient-Generated Subjective Global Assessment Short Form; SGA: Subjective Global Assessment

Body composition includes a visual examination of muscle and fat depletion

Metabolic stress includes questions regarding use of corticosteroids or the presence of fever

Neuropsychological problems include depression and dementia
The various methods used in nutritional assessment of cancer patients differ with regard to which nutritional aspects being emphasized. Sealy and colleges investigated to which extent commonly used methods for nutritional screening and assessment in cancer patients adequately cover the dimensions of the malnutrition definitions by ESPEN and ASPEN, respectively [69]. Based on the malnutrition definitions, four dimensions were defined; A) Assessment of nutrient balance B) Assessment of body weight, body area and body composition C) Assessment of muscle, immune and cognitive function and D) Measurement of inflammatory factors. They systematically reviewed the largest databases for studies including assessment of malnutrition, between 1998 and 2013. The authors discovered 37 different methods used for malnutrition assessment. Only four out of 37 methods covered all dimensions of malnutrition, namely the Nutritional Screening Questionnaire (NSQ), MNA, SGA and PG-SGA, of which SGA and PG-SGA were the most frequently used (Figure 8).
Figure 8. Frequency of use and content of domains within the definition of malnutrition. BIA, Bio-Impedance Measurement; BMI, body mass index; CHI, Creatine Height Index; GNS, General Nutritional Status; HAS, French National Authority for Health; MNA, Mini Nutritional Assessment; MSTC, Malnutrition Screening Tool for Cancer; MUST, Malnutrition Universal Screening Tool; NRI, Nutritional Risk Index; NSQ, Nutritional Screening Questionnaire; NST, Nottingham Screening Tool; PNI, Prognostic Nutritional Index; PINI, Prognostic Inflammatory and Nutritional Index; PG-SGA, Patient-Generated Subjective Global Assessment; SGA, Subjective Global Assessment; SNAQ, Short Nutritional Assessment Questionnaire. Figure is printed with permission from Content validity across malnutrition assessment in patients with cancer is limited, Journal of Clinical Epidemiology [69].
The selection of assessment tool to identify malnutrition has implications for whether cancer patients are adequately recognized as malnourished and subsequently receive appropriate nutritional therapy. For instance, it is not given that assessment tools originally designed to identify malnutrition in cancer patients in the lower range of BMI are suitable to detect malnutrition in cancer patients with overweight or obesity. The growing prevalence of overweight and obesity among cancer patients challenges the use of the traditional markers of malnutrition such as low BMI and weight loss. For instance, recent studies have demonstrated that substantial loss of muscle mass may be masked in overweight and obese cancer patients [40, 70], and cancer patients with identical BMI may have significantly different levels of skeletal muscle [71]. By using an assessment tool designed to capture changes in body weight without taking the aspect of body composition into consideration, important information may be missed since the weight change may consist of increases in fat and not muscle mass [72]. Hence, nutrition assessment tools in oncology practice should be adequately designed to identify malnutrition in patients with muscle mass depletion, particularly in overweight and obese cancer patients. Although the nutritional assessment tools recommended by ESPEN and by the Academy of Nutrition and Dietetics are validated in many cancer populations, they are not sufficiently validated in cancer populations with these nutritional concerns.

In the current thesis, PG-SGA was selected as nutritional assessment tool to evaluate its ability to detect low FFM. BIA and DXA were selected as methods to measure FFM. These methods are described in more details in the chapter General Discussion.
1.5 The role of diet after CRC diagnosis

Malnutrition and sarcopenia are common features of CRC. However, the role of malnutrition and dietary interventions after CRC diagnosis is not well investigated. Current available data on the impact of diet on CRC recurrence and survival in this patient population, are based on two large US cohorts, the CALGB (Cancer and Leukemia Group B) Diet and Lifestyle Companion Study [73] and the American CPS (Cancer Prevention Study) II Nutrition Cohort [74].

Meyerhardt and coworkers analyzed dietary patterns in stage III colon cancer patients treated by surgery and chemotherapy, and found that a western dietary pattern, i.e. a diet characterized by high intakes of red and processed meat, fat, refined grains and dessert, was associated with increased risk of cancer recurrence and mortality. In contrast, a prudent dietary pattern characterized by high intakes of fruits and vegetables, poultry and fish was associated to favorable outcome [75].

In participants recruited to the American CPS study, intake of red and processed meat after diagnosis was not associated with CRC-specific or all-cause mortality [76]. However, analyses from the same study revealed that individuals with high intakes of red and processed meat both before and after diagnosis had a 79 % increased risk of CRC-specific mortality compared with individuals with low intakes at both time points. Since the participants to a minor extent changed their intake of red and processed meat after diagnosis, the study did not provide data to evaluate the effect of change in dietary habits.

Glycemic load and total intake of carbohydrates related to CRC survival were analyzed from the CALGB study cohort. Both higher dietary glycemic load and total carbohydrate intake were associated to increased risk of recurrence and mortality in overweight or obese patients with colon cancer, but not in patients with BMI < 25 [77]. Moreover, patients who consumed two or more sugar-sweetened beverages per day after a colon cancer diagnosis had a 67 % increased risk of recurrence compared to patients who consumed less than two per month [78].

Taken together, these observational studies suggest that diet after a CRC diagnosis may affect risk of CRC recurrence and mortality. More data from additional cohort studies and
randomized controlled trials (RCTs) are however needed to be able to draw robust conclusions about the role of diet in CRC survivors. So far, only one RCT has been conducted in CRC patients to investigate the effect of a dietary intervention on survival. In this study, they aimed to test the effect of individualized dietary counseling with focus on symptom alleviation and/or arrest and adequate energy intake [45]. To date, no RCTs have tested the effect of a food-based dietary intervention on disease outcomes and survival in CRC patients. The CRC-NORDIET study was therefore designed to test the effect of a diet in accordance with the Norwegian food-based dietary guidelines (NFBGD) (Table 6). These guidelines were developed to prevent chronic diseases in the general population and are based on a comprehensive, systematic review of the evidence linking diet to the risk of chronic diseases [79].

Table 6. The Norwegian food-based dietary guidelines

<table>
<thead>
<tr>
<th>Dietary recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. A primarily plant-based diet is recommended, including plenty of vegetables, fruit, berries, wholegrain and fish, and limited quantities of red and processed meat, salt, added sugar and energy-rich foods</td>
</tr>
<tr>
<td>2. It is recommended to maintain a balance between energy intake and energy expenditure</td>
</tr>
<tr>
<td>3. Eat at least five portions of vegetables, fruit and berries every day</td>
</tr>
<tr>
<td>4. Eat at least four portions of wholegrain products every day</td>
</tr>
<tr>
<td>5. Eat the equivalent of two or three portions of fish per week</td>
</tr>
<tr>
<td>6. Low-fat dairy products should be included in your daily diet</td>
</tr>
<tr>
<td>7. It is recommended to eat lean meat and lean meat products, and limit the intake of red meat and processed meat</td>
</tr>
<tr>
<td>8. It is recommended to use cooking oil, liquid margarine or soft margarine</td>
</tr>
<tr>
<td>9. Water is recommended as the primary choice of drinks</td>
</tr>
<tr>
<td>10. Limit your intake of added sugar</td>
</tr>
<tr>
<td>11. Limit your intake of salt</td>
</tr>
<tr>
<td>12. Dietary supplements may be necessary to ensure an adequate intake of nutrients for some groups in the population</td>
</tr>
<tr>
<td>13. It is recommended that everyone participates in least 30 minutes of physical activity per day</td>
</tr>
</tbody>
</table>

For specific and detailed quantitative recommendations, see the Norwegian food-based dietary guidelines [79].
1.5.1 Factors affecting change in dietary habits

In order to be able to measure effects of dietary interventions, long-term compliance to dietary recommendations is essential. However, achieving long-term changes in dietary habits is complex and the individual’s ability or willingness to change diet is dependent on several factors. For instance, knowledge, beliefs and interest in nutrition may influence the motivation and susceptibility for change. Moreover, socioeconomic factors including financial conditions, literacy and support from family or spouse, and psychological factors such as depression and loneliness may also have impact on the motivation to make changes of dietary habits. In cancer patients, there may be additional barriers to change diet due to factors related to the disease and treatment, such as fatigue, pain, GI problems and reduced physical function. Thus, dietary interventions not taking into account these factors may be less likely to succeed in achieving compliance to the dietary advice.

There are several theories regarding human’s ability and potential to change behavior, including strategies to achieve these changes. The health belief model, social cognitive theory, theory of planned behavior and transtheoretical model of change are examples of theories focusing patient-centered factors such as self-efficacy, perceived control, barriers and benefits of making changes [59]. In the dietary counseling setting, various approaches based on these models may be used to enhance behavior change. Counseling targeting “behavior modification” is based on principles from behaviorism, a psychological theory founded by Watson [80]. According to this theory, it is essential to understand behavior by identifying the stimulus that triggers a certain response. For example, a patient eats more chocolate if chocolate is available in the kitchen. In this case, clinical dietitian according to a behavioristic approach will recommend the patient to avoid having chocolate in the kitchen and thus remove the stimulus that triggers the undesirable outcome. Within this approach, use of positive and negative reinforcements (i.e. feedbacks) are essential strategies to modify the patient’s behavior. One of the main limitations with this method is that effect of reinforcements may be temporary and that the desirable behavior terminates when the feedbacks disappear. In addition, the method has been criticized for leaving the responsibility of change to the counselor and not the patient and thus ascribing the patient a passive role in the process of change. Approaches emphasizing patient-driven behavior change such as cognitive approaches, take into account the relationship between thoughts,
emotions and behavior, in order to increase the patients` awareness of how they act in the way they do, and how their actions may influence the environment [80]. There are several related strategies with origin in cognitive theories. One of them, cognitive behavior therapy (CBT), seeks to identify behavior and thoughts leading to negative outcomes and includes strategies to change the behavior and thoughts. CBT is commonly used in treatment of several psychological and psychiatric disorders.

Several approaches used to facilitate a behavior change assume that the patient is motivated for changing behavior. However, lack of motivation may represent a major obstacle for achieving effects on desirable outcome. The motivational interviewing (MI) method developed by Miller and Rollnick, is a widely used and well documented [81] counseling approach where the primary focus is to enhance readiness for change by helping patients explore and resolve ambivalence. The unique core concept of MI is the patient-centered counseling with the motivation for change to be expressed by the patient and not argued by the counselor. The concept of MI was developed to treat alcohol abuse, but is now extensively used in several other areas for health behaviors. In the current thesis, MI was implemented in the nutrition intervention as part of the dietary counseling in the CRC-NORDIET study (paper 1) and will be further discussed in the General discussion.

Nutritional intervention is a central part of the Nutrition Care Process (NCP) model, which is the standardized process for nutritional care developed by the Academy of Nutrition and Dietetics (AND) [82]. The nutrition intervention aims to treat the nutritional problem and may consist of nutrition and food therapy, nutrition education and counseling. Nutrition education and counseling are both important parts of the nutrition intervention in order to achieve the treatment goals. Whereas nutritional education aims to help the patient to gain knowledge and skills needed to make desirable change, dietary counseling focuses on how to reach the goals, by identifying favorable conditions for change. The dietary intervention in the CRC-NORDIET study (paper 1) was therefore designed to include both education, i.e. educating the patients how to change their dietary habits according to the NFBDG, and dietary counseling with particular focus on individual needs, nutritional status and challenges with regard to symptoms and food intake as well as motivational status.
2 AIMS

In Norway, there has been a steady increase in survival of non-metastatic CRC patients the last decades. CRC patients have more comorbidities and reduced quality of life compared to the general population. To date, there is limited data on the role of diet after CRC diagnosis, and intervention studies investigating whether there exists a causal relationship between diet and disease outcomes and survival in these patients are urgently needed.

After CRC surgery, patients may experience nutritional problems leading to malnutrition, depletion of FFM and sarcopenia. It is suggested that malnutrition and FFM depletion may have impact on quality of life, physical function and survival in CRC patients, and hence appropriate nutritional interventions are needed to deal with these nutritional problems. PG-SGA is one of a few nutritional assessment tools targeting all dimensions of malnutrition, including depletion of fat and muscle. Moreover, the tool is recommended by The Oncology Evidence-Based Nutrition Practice Guideline for Adult from the Academy of Nutrition and Dietetics to use in cancer patients. However, the knowledge about its ability to identify low FFM is scarce.

The overall aims of this thesis focusing on non-metastatic CRC patients were to:

1. Develop and establish a RCT with individualized nutrition intervention
2. Evaluate the ability of the nutritional assessment tool PG-SGA to identify low FFM
3. Determine the prevalence of malnutrition, low FFM and sarcopenia
4. Evaluate the ability of two different BIA devices to assess FFM
3 SUMMARY OF PAPERS

3.1 Paper 1

This paper presents the study protocol of the Norwegian Dietary Guidelines and Colorectal Cancer Survival study. Men and women aged 50-80 years diagnosed with primary invasive CRC (Stage I-III) are invited to this randomized controlled, parallel two-arm trial 2-9 months after curative surgery. The intervention group (n=250) receives an intensive dietary intervention lasting for 12 months and a subsequent maintenance intervention for 14 years. The control group (n=250) receives no dietary intervention other than standard clinical care. Both groups are offered equal general advice of physical activity. Patients are followed-up at 6 months and 1, 3, 5, 7, 10 and 15 years after baseline. The study center is located at the Department of Nutrition, University of Oslo, and patients are recruited from two hospitals within the South-Eastern Norway Regional Health Authority. Primary outcomes are disease-free survival and overall survival. Secondary outcomes are time to recurrence, cardiovascular disease-free survival, compliance to the dietary recommendations and the effects of the intervention on new comorbidities, intermediate biomarkers, nutrition status, physical activity, physical function and quality of life.

The current study is designed to gain a better understanding of the role of a healthy diet aimed at dampening inflammation and oxidative stress on long-term disease outcomes and survival in colorectal cancer patients. Since previous research on the role of diet for colorectal cancer survivors is limited, the study may be of great importance for this cancer population.
3.2 Paper 2

The aim of this study was to investigate the ability of PG-SGA to detect low FFM in patients with non-metastatic CRC. Ninety-seven patients were included and categorized as well nourished (PG-SGA A, n=67) or malnourished (PG-SGA B, n=30). BIA was used to assess FFM. Low FFM was defined as low FFMI according to cut-off values recently proposed by ESPEN. Twenty-nine percent of the patients were identified with low FFMI. The proportion with low FFMI was significantly higher among patients classified as malnourished by PG-SGA compared to well nourished (p=0.015). The sensitivity was however low, as the PG-SGA categorization classified only 50.0 % of the patients with low FFMI as malnourished (PG-SGA B). Moreover, 60.7 % of the patients with low FFMI had a total PG-SGA score indicating need for a nutritional intervention (i.e. >4 points). Physical examination in the PG-SGA identified only 64.3 % of the patients with low FFMI as muscle depleted. In conclusion, our results indicate that the PG-SGA does not identify with sufficient sensitivity patients with low FFMI among patients with non-metastatic CRC. In clinical practice, PG-SGA should be accompanied by muscle mass assessments by BIA or other methods in order to detect low FFM in this patient group.
3.3 Paper 3

The aim of this study was to validate a whole-body and a segmental BIA device against DXA in CRC patients, and to investigate the ability of different empiric equations for BIA to predict DXA FFM ($\text{FFM}_{\text{DXA}}$).

Forty-three non-metastatic CRC patients (aged 50-80 years) were enrolled in this study. Whole-body and segmental BIA FFM estimates ($\text{FFM}_{\text{whole-bodyBIA}}$, $\text{FFM}_{\text{segmentalBIA}}$) were calculated using 14 empiric equations, including the equations from the manufacturers, before comparison to $\text{FFM}_{\text{DXA}}$ estimates.

Strong linear relationships were observed between $\text{FFM}_{\text{BIA}}$ and $\text{FFM}_{\text{DXA}}$ estimates for all equations ($R^2=0.94-0.98$ for both devices). However, there were large discrepancies in FFM estimates depending on the equations used with mean differences in the ranges -6.5-6.8 kg and -11.0-3.4 kg for whole-body and segmental BIA, respectively. For whole-body BIA, 77 % of BIA derived FFM estimates were significantly different from $\text{FFM}_{\text{DXA}}$, whereas for segmental BIA, 85 % were significantly different. For whole-body BIA, the Schols* equation gave the highest agreement with $\text{FFM}_{\text{DXA}}$ with mean difference $\pm$ SD of $-0.16 \pm 1.94$ kg ($p=0.582$). The manufacturer’s equation gave a small overestimation of FFM with $1.46 \pm 2.16$ kg ($p<0.001$) with a tendency towards proportional bias ($r=0.28$, $p=0.066$). For segmental BIA, the Heitmann* equation gave the highest agreement with $\text{FFM}_{\text{DXA}}$ ($0.17 \pm 1.83$ kg ($p=0.546$)). Using the manufacturer’s equation, no difference in FFM estimates was observed ($-0.34 \pm 2.06$ kg ($p=0.292$)), however, a clear proportional bias was detected ($r=0.69$, $p<0.001$). Both devices demonstrated acceptable ability to detect low FFM compared to DXA using the optimal equation.

In a population of non-metastatic CRC patients, mostly consisting of Caucasian adults and with a wide range of body composition measures, both the whole-body BIA and segmental BIA device provide FFM estimates that are comparable to $\text{FFM}_{\text{DXA}}$ on a group level when the appropriate equations are applied. At the individual level (i.e. in clinical practice) BIA may be a valuable tool to identify patients with low FFM as part of a malnutrition diagnosis.
4 General Discussion

4.1 Study designs

This thesis includes a study protocol for the RCT “The Norwegian dietary guidelines and colorectal cancer survival (CRC-NORDIET) study: a food-based multicenter randomized controlled trial” (paper 1) and two cross-sectional studies (paper 2 and 3) with populations drawn from the patients included in the CRC-NORDIET study.

The RCT was established due to paucity of data on the role of diet after CRC diagnosis, and more specifically, lack of intervention studies investigating whether a causal relationship exists between diet and disease outcomes and survival in colorectal cancer patients. Current evidence supporting diet after CRC diagnosis is based on epidemiological studies indicating that diet is of importance for CRC survivors, however, RCTs are needed to confirm that diet improves survival and disease outcomes in this population. RCTs are considered the most rigorous method for investigating the cause-and-effect relationship between a dietary intervention and outcome [83]. The main strength of this study design is the random allocation to treatment group(s) and control group(s), ensuring that the potential confounding variables, both known and unknown factors, are equally distributed among intervention subjects and controls.

The evidence for the impact of diet on disease in cancer prevention is based on observational and biological studies, since long-term RCTs are not appropriate for this purpose in a healthy population. Cancer patients are, however, suitable as a target for RCTs, since these patients are more likely to develop new morbidities and have increased risk of death, compared to people without a cancer diagnosis. Moreover, RCTs allow for the opportunity to strongly influence the participants’ diet, and thus generate differences between intervention subjects and control subjects that are sufficiently large to observe effects of the dietary intervention on the outcomes of interest.

In paper 2 and 3, we used a cross-sectional study design. Cross-sectional studies are often described as taking a “snapshot” of a group of individuals, since data is collected from one time point and thus reflects the situation at this particular time point [84]. Cross-sectional
studies are particularly useful to estimate prevalence of various conditions or outcomes, and factors associated with the outcomes. Since measurement of exposure and outcomes are performed simultaneously, no conclusions about temporal relationship may be drawn from this type of studies. Moreover, one limitation with these studies is that the outcome of measurements may vary from one point of time to another, and hence influence the generalizability of the results. The prevalence of low FFMI and malnutrition (e.g. PG-SGA B) assessed in paper 2 and 3 could have been different if measured at another point of time. In paper 2, were we aimed to investigate the ability of PG-SGA to detect low FFMI in patients with non-metastatic CRC, another prevalence of PG-SGA B and low FFMI would affect the results of these analyses.

The patients included in paper 3 were CRC patients who had undergone surgery for CRC within the last 4 years. Hence, the population consisted of patients enrolled in this study within some months after surgery as well as patients who were enrolled more than 3 years after surgery. Variability in timing of entry to the study may have implications for the prevalence of malnutrition and presence of symptoms related to surgery and cancer treatment. Since these factors may influence the validity of the results, we cannot rule out the possibility that our results would be different if the measurements were performed in a CRC patient population with for instance a higher proportion of patients recruited shortly after surgery.
4.2 The Norwegian dietary guidelines and colorectal cancer survival (CRC-NORDIET) study: a food-based multicenter randomized controlled trial

Paper 1 presents the study design of The Norwegian dietary guidelines and colorectal cancer survival (CRC-NORDIET) study. The CRC-NORDIET study is a multicenter RCT with two parallel study arms (Figure 9). The intervention group receives an intensive dietary intervention and general advice on physical activity, whereas the control group receives standard dietary advice and general advice on physical activity. The intervention starts 2-9 months after surgery (i.e. baseline) and consists of two periods; an intensive period that lasts 12 months, and a subsequent maintenance period which lasts for additional 14 years. The details of the intervention are given in the section “Methods and design" of paper 1. Some of the aspects of the study design are discussed in the next sections.

Figure 9. The CRC-NORDIET study design
4.2.1 Study participants

Patients eligible for the CRC-NORDIET study are men and women, 50-80 years, newly diagnosed with primary CRC, staged I-III according to the TNM system [13]. Precise inclusion and exclusion criteria are given in paper 1. Patients with stage IV are excluded from the study due to the short expected survival, as the study aims to test the long-term effects of diet. However, by including stage I-III we increase the generalizability of our findings since patients with these stages constitute the majority of the CRC patients.

The two patient populations included in paper 2 and 3, respectively, were recruited from the ongoing CRC-NORDIET study. Table 7 shows the distribution of sites and stages in the study populations from paper 2 and paper 3, compared to a reference population, i.e. patients with stage I-III treated by surgery for primary tumor Norway based on data from the latest report from the Norwegian Colorectal Cancer Registry. The distribution of colon, rectosigmoid and rectum cancer in the study populations in paper 2 and 3, respectively, was similar to the reference population. However, there were also some differences between the study populations and the reference population. For instance, the proportion of patients with stage I in paper 2 and the proportion of stage III patients in paper 3 were low compared to the reference population.
Table 7. Study populations in the CRC-NORDIET study vs CRC reference population in Norway

<table>
<thead>
<tr>
<th>Study population, paper 2 (n=97)</th>
<th>Study population, paper 3 (n=43)</th>
<th>CRC reference population* (n=2878)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years (median)</td>
<td>67 (CRC)</td>
<td>73 (colon) / 69 (rectum)</td>
</tr>
<tr>
<td>Women</td>
<td>46 (47.4%)</td>
<td>26 (60.5%)</td>
</tr>
<tr>
<td>Men</td>
<td>51 (52.6%)</td>
<td>17 (39.5%)</td>
</tr>
<tr>
<td>Colon cancer</td>
<td>54 (58.7%)</td>
<td>26 (60.5%)</td>
</tr>
<tr>
<td>Rectosigmoid cancer</td>
<td>6 (6.5%)</td>
<td>2 (4.7%)</td>
</tr>
<tr>
<td>Rectum cancer</td>
<td>32 (34.8%)</td>
<td>15 (34.9%)</td>
</tr>
<tr>
<td>Stage I</td>
<td>10 (11.2%)</td>
<td>15 (37.5%)</td>
</tr>
<tr>
<td>Stage II</td>
<td>46 (51.7%)</td>
<td>18 (45.0%)</td>
</tr>
<tr>
<td>Stage III</td>
<td>33 (37.1%)</td>
<td>7 (17.5%)</td>
</tr>
</tbody>
</table>

CRC: Colorectal cancer

*Numbers are taken from the latest report from the Norway Colorectal Cancer Registry (2015) [18]. In paper 2, localization (colon/rectosigmoid/rectum cancer) was available for 92 out of 97 patients, while TNM stage was available for 89 out of 97 patients. In paper 3, localization (colon/rectosigmoid/rectum cancer) was available for all patients, while TNM stage was available for 40 out of 43 patients.

Patients included in paper 2, were enrolled between August 2013 and March 2015. A total of one hundred and six patients with CRC stage I-III were assessed with the PG-SGA tool. Of these, nine patients were excluded from the analyses due to lack of data needed to determine FFMI. The patients included in paper 3 were enrolled between December 2015 and February 2016. Forty-five patients completed the DXA scans. Of these, two patients were excluded from BIA assessments due to pacemakers. Furthermore, two patients were excluded from the segmental BIA assessments due to upper extremity amputations and technical issues during the assessments, respectively. Thus, 43 completed whole-BIA assessment and 41 completed segmental BIA assessment.

In contrast to previous validation studies, we chose not to exclude patients with abnormalities in body shape, obesity, orthopedic prosthesis/implants, chronic diseases and fluid disturbances (presence of oedema) in order to increase generalizability. Patients with these features have often been excluded due to possible interference with the BIA.
measurements, resulting in highly selected patient populations. By including a broad spectrum of patients, we were able to explore the impact of these features on the agreement of the estimates.

4.2.2 The control group

The control group in the CRC-NORDIET study does not receive placebo in the sense of placebo as defined by the English Oxford dictionary as “a medicine or procedure described for the psychological benefit to the patient rather than for any physiological effect”. Placebo-controlled RCT is most often not possible when studying food-, or exercise-based interventions, since placebo-foods or placebo-exercise do not exist. We decided that the control group should receive standard clinical care as well as a booklet with standard dietary advice for ethical reasons. There is a risk that the control patients will improve their dietary habits, particularly the most health-conscious participants. We suggest that providing these dietary advice as a booklet is not sufficient to generate the same effects as the intensive dietary intervention with all the selected strategies included in this study. Hence, it is not expected that it will preclude the interpretation of results. Furthermore, we decided to give the control subjects, identically with the intervention group, free access to the training studio and general advice physical activity, as well as health reports following every visit at the study center and invitation to group meetings. We believe that all these aspects are important in order to increase the motivation to participate in our study as well as to reduce drop out in the long run.

4.2.3 Blinding

In RCTs, “blinding” refers to the procedure of ensuring that participants, health-care professionals, data collectors or data analyzers are not informed about which intervention is received [85]. The purpose with blinding is to reduce the risk that knowledge about the intervention received affects the outcome measurements. Because of the nature of the intervention in the CRC-NORDIET study, blinding of participants and health professionals involved in the assessments and the different activities with the patients (e.g. group meetings and dietary counseling), is not feasible. However, laboratory personnel and those who are involved in analysis and interpretation of data are blinded to the group assignment.
The effect of blinding or lack of blinding on intervention effects have been investigated [86]. Some studies suggest that lack of double-blinding may lead to overestimation of intervention effects compared to adequately blinded trials [87-89], however others have not found the same results [90]. Wood et al found that the bias associated with lack of blinding were restricted to studies with subjectively assessed outcomes, whereas there was no evidence for bias in studies with objective outcomes [86]. The primary outcomes in CRC-NORDIET study are objective outcomes, however, self-reporting questionnaires used in some of the analyses for the assessment of the secondary endpoints may be a possible source for performance bias.

4.2.4 The impact of physical activity

Physical activity is one of the established protective factors for CRC and furthermore, several observational studies suggest that being physical active after a diagnosis of CRC may play an important role for survival. In a recent meta-analysis of six large prospective cohort studies by Schmid and coworkers it was found that high vs low physical activity after CRC diagnosis was associated with a 42 % lower risk of total mortality (Hazard ratio (HR):0.58, 95 % CI, 0.48-0.70) and a 39 % lower risk of CRC-specific mortality (HR: 0.61, 95 % CI 0.40, 0.92) [91]. For the present, no RCTs have been completed to draw any conclusions on the effect of physical activity on risk for mortality in patients after CRC diagnosis. One ongoing trial, the CHALLENGE (Colon Health and Life-Long Exercise Change) trial is designed to investigate whether supervised physical activity have impact on survival in patients with high-risk stage II and III colon cancer [92]. Since physical activity probably is important to improve survival in patients diagnosed with cancer, we chose to give identical advice on physical activity to both intervention and control subjects, in order to isolate the effect of diet. Previous RCTs conducted in CRC survivors [93-95] have studied the effect of diet in combination with physical activity and thus, no conclusions for the role of diet can be made based on these studies.

Ideally, there should be no skewness with regard to physical activity between the two groups in our study design. However, in order to control for the confounding effects of physical activity, we have included several methods to monitor physical activity.
4.2.5 Long-term follow-up

When conducting an RCT to study the effect of diet on disease outcomes, it is difficult to estimate the time between dietary exposure and expected change in incidence of disease. Hence, RCTs should ideally be of a long duration. In our study, patients are followed until 15 years after baseline (see power calculations at 5, 10 and 15 years in paper 1). Hence, we can expect that the outcomes of interest (i.e. local recurrence, metastasis, new cancers, new morbidity or death) may occur within the time frame of follow-up. Based on data from the Norwegian cancer registry for the period 2013-2015, the 5-year relative survival for CRC patients stage I-III was reported to be 84 % and 85 % and for colon and rectum cancer respectively, meaning that 16 % and 15 %, respectively, died during the first 5 years after surgery [18]. Data from the same period showed that metastasis occurred in 16 % and 22 % of the patients with colon and rectum cancer, respectively and local recurrence occurred in 5% of the patients with rectum cancer within the first 5 years.

4.2.6 The dietary intervention

The recommended diet in the CRC-NORDIET study is in accordance with the NFBDG (Table 6). These guidelines were developed to prevent diet-related chronic diseases in the general population [79] and are based on a comprehensive, systematic review of the evidence linking diet to the risk of chronic diseases. Dampening of chronic low-grade inflammation and oxidative stress are likely mechanisms mediating these effects. The rationale for testing NFBDG in a CRC population is based on the hypothesis that a diet in accordance with these guidelines will have beneficial effects in CRC progression and diet-related comorbidities mediated through dampening of inflammation and oxidative stress. Chronic inflammation plays an important role in CRC-related comorbidities such as cardiovascular diseases, metabolic syndrome, obesity, type 2 diabetes and sarcopenia [96]. Moreover, experimental and clinical research demonstrates that inflammation profoundly affects all phases of cancer, from the initiation of cancer to early growth, progression and the process of metastases. Inflammation favors the processes leading to cancer and create a tissue microenvironment that allow the tumor to grow and metastasize [97]. Hence, we hypothesize that adherence to our dietary recommendations, will modulate the
inflammatory processes and thereby reduce cancer recurrence and development of new chronic diseases.

Within the NFBDG, we emphasize specific anti-inflammatory and antioxidant-rich foods and drinks (details given in paper 1), previously identified by our research group through clinical trials and in vivo and in vitro models [96, 98-104]. The potential of diet to reduce inflammation and oxidative stress has also been investigated by others. In the PREDIMED study, adherence to the Mediterranean diet (MD) (i.e. a diet rich in vegetables, fruits, whole grains, legumes, olive oil and fish, and limited intake of saturated fat and red meat), which is similar to the NFBDG, was demonstrated to increase the total antioxidant capacity [105]. The MD is furthermore shown to reduce the expression of genes related to inflammation (e.g. NF-kB, monocyte chemoattractant protein 1, TNF-α, and IL-6) in older people as demonstrated by Camargo et al [106]. High intakes of fruits and vegetables provide high levels of different polyphenols with both antioxidant and anti-inflammatory properties. For instance, polyphenols from olive oil are shown to modulate enzymes in the eicosanoid pathway leading to a reduced production of inflammatory mediators such as metabolites of arachidonic acid (i.e. prostaglandins and leukotrienes) [107]. In addition, olive oil polyphenols reduce inflammatory angiogenesis, which is central in the pathogenesis of cancer and atherosclerosis [108]. With regard to other components with anti-inflammatory properties, dietary fibre is shown to decrease inflammatory mediators such as CRP, IL-6 and TNF-α [109-111] whereas high intakes of fatty acids from marine fish and nuts (i.e. omega 3 fatty acids) are demonstrated to balance the pro-inflammatory omega-6 fatty acids, and hence modulate inflammation and blood coagulation [112]. For review of the impact of MD on inflammation, see the review paper published by Ostan et al [96].

The CRC-NORDIET study tests the effect of a whole-diet based on the assumption that a whole diet approach provides more beneficial effects than single nutrient interventions. It is previously shown in our research group that various plant compounds have the ability of creating synergistic effects in in cell experiments using human cancer cells, through modulation of NF-kB [101]. The hypothesis that a healthy dietary pattern may be beneficial for cancer patients is supported by epidemiological studies showing that a prudent dietary pattern (e.g. high intakes of vegetables, legumes, fruits, fish and low intakes of red and
processed meat) after cancer diagnosis is associated with reduced risk of recurrence and mortality.

4.2.7 Implementation of dietary intervention

The dietary intervention in the CRC-NORDIET study is designed to attain long-term compliance to the NFBDG. One of the major challenges in lifestyle intervention studies is to achieve sustainable changes in their daily life habits, including diet. In cancer patients, there may be additional barriers to change diet due to different conditions and consequences related to the disease and treatment, such as fatigue, pain, gastrointestinal problems, and depression. However, studies have reported that cancer patients may be particularly motivated to change diet immediately after diagnosis [4, 113, 114]. We have therefore designed our intervention to be initiated short time after diagnosis, i.e. within the time frame where the patients are most likely to be interested in changing diet. Furthermore, we have chosen multiple strategies to implement the dietary recommendations. All the strategies were carefully selected to achieve compliance to the dietary intervention; individual counseling by registered clinical dietitians (RCDs) (both face-to-face and telephone based), delivery of free foods, grocery discount cards, group meetings, cooking course, printed materials and access to a CRC-NORDIET web page. To our knowledge, no previous intervention studies conducted in CRC patients have included so many strategies to increase compliance to the dietary advice. Some of these strategies will be discussed in the next section.

4.2.8 Individualized nutritional counseling by a registered clinical dietitian

The nutritional face-to-face counseling aims to meet the individual needs as well as to educate the patients on how to change their diet in accordance with the NFBDG. Individualized dietary advice is given based on comprehensive evaluation of dietary intake, nutritional status assessed by PG-SGA and weight history as well as characterization of individual needs and problems (e.g. loss of appetite, gastrointestinal symptoms and fatigue). Furthermore, dietary advice is individualized by taking into account the patient’s personal eating habits and preferences.
Interventions with individualized dietary counseling are previously reported to be effective in achieving adherence to dietary advice in cancer patients [51, 115-117]. In a RCT by Ravasco and coworkers, CRC patients receiving dietary counseling during radiotherapy weekly for 6 weeks significantly increased their energy intake compared to patients with no dietary counseling [51]. Similar to our intervention, dietary advice was individualized and based on a thorough evaluation of clinical and nutritional status, adjusted to personal eating patterns and treatment-related symptoms. Evidence for the effect of individualized dietary counseling on dietary intake is also supported by RCTs conducted in patients with head and neck cancer [115, 117] and lung cancer [118, 119].

4.2.9 Telephone and web-based approach

Patients in the intervention group receive face-to-face dietary counseling at the study center three times the first year, and five times during the maintenance period (year 2-15). In addition, the patients receive telephone-based counseling in between the meetings at the study center, consisting of three phone calls during the first year, and then every second year during the maintenance period (year 2-15). The primary purpose with this follow-up is to monitor the patients’ body weight status, dietary pattern and motivational status. Good et al recently published a review evaluating the efficacy of telephone delivered interventions in cancer survivors [120]. Most of the 27 included RCTs in this review aimed to achieve changes in physical activity, or a combination of physical activity and diet, whereas five targeted weight control. Approximately 75 % of the reported trials were rated as successful (i.e. at least one significant end-of-intervention effect was observed). Evidence supporting the telephone-based approach is also published by others [121].

Two RCT’s performed in CRC patients which at least partially, succeeded in achieving changes in dietary behavior are the CanChange trial [122] and the Reach out to Enhance Wellness (RENEW) trial [95] in which both were based on interventions delivered by telephone. In the CanChange trial, 410 CRC survivors were randomized to either health coaching focusing at diet, physical activity, weight control, alcohol and smoking, or usual care. The intervention was delivered as telephone sessions biweekly over 6 months. At 12 months, significant intervention effects were observed for moderate physical activity, BMI, energy from total fat and energy from saturated fat. No significant effects were observed for
fruits, fiber, alcohol or smoking. In the RENEW study [95] including 641 older overweight or obese survivors of breast, colorectal and prostate cancer, the 12 months home-based intervention consisted of 15 telephone sessions and 8 automated prompts during 12 months. Significant intervention effects were found for almost all the targeted behaviors; intake of fruits and vegetables, saturated fat, weight loss and physical exercise.

Time and resources to travel are reported barriers for participation in clinical trials [123-125], and the telephone-based approach is hence an easily accessible way to participate in a trial. In addition, the patients may communicate with the RDNs by e-mail and they have access to a dynamic website. Internet-based approaches are effective channels to obtain frequent contact with the participants, and potentially to maintain behavior change. However, evidence supporting newer modalities such as web sites, mail, SMS, mobile/smart phone applications and blogs is currently limited [120] in cancer patients. When choosing effective strategies to communicate with the patients it is important to bear in mind the age group in the study population. Since the patient population in our study is in the range 50-80 years, we chose a combination of up to date modalities, such as mail, SMS and web site and more “old-fashion” modalities such as printed materials and telephone-based counseling. All information provided by web/mail/SMS is also given as printed materials to ensure that all patients in the intervention group have access to the same information.

4.2.10 Motivational interviewing

In the CRC-NORDIET study, the RCDs use principles from motivational interviewing (MI) to explore the potential to increase motivation. The degree of motivation (i.e. “very motivated”, “motivated”, “less motivated” and “not motivated”) is taken into account in each of the counseling sessions. Since the intervention in the CRC-NORDIET study lasts for 15 years, we have to expect that motivation may change during participation and it is likely that the participants will face challenges to maintain a healthy diet. We suggest that it is more strategic to focus on a few realistic goals instead of aiming at changing the whole diet. Each counseling session is therefore intended to result in a few dietary goals, and it is emphasized that the patient defines his or her own goals. Both the previously mentioned CanChange study and the RENEW study and several others [114] have based their interventions on theoretical frameworks and theories, such as the social cognitive theory [126] and MI [127]
in order to facilitate changes in lifestyle habits. Lifestyle trials anchoring their interventions into solid theories for behavioral change are reported to be more likely to achieve success [114].

4.2.11 Long-term adherence to the dietary intervention

Since most of the RCTs conducted in CRC patients are designed to investigate effects on short-term outcomes such as quality of life and physical function, studies investigating long-term adherence to dietary recommendations are limited in these patients. The earlier mentioned study by Ravasco and coworkers demonstrated that CRC patients receiving individualized dietary intervention during radiotherapy maintained their dietary intake in accordance to the recommendations more than 5 years after completion of radiotherapy [45]. The 6-week long nutritional intervention in this trial was delivered as weekly counseling sessions with registered dietitians and aimed to educate patients how to modify their nutritional intake to ensure sufficient energy and nutrient intake, as well as to select appropriate foods to target symptoms from radiotherapy. Our CRC-NORDIET study is designed to provide new knowledge about the long-term effects on adherence to the dietary recommendations. To increase the chances to obtain sustainable effects on dietary behavior, we have chosen to extend the intervention with a maintenance period of 14 additional years after the first intensive period of 12 months. During this maintenance period, intervention subjects receive individual counseling (face-to-face and telephone-based) once a year, invitation to several inspiration meetings and access to the CRC-NORDIET web site throughout the entire study period. Moreover, patients receive reports with feedbacks from the non-biological assessments (e.g. body weight, body composition analysis, blood pressure) after the assessments at study center and this may serve as additional inspiration for participation and retention in the study. Compliance to the intervention is fundamental in order to assess any effects of an intervention. We have therefore developed a specific questionnaire for assessment of compliance to the intervention, and this questionnaire will be validated within the first period of the study. Compliance to the intervention will also be measured by objective measures such as biological markers of food intake (e.g. carotenoids, fatty acids) which will be assessed at regular time points during the study period.
4.2.12 Endpoints and power calculations in the CRC-NORDIET study

The primary endpoints in the CRC-NORDIET study are

1. Disease-free survival (DFS) (events defined as detection of local recurrence or metastasis or any second primary other cancer or death from any cause)

2. Overall survival (OS) (event defined as death from any cause)

DFS was defined in agreement with the proposed guidelines by Punt el al [128]. The primary endpoints will be assessed 5, 10, and 15 years, respectively, of follow up after baseline.

The 16 secondary endpoints in the study are presented in paper 1. Time to recurrence and all endpoints including survival will be assessed 5, 10, and 15 years of follow up after baseline. The remaining secondary endpoints will be assessed after 6 months, 1 year and 3 years after baseline.

The sample size was calculated with a logrank test, based on a statistical power of 80 % and a significance level of 5 %. Five-year OS data from the Norwegian cancer registry report from 2013 [20], 68%, was used as the expected OS for the control group in the study. A sample size of 250 participants in each study group was needed to detect a 25% reduction in mortality in the intervention group, with a statistical power of 80 % (corresponding to a hazard ratio of 0.71). To achieve a 25 % reduction in events of DFS 5 years after baseline, 190 patients were needed in each study group corresponding to 80 % power, to detect a HR of 0.70 (Paper 1). An expected reduction in mortality of 25 % was based on observational studies comparing high vs low adherence to a healthy diet and effects on survival from cancer [20, 76, 129-132], due to limited number of RCTs in this field.
4.2.13 Status of the ongoing CRC-NORDIET study

Recruitment of patients to the CRC-NORDIET study was initiated in 2012. Table 8 shows the number of patients enrolled and as well as the number of patients lost from the study due to voluntary quit, exclusion or death. At the end of January 2018, 340 patients were enrolled, and out of these, 324 patients are still participating in the study. Of the 324 patients who are still in the study, 175 and 165 are intervention patients and controls, respectively.

It is estimated that calculated sample size of 500 participants will be reached by the end of 2020. Of the 340 patients enrolled in the study so far, only 16 patients (4.7 %) have voluntarily quit. Based on these observations we suggest that study participation is conceived as feasible by the patients.

Table 8. Status of participation in the CRC-NORDIET study

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enrolled in the study</td>
<td>340</td>
<td>175</td>
</tr>
<tr>
<td>Voluntary quit</td>
<td>16</td>
<td>10</td>
</tr>
<tr>
<td>Excluded*</td>
<td>11</td>
<td>7</td>
</tr>
<tr>
<td>Dead</td>
<td>9</td>
<td>2</td>
</tr>
<tr>
<td>Total lost</td>
<td>36</td>
<td>19</td>
</tr>
</tbody>
</table>

Status of study participation was analyzed the 31 th of January 2018.

*See description of inclusion and exclusion criteria in paper 1.

A: Intervention group
B: Control group
4.3 Nutritional assessment by PG-SGA

PG-SGA was originally developed to evaluate nutritional risk or presence of malnutrition in an oncologic setting [133]. It was modified from the original SGA [65] with additional cancer-related nutritional impact symptoms, assessment of recent weight loss (i.e. weight loss the recent two weeks) reflecting anabolic or catabolic state and a change from clinician-generated to patient-generated approach (i.e. patients completing the first part of the tool instead of the clinician). See paper 2 for details. The original PG-SGA was developed to categorize the patient as PG-SGA A; no nutritional risk/well nourished, PG-SGA B; moderate risk of malnutrition/malnourished, or PG-SGA C; severe risk of malnutrition/severe malnourished. The scored PG-SGA was later introduced to facilitate monitoring of subtle changes in nutritional status as well as effects of nutritional interventions, to be utilized both in the clinical and research settings. More recently, several studies have evaluated the shored version of PG-SGA (i.e. the PG-SGA Short Form, the patient-generated part of the tool) that is meant to be used as a screening tool. Hence, the PG-SGA is considered to be a “4-in-1 instrument” to be used both to the purpose of screening, assessment, monitoring and triaging for interventions [134].

PG-SGA is now widely used, and the full version is recommended by the Academy of Nutrition and Dietetics as one of the nutritional assessment tools to use in clinical oncology. Since the patients may complete the patient-centered part of the tool prior to consultation with the clinical dietitian, valuable time may be saved. The complete assessment takes only approximately 5 minutes and gives a detailed overview of the patient’s nutritional status immediately. The PG-SGA scoring is useful in the follow-up of these patients, by using the scores to monitor changes during and after nutritional therapy. Hence, in the CRC-NORDIET study, changes in PG-SGA scores will be analyzed and compared between the intervention group and control group, to investigate the effect of the intervention on nutritional status. Importantly, PG-SGA has been found to predict clinical outcomes such as length of hospital stay [135, 136], rehospitalization [137], postoperative complications [138], quality of life [139] and survival [136, 140-143].
In the recent publication by Sealy and colleges, it was demonstrated that PG-SGA is one of few assessment tools in oncology that include all domains within the malnutrition definitions by ESPEN and ASPEN [69]. One of these domains is assessment of body weight, body area and body composition. The physical examination in the PG-SGA consists of visual inspection and palpation of muscles, subcutaneous fat and edema and seeks to evaluate nutritional depletion. To which degree this examination is able to detect low muscle mass/FFM in comparison to a reference method is however not well investigated.

To the best of our knowledge, no studies have investigated the ability of the PG-SGA tool to detect nutritional depletion in patients with non-metastatic CRC. Hence, the primary aim of paper 2 was to examine the ability of the PG-SGA to detect low FFM in these patients, with BIA as reference method for assessment of FFM (see section 4.6). Although PG-SGA scores previously are shown to be associated nutritional measures such as BMI and weight loss, nutritional deficits may occur across the categories of BMI, and malnutrition may be present in patients with absence of weight loss. With the growing prevalence of overweight and obesity for several cancer sites, it is important to investigate whether the PG-SGA tool is capable of detecting nutritional depletion also in these patients. Several studies aimed to explore the concurrent validity of PG-SGA have used SGA or malnutrition ICD-10 codes as reference or “gold standard”. In these studies PG-SGA score have demonstrated strong diagnostic accuracy with high sensitivity and specificity [135, 137, 144]. However, neither SGA nor ICD-10 classification of malnutrition are appropriate as measures of nutritional deficits. Both methods reflect the same risk factors as evaluated by the PG-SGA such as reduced food intake and weight loss. Furthermore, the ICD-10 codes for malnutrition emphasize low BMI, even though nutritional deficits also may be present in individuals with normal or high BMI.
4.4 Assessment of fat-free mass

4.4.1 Methods for assessment of FFM

The field of body composition in medical research has grown rapidly the last decades and brought valuable insight about the impact of body composition on morbidity and mortality in cancer patients. To date, body composition methodology encompasses a large number of methods that differ with regard to precision, costs and availability. Advanced imaging techniques such as DXA, CT and MRI are considered gold standard methods for assessment of FFM due to their ability to precisely measure different tissues within FFM, such as skeletal muscle, fat, bone and organs. However, access to these methods is often limited in clinical practice and BIA is considered a more available alternative to assess FFM. Since BIA estimates FFM indirectly, it is less precise compared to the gold standard methods. In the current work, BIA and DXA were chosen to assess FFM, and certain methodological aspects regarding these methods will be discussed in the next sections.

Although all are considered gold standard methods, the imaging techniques DXA, CT and MRI have their strengths and limitations, and it is important to note that they measure different compartments of the human body. In order to discuss DXA as a reference method for FFM assessments, it is relevant to compare the method in light of the other gold standard methods. CT and MRI allow for segmentation and quantification of different tissues from either cross sectional area in single images or series of images that encompass entire organs. Within these cross sectional images, the amount of skeletal muscle (including psoas, paraspinal muscles, transversus abdominus, external and internal obliques and rectus abdominus) and adipose tissues (including the subcutaneous, visceral and intramuscular adipose tissues) can be precisely determined. For CT, images from the third lumbar (L3) vertebra has been defined as the landmark of interest since the composition of skeletal muscle and adipose tissue at this area is found to be highly representative for whole-body tissue quantities [33, 34]. The cross-sectional areas (m²) can be computed for each tissue by the use of specific CT imaging software, and further incorporated into prediction equations to estimate whole-body skeletal muscle mass and adipose tissue [145-147]. However, use of CT in body composition assessment is limited due to the high radiation exposure, and only images taken as part of routine care may be utilized for this purpose. Furthermore, the
estimation of whole-body tissue quantities is based on regression equations developed in a selected patient population and may hence not be valid in all populations. In contrast to CT, MRI is based on for whole-body assessments and repeated measurements than CT. However, there are also some limitations related to the use of MRI in clinical practice. It is less available, requires high technical competence and the analysis is time consuming.

4.4.2 DXA

DXA was originally developed to measure bone density and is primarily used to diagnose osteoporosis. DXA is now also used to characterize body composition as the method also allows for whole-body and regional determination of FFM, bone mass, and FM. The sum of lean soft tissue (i.e. FFM without bone) in arms and legs is referred to as total appendicular skeletal muscle. With its high precision is considered one of the gold standard methods for measurement of fat and lean mass. In contrast to CT, the radiation exposure is very low, and DXA is thus more suitable for repeated measurements. The repeatability is found to be very high [148]. Since we had access to a DXA device in our research setting and considered DXA as the most suitable imaging method for assessment of fat-free mass, DXA was used as reference method in the validation study in paper 3. There are, however, some methodological aspects related to DXA that need to be further discussed. Similar to BIA, also DXA is based on an assumption that lean soft tissue is constantly hydrated at 73 %. Thus, the DXA estimates may be less reliable in patients with fluid and electrolyte disturbances. Small variations in hydration status (i.e. 68-78 %) are however not considered to significantly influence the estimates [149]. On the other hand, severe overhydration such as ascites and edema, may have substantial effect on the measurements. In paper 3, only 12 % of the patients were observed with edema and only four of the patients had a hydration status exceeding the normal range for the ration between total body water (TBW) measured with BIA (i.e. the segmental BIA) and FFM measured with DXA (data not shown). Hence, severe overhydration was not a major concern in our study and had minimal impact on the interpretation of our results.
4.4.3 BIA

Compared to imaging techniques such as CT, MRI and DXA that directly measure lean body mass with high precision, BIA measures these compartments indirectly by measuring the impedance of a low-voltage current passing through the body. The impedance consists of two components, the resistance, which is the opposition of an ionic solution in both intra- and extracellular spaces and the reactance representing the capacitance from the cell membranes [37]. The resistance and reactance values are further incorporated in linear regression equations to calculate TBW or FFM.

These equations have been developed in different populations and combine BIA impedance data with variables such as height, weight data, age and gender to calculate the various body compartments. One of the main limitations with BIA is related to the complexity of these equations. Although a prediction equation is developed and validated in a specific population it will not necessarily suit other populations. In addition to the equations incorporated in the various BIA devices, there are a large number of published equations available in the literature. It is therefore confusing for the clinicians to know which equation should be used in which patient. Moreover, BIA relies on several assumptions such as normal body composition and normal fluid and electrolyte status. Since the validity of BIA is mainly tested in healthy populations where these assumptions are met, the clinical value of BIA in patient populations has in general been considered limited.

The existing BIA devices are either based on a whole-body approach or a segmental approach. With the whole-body BIA approach, the human body is viewed as cylindrical conductor with a uniform cross-sectional area. This model is demonstrated to be valid in healthy individuals with BMI in the range 16.0-34.0 kg/m$^2$, provided that hydration is normal and the BIA equation used is applicable to the population studied [150]. In contrast to the whole-body BIA, the segmental BIA devices measure impedance in the various segments, such as arms, legs and trunk.

BIA has several advantages compared to DXA, CT and MRI. It is relative cheap, requires minimal operator training and provides the results immediately. Some BIA devices are portable and may be used in settings where DXA, CT and MRI is not available, such as in
primary care, hospital wards and institutions such as elderly care and nursing homes. BIA may unquestionably be an easy accessible and useful tool for body composition analysis in clinical practice, given that the various devices and equations are valid.

4.5 The ability of PG-SGA to identify low FFM

In paper 2, 69% were evaluated as well nourished (PG-SGA A) and 31% as malnourished (PG-SGA B). No patients were classified as severely malnourished (PG-SGA C). The prevalence of low FFM and sarcopenia in this population was 29% and 22%, respectively. The primary aim of the study was to examine the ability of the PG-SGA to identify low FFM. Our results showed that despite that almost one third of the patients were found to have low FFM, PG-SGA did not identify these patients with sufficient sensitivity.

In paper 2, only half of the patients with low FFMI were evaluated as malnourished by the PG-SGA (i.e. the sensitivity of the PG-SGA categories). When we analyzed the various components of PG-SGA in patients with low FFMI, we observed that the majority of these patients were weight stable or gaining weight at the time of assessments, accompanied by a normal food intake (i.e. stable or increased) and no symptoms affecting food intake.

According to the developers of PG-SGA, the fundamental concept of the PG-SGA assessment is to consider the patient in terms of anabolic vs catabolic [134]. They recommend the weight history component of PG-SGA to be used as an indicator of anabolism or catabolism, and by using recent weight change (weight loss the last month and/or the last 2 weeks), improvements or deterioration may be detected. However, since increase in body weight may consist of FM rather than FFM, monitoring only body weight will not precisely reveal the specific shifts between lean tissues and adipose tissues.

In both study populations (i.e. paper 2 and 3, respectively), we observed a high prevalence of overweight and obesity. In paper 2, fifty-nine percent of the patients had BMI above the normal range, whereas only 8% were underweight. These findings are in line with the findings by Thoresen in Norwegian metastatic CRC patients [47] and by several others [39, 41, 42, 46, 151]. Moreover, thirty-nine percent of the women and 41% of the men in paper 3 were classified as abdominally obese (waist circumference ≥88 cm for women and ≥102 cm

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for men). These findings were not unexpected, since body fatness and abdominal fatness are established risk factors for CRC.

In paper 2 we demonstrated that 29% of the patients were identified with low FFMI assessed with BIA. About 32% of these patients had BMI in the overweight range. Interestingly, we observed that PG-SGA physical examination (i.e. visual inspection and palpation of muscles in temporal areas, deltoids and quadriceps) detected only 64% of the patients with low FFMI (as assessed with BIA), implying that 36% of the patients were not identified. The physical examination part of the PG-SGA is recognized as challenging, and it is emphasized that training is necessary both to increase the precision as well as to ensure reliability [134]. However, it is also acknowledged that muscle mass depletion may be difficult to detect in the clinical setting. Whereas clinical signs of wasting may be obvious in patients with BMI in the lower range, it may be masked in patients with normal or high BMI. In paper 2 we observed that mean BMI was found to be significantly higher in those patients who were not identified as muscle depleted by the PG-SGA. Furthermore, we observed a higher proportion of overweight patients among these patients compared to those who were captured as depleted. These findings indicate that PG-SGA is not sensitive enough to detect low FFM among patients with non-metastatic CRC, particularly in overweight and obese patients. To the best of our knowledge, only one study has previously examined the ability of nutritional risk screening and nutritional assessment tools to detect malnutrition according to low FFMI. In this study, Elkan and coworkers compared the sensitivity and specificity of BMI, SGA, MNA, MUST and NRS-2002 in patients with rheumatoid arthritis [152]. In line with our findings, SGA showed poor sensitivity (46%) to detect low FFM in these patients.

Although PG-SGA is a recommended tool for nutritional assessments in oncology practice, there is lack of research concerning the ability of this tool to identify malnourished patients in terms of low FFM, and our results contribute to new knowledge within this field.
4.6 The ability of BIA to assess FFM

In paper 3, we tested the ability of two different BIA devices to assess FFM compared to DXA as reference method. Main finding from this study were that both devices showed good agreement compared to DXA, when using the appropriate BIA equation.

In paper 2, a whole-body BIA device was used to assess FFM. Since the whole-body BIA approach relies on the assumptions on normal body composition and hydration status, the estimates of FFM could potentially be unreliable in our population consisting of CRC patients. Hence, we performed a validation study by testing the validity of the same whole-body BIA device used in paper 2 against DXA as a reference method in a separate population of CRC patients. The reason why this validation was not performed in the same patient population as those included in paper 2 was that DXA was not available at that time. Further, we compared the whole-body BIA device with a segmental BIA device. It has been suggested that a segmental BIA is better suited than a whole-body BIA in patients with overweight and obesity since the device measures impedance in the various segments and takes into account that the trunk only contributes in a minor extent to the whole-body resistance. However, there is limited data supporting that this approach is superior to the whole-body approach. The results from this validation study published in paper 3 showed that both devices, the whole-body and the segmental BIA device, demonstrated good agreement with DXA, when the appropriate BIA equation was used. Hence, we could not confirm the superiority of segmental BIA over whole-body BIA in estimation of FFM in this patient population.

As part of this validation study, we tested a set of BIA equations, both the equations incorporated in the BIA software of each of the devices (i.e. the manufacturer’s equation) as well as a selection of equations from the literature. Our results showed that use of the various equations resulted in significant different estimates of FFM. These results are in line with the findings reported in a recently published review by Haverkort based on studies conducted in surgical and oncological patients [153]. Both our study and the review by Haverkort hence conclude that selection of equation in BIA analysis has significant implications for the accuracy of estimates. Since the BIA equations are developed in specific populations based on reference methods, they are specifically suited for these populations.
The equations selected in our study were a number of equations mainly developed in healthy populations and previously tested in cancer patients [154-163]. One of the equations was the Geneva equation [158] which is recommended by ESPEN. Interestingly, we found that two of the existing equations, the Schols and the Heitmann equations, applied in the whole-body and segmental BIA respectively, gave the highest agreement with the estimates from DXA. The Schols equation was developed in patients with chronic pulmonary disease [163], while the Heitmann equation was developed in a healthy population consisting of adult Danes 35-65 years of age [157]. Few others have validated these equations in patient populations. Mourtzakis compared FFM estimates calculated with Schols equation with DXA as reference in a mixed population consisting of 51 patients with locally advanced or metastatic non-small cell lung and colorectal cancer [164]. Similar to our population, the patient population in this study was dominated by overweight and obese patients. However, in contrast to our results, they observed large discrepancy between FFM calculated with Schols equation and and DXA. The Heitmann equation was validated in a study with 26 patients undergoing major abdominal surgery, and similar to our findings, they found good agreement between BIA and DXA estimates [165].

The manufacturer`s equations in the whole-body and the segmental BIA, respectively, were not the most suitable equations. Using the manufacturer`​s equation in the whole-body BIA gave a small overestimation of FFM by 1.5 kg with a tendency towards proportional bias. Hence, by using estimates based on this equation in paper 2, it is possible that the prevalence of patients with low FFM would be higher if the optimal equation (i.e. the Schols*) was used. In paper 3 we found that the prevalence of low FFM was 33 % based on DXA estimates compared to 26 % and 35 % by the use of the manufacturer`​s and the Schols equations, respectively.

On the individual level, FFM estimates were within -2.8-5.7 kg and -4.4-3.7 kg, using the whole-body and segmental BIA device, respectively, for approximately 95 % of the patients. Moreover, we observed that both devices resulted in proportional bias. The clinical implications of these findings are therefore that FFM estimates may be less accurate in individuals with increased FFM, and that single measurements should be interpreted with
Repeated measurements in clinical practice, with the same BIA advice and equation may however, contribute with important insight into changes in FFM, and FM.

Our results showed that despite including patients with abnormal body shapes, obesity, presence of chronic diseases and orthopedic prosthesis/implants, we observed high agreement between FFM estimates from both BIA devices and DXA. These findings support BIA as a good alternative to DXA.

4.7 The clinical implications of this work

The CRC-NORDIET study is designed to gain a better understanding of whether diet after diagnosis has impact on long-term outcomes, risk of recurrence and survival in patients with CRC. Due to lack of studies, CRC patients currently have no specific dietary recommendations, other than the general recommendations for people who are not diagnosed with cancer. Although observational studies suggest that diet after diagnosis may play a role, RCTs are urgently needed to confirm the potential of diet to improve outcomes in this patient population. Our study will therefore be of great importance for this group of patients.

The majority of the patients included in this work underwent nutritional assessments at a time point where cancer treatment was completed. Existing literature shows that malnutrition is prevalent in CRC patients at the time of diagnosis, however, the high prevalence of malnutrition, FFM depletion and sarcopenia found in our population post-surgery, suggest that a significant proportion of these patients are still in need for nutritional intervention. Persistent nutritional problems may lead to continued loss of body weight and further deterioration of nutritional status, which may influence the further course of survivorship. Although the cancer disease is cured and the patients are predicted good prognosis, decline in nutritional status may negatively impact quality of life, functional capacity and survival, as shown by Ravasco and coworkers who demonstrated that CRC patients who did not receive intensive nutritional counseling experienced decline in nutritional status, quality of life and survival compared to the patients who received dietary counseling [45].
Most of the studies demonstrating associations between malnutrition and sarcopenia, and clinical outcome measurements and survival in CRC patients have included high proportions of patients with locally advanced or metastatic disease. The current work provides new knowledge about the high prevalence of malnutrition and sarcopenia in a CRC population without metastatic cancer. The relationship regarding low FFM and sarcopenia and clinical outcomes and survival will be investigated in future analyses in the CRC-NORDIET study.

In the current work, we demonstrated that by using the PG-SGA categories, only half of the patients with FFM depletion and less than half of the patients with sarcopenia were identified by this tool. Consequently, a great proportion of the patients would incorrectly be evaluated as “well nourished” if PG-SGA was used as the only assessment tool for nutritional assessment. Based on our findings, we recommend that body composition analysis should be performed in addition to the PG-SGA.

PG-SGA was developed to evaluate the patient in terms of anabolic or catabolic, with emphasis on recent changes in body weight. However, in the context of the increasing prevalence of overweight and obesity in cancer patients, and the support from studies demonstrating that lean mass depletion may be masked by weight increase and increased BMI, monitoring only body weight will exclude important information regarding body composition. For example, if the patient continues to gain weight in terms of fat at the expense of lean body mass, it may lead to increased risk of sarcopenic obesity with all the health risks associated. Although the literature is scarce in non-metastatic CRC patients, preliminary data suggest that sarcopenia is associated with reduced survival [57], and hence, these individuals should be identified and monitored.

In clinical practice, access to sophisticated instruments such as DXA may be limited for this purpose. In the current work, it was demonstrated that use of BIA resulted in estimates of FFM that were comparable to DXA on group level when the most appropriate equations were used. Interestingly, our work demonstrated that use of the various equations resulted in significant different estimates of FFM, and moreover, that the two BIA devices gave significant differences in FFM estimates, when using the same equation. These findings imply that BIA devices should not be used interchangeably in the clinical setting.
The results in paper 3 showed that the proportion of patients identified with low FFM according to the cutoff values by ESPEN, varied substantially depending on BIA device and equation used. Thus, some patients will not be correctly identified with low FFM as part of the malnutrition diagnosis if a suboptimal equation is used.
5 CONCLUSIONS

The present thesis concludes with the following:

- The CRC-NORDIET study was developed and established in 2012. At the end of January 2018, 340 patients were enrolled in the study. It is estimated that the calculated sample size of 500 participants will be reached by the end of 2020. Of the 447 patients enrolled in the study so far, only 16 patients (5%) have voluntary quit. Based on the high retention rate in our study we suggest that the study design is conceived as feasible by the patients.

- In our population with non-metastatic CRC patients, 69% were evaluated as well nourished (PG-SGA A) and 31% patients were categorized as malnourished (PG-SGA B). No patients were evaluated as severely malnourished (PG-SGA C). Based on BIA assessments, low FFM was identified in 29% of the patients. Twenty-two percent were diagnosed with sarcopenia. Our findings indicate that a significant portion of the patients were in need for nutritional intervention although most of the patients had already completed their cancer treatment.

- Despite that almost one third of the patients were found to have low FFM, PG-SGA did not identify these patients with sufficient sensitivity. The PG-SGA categorization classified only 50% of the patients as malnourished (PG-SGA B). Moreover, only 64% of the patients with low FFM were evaluated as muscle depleted by the physical examination in the PG-SGA.

- Both BIA devices showed good agreement compared to the reference method DXA, when using the appropriate BIA equation. Hence, BIA may be a useful tool to identify patients with low FFM and should accompany PG-SGA in the nutritional assessment of CRC patients. It is however, important to note that selection of BIA device and BIA equation may result in significantly different estimates of FFM, and the same BIA device and equation should be used when assessments are repeated during follow-up of the patient.
6 Future Perspectives

Future analyses from the CRC study will examine the ability of the CRC-NORDIET intervention to improve nutritional status as well as to maintain or increase FFM. In cancer patients with metastatic disease, it is well established that the catabolic state favors an ongoing loss of skeletal muscle, a process that cannot be reversed by conventional nutrition care. In contrast, there is limited data concerning the ability of nutritional interventions to improve nutritional status and preserve or improve muscle mass in patients with localized cancer. The dietary intervention in the CRC-NORDIET study was not specifically designed to achieve beneficial outcomes in terms of muscle mass. However, the dietary counseling aims to ensure adequate energy and protein intake, which is of importance to prevent weight loss and depletion of fat-free mass. Moreover, the recommended diet in the CRC-NORDIET targets a healthy body composition, including prevention of overweight and obesity. Future analyses from the CRC study will examine changes in fat-free mass assessed by BIA and DXA from baseline and during follow-up. Comparison of BIA and DXA at several time points will provide interesting information regarding the agreement between methods.

Although this work suggest that BIA should be applied in combination with PG-SGA to sufficiently identify patients with FFM depletion, it will be interesting to investigate whether the combination of PG-SGA and BIA will predict morbidity and mortality better than each method separately.
7 REFERENCES


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The Norwegian dietary guidelines and colorectal cancer survival (CRC-NORDIET) study: a food-based multicentre randomized controlled trial

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Abstract

Background: Colorectal cancer survivors are not only at risk for recurrent disease but also at increased risk of comorbidities such as other cancers, cardiovascular disease, diabetes, hypertension and functional decline. In this trial, we aim at investigating whether a diet in accordance with the Norwegian food-based dietary guidelines and focusing at dampening inflammation and oxidative stress will improve long-term disease outcomes and survival in colorectal cancer patients.

Methods/design: This paper presents the study protocol of the Norwegian Dietary Guidelines and Colorectal Cancer Survival study. Men and women aged 50–80 years diagnosed with primary invasive colorectal cancer (Stage I-III) are invited to this randomized controlled, parallel two-arm trial 2–9 months after curative surgery. The intervention group (n=250) receives an intensive dietary intervention lasting for 12 months and a subsequent maintenance intervention for 14 years. The control group (n=250) receives no dietary intervention other than standard clinical care. Both groups are offered equal general advice of physical activity. Patients are followed-up at 6 months and 1, 3, 5, 7, 10 and 15 years after baseline. The study center is located at the Department of Nutrition, University of Oslo, and patients are recruited from two hospitals within the South-Eastern Norway Regional Health Authority. Primary outcomes are disease-free survival and overall survival. Secondary outcomes are time to recurrence, cardiovascular disease-free survival, compliance to the dietary recommendations and the effects of the intervention on new comorbidities, intermediate biomarkers, nutrition status, physical activity, physical function and quality of life.

Discussion: The current study is designed to gain a better understanding of the role of a healthy diet aimed at dampening inflammation and oxidative stress on long-term disease outcomes and survival in colorectal cancer patients. Since previous research on the role of diet for colorectal cancer survivors is limited, the study may be of great importance for this cancer population.

Trial registration: ClinicalTrials.gov Identifier: NCT01570010.

Keywords: Colorectal cancer, Disease-free survival, Overall survival, Time to recurrence, Cardiovascular disease-free survival, Comorbidity, Inflammation, Oxidative stress, Antioxidant-rich foods, Food-based dietary guidelines

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Background

The incidences of colorectal cancer (CRC) are 5–10 times higher in Europe, North America and Oceania than in countries in Africa, south Asia and Central America [1], and the incidence in Norway is among the highest in the world [2]. Established risk factors for CRC are age, family history of CRC, inherited syndromes (Familial adenomatous polyposis, Lynch syndrome) and inflammatory bowel disease. In addition, several modifiable lifestyle-related risk factors are associated with CRC. Those include smoking, body fatness, abdominal fatness, diabetes, physical inactivity and an unhealthy diet (high consumption of alcohol, red and processed meat, and low consumption of foods containing dietary fibre) [3, 4]. World Cancer Research Fund (WCRF)/American Institute for Cancer Research (AICR) estimates that about 45% of all CRC cases could be prevented by improved lifestyle [3].

About 40% of CRC patients [5] have at least one concomitant disease (e.g. hypertension, cardiovascular disease (CVD), diabetes, chronic obstructive pulmonary disease or other malignancies) at the time of diagnosis and increased risk of developing additional comorbidities after CRC diagnosis [6–10]. These comorbid conditions may preclude or reduce effect of treatment, and consequently reduce disease-specific and total survival [8, 11, 12].

While it is well established that an unhealthy diet increases risk of CRC (e.g. see the latest update from World Cancer Research Fund, 2011 [4]) there are few studies that have focused on the effect of diet on disease outcomes and survival [13–15]. In paucity of data, health authorities in most countries recommend the same diet to CRC survivors (i.e. patients living with a CRC diagnosis, including those who have recovered) as to people without a cancer diagnosis [3].

Inflammation and oxidative stress are central underlying disease mechanisms in cancer and several other chronic diseases. Recent research suggests that there are two major molecular pathways leading to CRC, both of which involve inflammation and oxidative stress as major driving forces. The majority of CRC cases may be due to molecular events that result in chromosomal instability, while about 20-30% of CRCs are due to gene hypermethylation (called CpG island methylator phenotype (CIMP)) [16–18]. A large proportion of the CRC cases due to CIMP display microsatellite instability [18, 19]. In total, about 70 mutations in different genes have been identified as relevant for these two pathways to CRC, and it is assumed that each individual CRC tumor accumulates an average of 9 CRC pathogenic mutations out of this total pool of 70 mutations [16].

The heterogeneous pathogenesis of CRC comply with the hallmarks of cancer defined by Hanahan and Weinberg [20] and the cancer genome landscape as defined by Vogelstein et al [21]. Underlying these hallmarks of cancer, Hanahan and Weinberg proposed that genome instability and inflammation are two underlying driving forces [20]. These two processes or mechanisms are closely intertwined, since inflammation is a major cause of oxidative stress, and oxidative stress is a major cause of genome instability. Although inflammation and oxidative stress ultimately may be related to all CRC cases, the degree of inflammation and oxidative stress may vary significantly with the molecular signature present in the individual CRC patient [22].

In clinical trials and various models systems, we have identified a number of plant foods (e.g. berries, nuts, spices, coffee and specific fruits and vegetables) with the potential of dampening inflammation and oxidative stress [23–29]. Furthermore, a number of studies have also suggested that adherence to a prudent diet (e.g. Mediterranean diet) reduce inflammation and oxidative stress [30, 31]. We suggest that a prudent diet rich in specific plant-foods may be beneficial for CRC patients, especially those CRC cases with molecular signatures creating major inflammation and oxidative stress.

No intervention studies have investigated the role of diet in disease outcomes and survival in CRC-patients after diagnosis. Furthermore, no previous diet intervention study has focused on dampening inflammation and oxidative stress in this cancer population. This paper presents the background and design of a randomized controlled food-based diet intervention that examines the effects on disease outcomes and survival in CRC survivors. The diet intervention includes foods and drinks that have been suggested to dampen inflammation and oxidative stress. While specific anti-inflammatory and antioxidant-rich foods are emphasized in each food category, the complete intervention is fully in accordance with the prudent diet recommended by the Norwegian food-based dietary guidelines (NFBG) [32] (i.e. a diet similar to the Mediterranean diet).

Objectives

Outcomes are inconsistently defined in many clinical cancer trials [33, 34]. For the primary outcomes, we have used the proposed guidelines for outcomes as described by Punt et al [34]. The two primary outcomes are (to be assessed when all patients have completed 5, 10, and 15 years, respectively, of follow-up after baseline):

1. Disease-free survival (DFS) (events are defined as detection of local recurrence or metastasis or any second cancer or death from any cause)
2. Overall survival (OS) (event is defined as death from any cause)
Secondary outcomes are:

I. Time to recurrence (events are defined as detection of local recurrence or metastasis)
II. CVD-free survival (events of CVD (ICD-10; chapter I) or death from any cause)
III. CRC-specific survival (death due to CRC)
IV. Total cancer-specific survival (death due to CRC or any other cancer)
V. Inflammatory disease-specific survival (death due to inflammatory disease)
VI. Cardiovascular (CVD)-specific survival (death due to CVD)
VII. New morbidity of other diet-related chronic diseases (e.g. ischemic coronary heart disease, cerebrovascular disease, thromboembolic disease, type 2 diabetes, obesity, hypertension and chronic obstructive pulmonary disease)
VIII. Dietary intake and nutritional status
IX. Physical activity and function
X. Nutrition biomarkers (e.g., carotenoids, fatty acids, 25-hydroxy vitamin D)
XI. Body composition
XII. Anthropometric measures (e.g. weight, waist and hip circumference)
XIII. Biomarkers for inflammation and oxidative stress (e.g. isoprostanes, cytokines)
XIV. Transcription- and epigenetic profiles
XV. Biomarkers for cardiovascular disease, metabolic syndrome, type 2-diabetes, thromboembolic disease and cancer (e.g. blood pressure, total/LDL-cholesterol, HbA1c, CRP, IL-6, IL-10, TNFα)
XVI. Health related quality of life and fatigue

The secondary outcomes will be assessed after 5, 10, and 15 years and described in detail in subsequent reports. In addition, intervention effects on secondary outcomes VII-XVI will also be assessed at 6 months, 1 year and 3 years follow-up.

Methods and Design

Study design
The CRC-NORDIET study is a multicentre, randomized controlled trial (RCT), with two parallel study arms. The intervention group receives an intensive dietary intervention and general advice on physical activity (see below), whereas the control group only receives standard general dietary advice and general advice on physical activity. Newly diagnosed CRC patients undergoing surgery are recruited to the study. In addition, an age-matched CRC-free reference group (will be published elsewhere) will also be included. The intervention starts 2–9 months after surgery (i.e. baseline), and consists of two periods: an intensive period that lasts 12 months, and a subsequent maintenance period which lasts an additional 14 years. Patients are invited to the study centre, situated at the Department of Nutrition, University of Oslo, at baseline, 6 and 12 months after baseline, and 3, 5, 7, 10 and 15 years after baseline. Additional follow-ups by regular mail, phone and e-mail, occur throughout the study. The study flow diagram is presented in Fig. 1. The design and handling of data of the CRC-NORDIET study is in fully agreement with the CONSORT statement [35].

Patients and eligibility
Men and women 50 to 80 years of age with newly diagnosed primary invasive colorectal cancer (ICD-10 18-20), staged I-III (TNM-staging system [36]) are eligible for the study. The patients must be able to read and understand Norwegian and to provide a signed informed written consent. Patients unable to perceive information and understand the intervention due to diagnosed dementia, or altered mental status as well as patients participating in other RCTs in conflict with our trial are excluded from the study. Precise inclusion and exclusion criteria are presented in Table 1.

Recruitment and randomization
Patients are recruited from Oslo University Hospital and Akershus University Hospital within the South-Eastern Norway Regional Health Authority. Screening for eligible patients is performed by research investigators in cooperation with hospital personnel by monthly reviews of surgery lists and medical records. Eligible patients are invited within 9 months from surgery.

Patients accepting the invitation sign an informed consent. Signed informed consent gives permission to the study personnel to take biological samples, perform physical measurements, and retrieve information from medical records, health registries and questionnaires. Information about storage of biological materials and use of individual data retrieved during the whole study for analysis and publishing purposes is also included in the informed consent letter.

Prior to baseline of the intervention, patients are randomized to either intervention group A or control group B in blocks of four. The random number sequence is computer-generated for each hospital. The person who generates the allocation sequence is neither the same person who determines eligibility nor the person that informs patients about their allocated study group. The patients are informed about the study group assignment at the baseline visit. Due to the nature of the intervention, neither the registered dietitians, nor the other research coworkers who meet the patients at the study centre, nor the patients themselves are blinded to group allocation.
**Intensive period of intervention**

The CRC-NORDIET study offers an extensive intervention program for patients in group A, consisting of individual counselling on nutrition and physical activity, grocery discount cards, delivery of free food items, group meetings, printed materials, access to a CRC-NORDIET webpage and contact by telephone and e-mail. The patients in group B are offered the same individual counselling on physical activity as group A, as well as general group meetings. An overview of the intervention program and the instruments used are presented in Table 2 and Table 3, and in Additional file 1.

**Group A: diet intervention**

Colorectal cancer patients experience different disease courses due to different stages at diagnosis, location of tumor, surgical procedure and adjuvant treatment. The diet intervention is therefore designed to meet the patients’ individual needs after surgery. In the initial phase, when symptoms related to cancer and cancer treatment are most common, the dietary focus is mainly on recovery and treatment of symptoms and progressive weight loss. Later, when symptoms and weight loss are treated and under control, and the disease conditions are more stable, the major focus is long-term disease-free living and secondary preventions. In this phase, we emphasize a diet which may dampen chronic inflammation and oxidative stress, fully in accordance with the NFBDG. A number of strategies are implemented to improve compliance to the recommended diet of the CRC patients in group A (see below).

**The dietary recommendations in the CRC-NORDIET intervention**

The NFBDG, published in 2011, was developed to prevent chronic diseases in the general population [32]. These guidelines are based on a comprehensive, systematic review of the evidence linking diet to risk of chronic diseases, including cancer. The guidelines do not provide a detailed diet plan, but define major aspects of the diet (Additional file 2). In the current study, the particular focus will be on the following NFBDG recommendations:

1. daily intake of fruits, berries and vegetables (≥500 g/day)
2. weekly intake of 300-450 g fish
3. daily intake of 70-90 g wholegrains
4. limiting red and processed meat to maximum 500 g/week
5. keeping body weight within normal range of body mass index (BMI)
6. reduce intake of added sugar to < 10 E%
7. reduce salt intake to less than 6 g/day
8. achieving an average of at least 30 min of moderate (3–6 metabolic equivalents (METs)) physical activity per day or 150 min of moderate physical activity per week

The NFBDG can be implemented in different ways. For example, the recommendations of eating 500 g fruits, berries and vegetables every day may include different selections of individual foods, all compliant
to the quantitative advice. However, not all of these foods may dampen inflammation and oxidative stress. Since inflammation and oxidative stress are ubiquitous as common basic pathogenic mechanism, we have selected to compose the intervention not only according to the NFBDG, but also by emphasizing those foods with strongest evidence for dampening low grade chronic inflammation and oxidative stress: We have identified foods and drinks that have high contents of redox-active compounds and/or have antioxidative effects individually or in combination in in vitro models, animal models, clinical trials and/or epidemiological studies [23–26, 28, 29, 37–56] (detailed list with references in Additional file 3):

- Drinks (e.g. coffee, black tea)
- Fruits and vegetables (e.g. onions, broccoli, tomatoes, carrots, pomegranates, garlic, oranges, olives)
- Berries (e.g. blueberries/bilberries, blackberries, and raspberries)
- Nuts (e.g. walnuts, almonds, and hazel nuts)
- Herbs and spices (e.g. thyme, oregano, clove, cinnamon, and rosemary)
- Whole grain (e.g. barley)
- Miscellaneous (dark chocolate)

Furthermore, we have also identified that the following foods and drinks may have anti-inflammatory effects individually or in combination in cell cultures, animal models, clinical trials and/or epidemiological studies (detailed list with references in Additional file 3):

### Table 1 Inclusion and exclusion criteria

<table>
<thead>
<tr>
<th>Inclusion criteria</th>
<th>Exclusion criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary adenocarcinoma colorectal cancer (ICD-10 C18-C20):</td>
<td>Colorectal adenoma, carcinoid, abdominal carcinomatosis or sarcoma</td>
</tr>
<tr>
<td>C18 Malignant neoplasm of colon</td>
<td>Unable to read and understand Norwegian</td>
</tr>
<tr>
<td>C18.0 Caecum</td>
<td>Unable to perceive information and understand the intervention as such due to dementia or altered mental status</td>
</tr>
<tr>
<td>C18.1 Appendix</td>
<td>Unable to follow the dietary intervention due to medical/clinical conditions e.g. total parental nutrition, permanently institutionalized</td>
</tr>
<tr>
<td>C18.2 Ascending colon</td>
<td>Participation in another study in conflict with the intention of the CRC-NORDIET study</td>
</tr>
<tr>
<td>C18.3 Hepatic flexure</td>
<td></td>
</tr>
<tr>
<td>C18.4 Transverse colon</td>
<td></td>
</tr>
<tr>
<td>C18.5 Splenic flexure</td>
<td></td>
</tr>
<tr>
<td>C18.6 Descending colon</td>
<td></td>
</tr>
<tr>
<td>C18.7 Sigmoid colon (sigmoid (flexure)</td>
<td></td>
</tr>
<tr>
<td>C18.8 Overlapping lesion of colon</td>
<td></td>
</tr>
<tr>
<td>C18.9 Colon, unspecified</td>
<td></td>
</tr>
<tr>
<td>C19 Malignant neoplasm of rectosigmoid junction</td>
<td></td>
</tr>
<tr>
<td>C20 Malignant neoplasm of rectum</td>
<td></td>
</tr>
<tr>
<td>TNM stage I-III</td>
<td></td>
</tr>
<tr>
<td>Age 50–80 years old</td>
<td></td>
</tr>
</tbody>
</table>

### Table 2 Instruments used to facilitate compliance in intervention group A during the first 12 months

<table>
<thead>
<tr>
<th>Instruments used to facilitate compliance</th>
<th>Baseline (at study centre)</th>
<th>1 month (at home)</th>
<th>3 months (at home)</th>
<th>6 months (at study centre)</th>
<th>9 months (at home)</th>
<th>12 months (at study centre)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nutritional counselling</td>
<td>Face to face individual</td>
<td>Phone call</td>
<td>Phone call</td>
<td>Face to face individual</td>
<td>Phone call</td>
<td>Face to face individual</td>
</tr>
<tr>
<td>Free-of-charge food</td>
<td>Delivered at the visit</td>
<td>Home delivery</td>
<td>Delivered at the visit</td>
<td>Home delivery</td>
<td>Delivered at the visit</td>
<td></td>
</tr>
<tr>
<td>Information/courses</td>
<td>Folder with information on the study and the study instruments</td>
<td>Inspiration day and Cooking course</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Discount card</td>
<td>Discount card (25% discount on healthy foods)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRC-NORDIET Webpage/e-mail</td>
<td>Login-restricted webpage access and e-mail communication</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Physical activity</td>
<td>Access to free training facilities (&quot;Pusterommet&quot;)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reports from non-biological measurements</td>
<td>Reports sent to the patients after every visit</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
During the 15 year intervention period, these foods and drinks are gradually implemented in the advice to group A.

While these antioxidant- and phytochemical rich foods are advised as part of a balanced diet according to the NFBDG, patients were advised not to take any antioxidant supplements [55, 57].

**Intervention strategies**

The following instruments are used to facilitate compliance to the intervention in group A.

1. Individualized nutrition counselling by a registered clinical dietitian.

The nutritional counselling aims to meet the individual nutritional needs as well as educate the patients on how to change dietary habits in accordance with the NGBDG. In order to individualize the dietary advice, the registered clinical dietitian performs a comprehensive evaluation in each of the meetings (Fig. 1). The Patient-Generated Subjective Global Assessment (PG-SGA) tool [58] is used to assess nutritional status and nutritional impact symptoms. Weight and height measured the same day is used to calculate BMI, and current weight is compared with previous weight measurements to calculate weight changes. The presence of stoma is recorded as well as treatment status (i.e. whether or not the patient receives adjuvant treatment). Dietary intake is assessed by 24-h recall (at baseline). In addition, the registered clinical dietitian characterizes the patient’s current diet in relation to the NFBDG, and record use of supplements.

When the nutritional evaluation is completed, the patient receives dietary advice based on nutritional status and weight history. If the patient is malnourished or at risk of malnutrition (i.e. PG-SGA category B or C), dietary counselling primarily focuses on improving nutritional status by treating symptoms, ensuring an adequate energy and protein intake, and to prevent further nutritional deterioration. In terms of progressive weight loss, patients with PG-SGA B or C with BMI >20 are recommended to stabilize their body weight. Patients with BMI < 20 are recommended to increase their body weight within the range of a normal BMI, determined in the current study as BMI 20–27 for patients aged 50–80 years [59, 60]. Well-nourished patients (i.e. PG-SGA category A) with BMI >27 are recommended to decrease their weight within normal BMI range. The recommended change (weight gain or weight reduction) is set to maximum 3 kg in 6 months to ensure an optimal change in body composition.

If the patient is evaluated as well-nourished (PG-SGA A), the dietary counselling primarily focuses on the NFBDG. Examples of week menus are used to illustrate examples of foods and amounts to be eaten in adherence with the NFBDG. Food alternatives are given to adjust the week menu to the patient’s personal eating habits and preferences.

Motivation to change dietary habits in accordance to the NFBDG is recorded by asking whether the patient considers herself/himself to be either “very motivated”, “motivated”, “less motivated” or “not motivated”. When one of the last two categories is present, the registered clinical dietitian explores the potential to increase motivation by using techniques from Motivational Interviewing (MI) [61]. The degree of motivation (“very motivated”, “motivated”, “less motivated” or “not motivated”) is taken into account in each of the counselling sessions.

Each of the nutritional consultations is intended to result in a few dietary goals in agreement with the patient. It is emphasized that the patient defines her/his personal goals to increase the chances that he or she will succeed in changing dietary habits. The registered clinical dietitian aims at encouraging the patient to achieve these goals and the goals will be revised at next session. The telephone-based counselling in between the meetings at the study centre focus at monitoring the patient’s body weight status, dietary pattern according to the predefined goals and motivational status. In addition to the scheduled consultations at the study centre and by telephone, the patients have the opportunity to contact the registered clinical dietitian by e-mail during the entire study period.
intervention period. The same registered clinical dietitian follows the patient during the entire intervention period, when possible.

2. Discount card (25% discount on healthy foods)

The patients in the intervention group are offered a discount card from the retailer company, "Norgesgruppen", which is Norway's largest enterprise within the grocery market, with a market share of 40%. The discount card can be used within the first year of the intervention and gives a 25% discount on all fresh vegetables, fruit, berries and fish and on all food items marked with the keyhole symbol, which is used by the health authorities to label food that is considered the most healthy within its food category [62]. The discount card can be used in all food stores and supermarkets within "Norgesgruppen".

3. Delivery of specific foods

The CRC-NORDIET is sponsored by several food producing companies with free food items, specifically selected in accordance with the anti-inflammatory and antioxidant-rich foods emphasized in this study, such as juice, garlic, tomato juice, fish, coffee, tea, cereals, whole grain bread, oils etc. At all visits to the study centre, the patients in group A receive a bag containing a mixture of these food items. In addition, they receive a box with free food items delivered to their homes two times during the intensive period of the intervention.

4. CRC-NORDIET website

The patients in the intervention group get access to a login-restricted, dynamic website with detailed information about the NFBDG, portion sizes of recommended intake of fruits and vegetables and whole grain, food recipes, examples of week menus, dietary advice for treatment-related symptoms and advice on physical activity. In addition, information about the CRC-NORDIET study and contact information for the study organizers are given. The website is continuously updated.

5. Printed materials

The patients in the intervention group receive printed materials at the first visit to the study centre and at all follow-ups to ensure that also patients who do not use the internet get all relevant information.

6. Cooking course

During the first 6 months of the intervention, each patient in group A is offered a one-day cooking course. This course is led by a registered clinical dietitian who follows a protocol developed for the CRC-NORDIET intervention. The aim of the cooking course is to give the patients practical experience in making healthy dishes and to introduce healthy choices when shopping for food. The course consists of a one hour lecture on the NFBDG and how to implement these guidelines in daily cooking. All recipes can also be found on the CRC-NORDIET web site.

7. Physical activity

The CRC-NORDIET study has an agreement with "Active against cancer" [63], a non-governmental non-profit organization founded in 2007. The organization operates a free training studio ("Pusterommet") for cancer patients at several hospitals in Norway. The physical therapists working at these studios are instructed to give individualized advice for exercises during and after cancer treatment. The CRC-NORDIET patients are encouraged to utilize this offer.

Moreover, the CRC-NORDIET patients are advised to practice moderate physical activity for at least 30 min per day, or 150 min per week, and they receive a booklet on how to be physically active in daily life. In addition, they are recommended to use local facilities, including swimming pool, health training centres and walks in their neighbourhoods.

8. Inspiration day

The patients in Group A are invited to an inspiration day within the first 6 months of the intervention. The day opens with a 45 min lecture about the aim and background of the CRC-NORDIET study by the project leader, with special focus on the NFBDG. The patients are shown examples of different portion sizes of fruits and vegetables, nuts, whole grain products, the food-dish-model, and have the opportunity to talk to registered clinical dieticians. The last part of the inspiration day focuses on physical activity, and starts with a lecture about physical activity incorporated in daily life. The patients also meet the physical therapists from "Pusterommet". The meeting ends with a lunch and a quiz about physical activity, and each patient receives a pedometer as an incentive to be physically active.

9. Written reports

The patients receive reports from the non-biological samplings (e.g. anthropometric measurements and blood pressure, described in detail in the following section) performed at the three time points during the intensive intervention period (baseline, 6 and 12 months after treatment).
baseline), as well as a one-year report showing the development during the last year. Reports from the physical activity monitors are given to the patients after the first intensive year of intervention.

**Group B: control group**

1. **Physical activity**

   Patients in the control group receive the same basic advice on physical activity as well as free access to the training studio as patients in the intervention group (see above).

2. **Inspiration day**

   The inspiration day is structured identically as for group A, except for the session focusing particularly on diet, which is excluded in the inspiration day for group B.

3. **Dietary information**

   The patients in group B receive a booklet with basic dietary advice at baseline. In contrast to the intervention group, the control group receives no individualized dietary advice adapted to their eating habits and preferences. If they seek counselling concerning symptoms related to cancer or cancer treatment, the registered clinical dietitians provide dietary advice based on information from booklets and other printed materials already available in the hospitals. This information and dietary advice is considered as part of the standard care.

4. **Written reports**

   The patients in group B receive written reports similarly as group A after all visits during the intensive intervention period.

**Moderate intervention during maintenance period (year 2–15)**

During the maintenance period, which starts after the first intensive year and lasts for 14 years, both groups receive reports (e.g. anthropometric measurements and blood pressure) following every visit at study centre (year 3, 5, 7, 10 and 15).

The patients in group A are invited to an inspiration day every year during moderate period of intervention. The aim of these meetings is to maintain the focus on foods dampening inflammation and oxidative stress and the NFBGD, and to encourage the patients to continue following the guidelines in a long-term perspective. In addition, group A are offered dietary counselling at each visit at the study centre, as well as a telephone counselling by the registered clinical dietitians once a year. They also have access to the CRC-NORDIET webpage which is continuously updated with information and encouragements (e.g. recipes, nutrition information, motivational tips and relevant popular reports from nutritional sciences) until the end of study participation. An overview of the instruments used during the maintenance period is presented in Table 4.

**Assessment of primary outcomes**

Several registries and medical records will be used for assessment of primary outcomes. The registries and time points for primary outcome assessment are summarized in Table 5.

**Questionnaires, biological samplings and measurements**

Group A and Group B are undergoing equal regimes of measurements and biological samplings at all visits (Additional file 4). All patients are also asked to complete several questionnaires regarding demographic information, dietary intake, health status and physical activity (described below) (Additional file 4). The questionnaires administered at baseline of intervention are also completed at 6 months and 12 months follow-up. After the first year, the patients are invited to the study centre for questionnaires, biological samplings and measurements 3, 5, 7, 10 and 15 years after baseline. In addition to the visits to the study centre during the maintenance period, finger prick blood sample equipment (dried blood-spot cards) and questionnaires are sent to the patients’ home at certain time points and subsequently returned to the study centre.

**Demographic information**

A short questionnaire is used to assess demographic characteristics including age, gender, marital status, ethnicity, level of education, working status, family history of CRC or other type of cancer.

**Table 4** Instruments offered to the respective groups during maintenance period of intervention

<table>
<thead>
<tr>
<th>Instruments</th>
<th>Time points</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dietary counselling at study centre (Group A)</td>
<td>2, 4, 6, 8, 9, 11, 12, 13, 14 years, 15 years</td>
</tr>
<tr>
<td>Dietary counselling by telephone (Group A)</td>
<td>X</td>
</tr>
<tr>
<td>Inspiration day with extended diet session (Group A)</td>
<td>X</td>
</tr>
<tr>
<td>CRC-NORDIET Website/e-mail (Group A)</td>
<td>X</td>
</tr>
<tr>
<td>Reports from non-biological measurements (Group A and B)</td>
<td>X</td>
</tr>
</tbody>
</table>
Assessment of dietary intake

Semi-quantitative food frequency questionnaire (FFQ)
The semi-quantitative 282-item FFQ used in CRC-NORDIET is designed to assess habitual diet over the preceding year, including both frequency of intake and portion sizes. The FFQ is described and validated elsewhere [64, 65].

Compliance questionnaire
The compliance questionnaire is a semi-quantitative short 63-item FFQ, developed within this study and designed to assess the dietary intake (grams per day) and physical activity (minutes per day) for the last 1–2 months. The questions correspond to the food groups and the recommendations regarding physical activity of the NFBDG. The questionnaire will be validated within the first period of study.

Food records
Food intake is recorded by using a 7-days weighed food record. The patients are provided with a food diary and a digital scale, and are instructed on how to weigh and record all foods and beverages consumed during a period of seven days. The food diary include all days of a week, and can either record seven consecutive days or be divided into two periods of three and four days of a week. The food records are performed in a subgroup of patients (will be published elsewhere).

24-h recall
A registered clinical dietitian performs a 24-h recall at baseline by asking the patients in the intervention group in details about the intake of foods and drink during the past 24-h period. The 24-h recall is performed only in intervention patients since it is an integrated part of the nutritional counselling.

Assessment of physical activity and function

Recording of daily physical activity
The physical activity monitor SenseWear Mini Armband (BodyMedia, Pittsburgh, Pennsylvania, USA) [66] is used to record daily physical activity, inactivity and energy expenditure during seven consecutive days among all patients in both study arms at all visits. The armband monitors physiological data such as heat flux, galvanic skin response, 3-axis accelerometer and skin temperature. All data are retrieved from the armband to the computer with the SenseWear Professional Software [66]. The participant are instructed how to use the armband, and return it in a stamped envelope to the CRC-NORDIET study at the end of the test period. The armband is pre-programmed with the co-predictors such as weight, height, age, gender, smoking status (smoker/non-smoker) and placed around the non-dominant arm.

Self-reported physical activity
The patients are asked to complete a questionnaire regarding frequency, intensity and duration of their daily physical activity, as well as duration of sedentary time. These questions are based on the questionnaire from the HUNT 3 study in Norway [67].

6-min walking test
Patients are invited to a 6-min walk test (6MWT) at several time points. The test is performed indoors, along a long, flat, straight, enclosed corridor with a hard surface. The walking course is 30 m in length, and cones mark the turnaround points. A countdown timer (or stopwatch) is used to record the time of the test. Prior to the test, the researcher measures the blood pressure of the patient. In addition, the pulse is monitored before, during and after the test. The patients are asked to grade its level of shortness of breath and the level of fatigue by using the Borg scale 6-20 before and after the test. Total length of walking (in meters) is recorded during 6 min of time.

Sit-to-stand test
The test is performed by the use of a straight back chair with a solid seat at the height of 44 cm. The patients are instructed to sit on the chair with arms folded across their chest, and then to stand up and sit down as quickly and frequently as possible within 30 s, keeping both arms folded across the chest. The number of stands during this period is counted.

Handgrip strength
Hand-grip strength is measured by the MAP 80 K1 Hand grip dynamometer (KERN & SOHN GmbH, Balingen, Germany) and measured as described in the manufacturer’s protocol [68]. The maximal strength of hand grip (kg) is recorded. For women and men, a 40 kg- and 80 kg-spring is used, respectively. The grip strength is measured with one punch and repeated three times on both hands. The maximum handgrip strength on both left and right hands are recorded.

Assessment of nutritional status

Patient-Generated Subjective Global Assessment (PG-SGA)
Nutritional status is measured by using the scored PG-SGA [58], a nutritional assessment tool specifically developed and validated for cancer patients. A
translated (Norwegian) version is used. Both the global categories well-nourished (A), moderate malnourished (B) and severe malnourished (C), as well as the numerical scoring system are used to characterize the nutritional status.

**Anthropometric measurements**

**Body weight** Body weight (kg) is measured by using a non-slip Marsden M-420 Digital Portable Floor Scale (Marshden, Rotherham, South Yorkshire, United Kingdom) or a digital wireless measuring station for height and weight, Seca 285 (Seca, Birmingham, United Kingdom) [69]. Measurements are performed with light clothes and without shoes. Body weight is recorded with 2 decimals and the kind of clothing is recorded.

**Height** Height (cm) is measured using either a mechanical height rod (Kern MSF-200, [68]) or a digital wireless stadiometer (Seca 285 [69]). The height is recorded with one decimal precision.

**Waist and hip circumference** Waist circumference is measured at the midpoint between the lower margin of the last palpable rib and the top of the iliac crest, whereas the hip circumference is measured around the widest portion of the hips. Waist and hip circumference are used to calculate the waist hip-ratio (WHR) which is a well-established indicator of abdominal fatness [70].

**Body composition analysis**

**Bioelectrical impedance analysis** (BIA) BIA is performed under standardized conditions by the use of BIA 101 (SMT Medical, Würzburg, Germany) that applies a current of 0.8 μA at a frequency of 50 kHz. Four skin electrodes are placed on hand and foot of the patients when lying in supine position. All measurements are conducted on the patients’ right side as instructed by the manual. Resistance (Rz) and reactance (Xc) are used in appropriate and validated equations to calculate body composition compartments such as fat mass, fat free mass and muscle mass. In addition, BIA is also performed with Seca mBCA515 (Seca, Birmingham, United Kingdom) [71]. Patients carrying a pacemaker are excluded from the BIA measurements.

**Dual-energy x-ray absorptiometry** (DXA) The Lunar iDXA (GE Healthcare Lunar, Buckinghamshire, United Kingdom) is used to measure bone mineral density and body composition, including quantification of visceral fat.

**Computed tomography** (CT) CT images taken routinely for clinical purposes are used for body composition analysis, i.e. quantification of fat (visceral, subcutaneous and intermuscular adipose tissue) and skeletal muscle. The images are analysed using the Sliceomatic software, version 4.3 (Tomovision, Montreal, Canada). The third lumbar vertebra (L3) is chosen as standard landmark since skeletal muscle, lean tissue mass and adipose tissue at this level are significantly correlated to whole-body tissue in healthy adults [72].

**Blood pressure** Blood pressure (BP) is measured with the digital blood pressure patient monitor CareScape V100 (GE Healthcare, Fairfield, USA) and performed by trained staff following the clinical procedure as described by the manufacturer [73]. After a 5 min resting period in a silent room, BP is measured four times on the non-dominant arm with intervals of one minute.

**Biobank** A variety of biological samples will be collected at different time points during the study and will be used for the purposes of measuring surrogate outcomes, biomarkers of food intake and for identification of phenotypes associated with different responses to the intervention.

**Venous blood samples** Overnight fasting blood samples are taken between 07.30 and 10.30 at the study centre by a trained technician. BD Vacutainer® (Becton, Dickinson and Co, Franklin Lakes, NJ, USA) tubes are used to collect ethylene diamine tetraacetic acid (EDTA) samples (no. 367861 and 366643), serum samples (no 368774), lithium heparin samples (no 367526), and citrate samples (no 369714).

Serum tubes are placed in room temperature for 30 min. Serum, EDTA and heparin samples are centrifuged at 1500 g, 10 min, 15 °C. Serum, plasma and red blood cells are aliquoted, and immediately stored in at −80 °C until further analysis. Whole blood from EDTA samples are also aliquoted for e.g. DNA extraction and DNA damage/repair analysis. The buffy coat from the heparin samples are either frozen at −80 °C for later analysis or used to obtain isolated peripheral blood mononuclear cells (PBMC) through Percoll centrifugation. The isolated PBMCs from heparin samples are used for ex vivo experiments. Two citrate tubes are kept 1 h respectively at 4 °C and room temperature before centrifugation (2500 g, 15 min, 4 °C) to obtain core plasma, plasma and red blood cell aliquots that are stored at -70 °C. One citrate tube is centrifuged (2500 g, 15 min, 4 °C) within 30 min of sampling, and core plasma is stored at −80 °C for further analysis of thromboembolic factors. The citrate buffy coats are used to obtain isolated PBMCs for the study of DNA repair and DNA damage. PAXgene Blood RNA Tubes (cat.no 762115, PreAnalytiX, Hombrechtikon, Switzerland) are used as source for total blood RNA. The tubes are kept 2 h at
room temperature before they are frozen at -20 °C for 24 h and subsequently transferred to -80 °C until time for RNA isolation.

**Isolation of buffy coats from EDTA samples** The EDTA buffy coats are re-solved in 9% NaCl (cat.no 586564, B.Braun Melsungen AB, Melsungen, Germany) solution before added on top of 4 ml Lymphoprep (cat.no 1114545, Axis-Shield, Oslo, Norway) in a 15 ml tube for centrifugation (20 min RT 400 g) to isolate PBMCs which are further used for a chromatin crosslinking procedure. The crosslink procedure for preparing the cell pellets for ChIP-chip analysis are performed as follows: Firstly, PBMCs are allowed to crosslink with 1% formaldehyde (final concentration) for 10 min at room temperature, adding glycine (0.125 M) for 10 min at room temperature to stop the crosslinking process. After washing the cell pellets twice with 10 mL of ice-cold 1 x PBS the pellets are immediately stored in 2 ml plastic tubes at -80 °C until proceeding further with protocols for Chip-on-Chip analysis at a later time point.

**Finger prick blood samples** Finger prick blood samples for analysis of e.g. biomarkers of dietary intake, oxidative stress and oxidative damage are collected by the dried blood spots (DBS) method as previously described [74]. DBS cards (2 cards per patient) are allowed to dry in room temperature for 2 h and are frozen at -80 °C in airtight aluminium bag with a desiccant until further analysis.

**Urine samples** Biomarkers of food intake, oxidative stress and other risk factors related to the progression of CRC will be measured in urine. Urine samples are collected from a subpopulation several times during the intervention by the methods as previously described [75–77].

**Faeces samples** Microbiota and biomarkers related to CRC will be measured in faeces samples which are collected from a subpopulation several times during the intervention. The patients will receive a specific faeces sample tool kit and are asked to collect the sample at home and mail it to the study centre. Sampling and analysing of the faeces samples will be performed by following the procedure as described by Naseribafrouei [78].

**Tumour tissue** Molecular signatures in CRC tumours that are linked to inflammation, oxidative stress and energy balance have been shown to predict response to lifestyle intervention. Characterization of tumor markers will be performed by immunohistochemistry, PCR, sequencing and q-PCR (to be published elsewhere). Furthermore, we will study whether tumor markers predict response to the dietary intervention. Samples of tumor tissue are collected at surgery in collaboration with the hospitals. Molecular signature data are also obtained from the CRC biobank project at the Oslo University Hospital.

**Oral glucose tolerance test** Prior to the oral glucose tolerance test, the patient is fasting for at least 8 h. Blood samples (serum and PAX tubes) are taken, and blood glucose is measured with a blood glucose meter [79]. The patients are asked to drink 75 g of glucose (D (+)-Glucose (product number: 1370485000, Merck-Millipore Corp, Darmstadt, Germany) in 4 dl of boiled water. The glucose liquid is expected to be consumed total within 5 min. Blood samples will be taken after 2 h. Exclusion criteria for oral glucose tolerance test are Diabetes Type I, use of insulin and/or fasting blood glucose level exceeding 10 mmol/l.

**Health related quality of life and fatigue** Quality of life will be self-reported and measured using the generic, multi-purpose-form questionnaire for Health related quality of life (HRQOL) called Short form (SF) health survey consisting of 36 items (SF-36) [80]. The 36 items are categorized into eight multi-item scales; 1) physical functioning, 2) role physical, 3) bodily pain, 4) general health, 5) vitality, 6) social functioning, 7) role emotional and 8) mental health as well as a single-item measuring health transition during the last year. The data will first be standardized in order to compare results across studies [80] and then recoded according to a syntax developed by Loge et al [81].

A validated generic fatigue questionnaire (FQ) is used to assess the patients subjective fatigue status (11 items) and the duration and extent of fatigue (2 items) [82]. The FQ asks about fatigue symptoms experienced during the last month compared to how the subject felt when she/he was last feeling well [82–85]. Each item has four response-choices [82]. The scoring of each response is based on a Likert- (0, 1, 2, 3) and a dichotomized (0, 0, 1, 1) scale. The latter is only used for case definition. The total sum of the Likert-scores is designated total fatigue (TF) where higher scores imply more fatigue.

**Assessment of new morbidity of diet-related chronic diseases and adverse events** New morbidity of diet-related chronic diseases arising after CRC diagnosis (e.g. ischemic coronary heart disease, cerebrovascular disease, thromboembolic disease, diabetes, hypertension and chronic obstructive pulmonary disease) will be collected from the national health registries in Norway, a comorbidity questionnaire developed for this study designed to assess comorbidity based on data from the third Norwegian population health study (HUNT 3) [67], and from medical records. These
data will be supplemented by data on drug use from the Norwegian Prescription Register. Adverse events are recorded based on the Common Terminology Criteria for Adverse Events (CTCAE, Version 4.0) [86].

Sample size

Calculation for primary outcomes

The sample size calculations are based on assuming a Weibull distribution for the survival times in both arms. We further assume a constant hazard ratio for the intervention effect over time and that we have the same follow-up of 5, 10, or 15 years, respectively, for all patients. Sample sizes required to achieve a statistical power of 80% and significance level of 5% were calculated with computer simulations using the spower function in R (version 3.2.0) package Hmisc version 3.17–0. Survival rates in the control group [2, 87] and expected reduction in mortality rates in the intervention group are taken from the literature (see Discussion, [88–93]). With a 68% 5-year OS in the control group, we have 80% power to detect a 25% reduction in mortality due to the intervention (corresponding to a hazard ratio of 0.71). The required total sample size is then 500 (250 in each study group) (Table 6).

Moreover, sample size calculation based on 25% reduction in events of DFS after 5 years of surgery (59% 5-year DFS in the control group), we have 80% power to detect HR of 0.70, with 190 patients in each group (Table 6).

Table 6 Sample size in each group (n) and hazard ratios (HRs*) for selected scenarios of reduction in mortality by intervention. The power is 80% and significance level 5%

<table>
<thead>
<tr>
<th>Primary outcome</th>
<th>Reduction in mortality by intervention</th>
<th>Survival rates in the control group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20%</td>
<td>25%</td>
</tr>
<tr>
<td>DFS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 years</td>
<td>320</td>
<td>190</td>
</tr>
<tr>
<td>10 years</td>
<td>240</td>
<td>140</td>
</tr>
<tr>
<td>15 years</td>
<td>180</td>
<td>120</td>
</tr>
<tr>
<td>OS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 years</td>
<td>390</td>
<td>250</td>
</tr>
<tr>
<td>10 years</td>
<td>280</td>
<td>180</td>
</tr>
<tr>
<td>15 years</td>
<td>210</td>
<td>140</td>
</tr>
</tbody>
</table>

* HR = hazard ratio of intervention versus control, which corresponds to the assumed survival rate in the control group and assumed reduction in mortality by intervention.

DFS disease-free survival, OS overall survival

Stratified and subgroup analysis

All of the power calculations are based on a heterogeneous population of CRC patients.

Since post-surgery treatment may vary, and colon versus rectum cancer may respond differently to the diet intervention, we will also perform stratified statistical analysis. It is not known whether treatment effects are different in these subgroups. These stratified analyses will be conducted with primary outcomes at the later time-points in the study and at all time-points to assess mean differences between the control and the intervention groups in biomarker analysis, as these data normally require fewer patients per group.

Statistical analysis

Data will be analysed using SPSS (IBM SPSS Statistic 22). For the survival outcomes (primary outcome 1 and 2, and secondary outcomes I-VI) tests will be performed to compare survival rates between the control and intervention groups at 5, 10, and 15 years after baseline. Survival probabilities will be estimated with the Kaplan-Meier method. Cox proportional hazards models will be used to identify prognostic and predictive biomarkers for survival outcomes.

For non-survival secondary outcomes, parametric or non-parametric tests for two-group comparisons will be used to assess group differences at individual time-points. In addition, mixed effect models for longitudinal data and regression models will be used to evaluate association and change over time in dietary intake, nutritional status, body composition, molecular tumor characteristics, physical function and activity, quality of life, fatigue and treatment related outcomes and to examine differences between the intervention and control groups. All statistical tests are performed as two-sided tests. Effects are considered statistically significant if \( p < 0.05 \).

Discussion

The primary aims of the CRC-NORDIET are to study whether a healthy diet rich in anti-inflammatory and antioxidant-rich foods and based on the NFBDG can improve DFS and OS in CRC patients. To our knowledge, this is the first randomized controlled trial designed to investigate the effect of a dietary intervention on these outcomes in CRC patients, and to investigate the potential role of diet in dampening of inflammation and oxidative stress in these patients. The multiple strategies used to achieve compliance to the intervention during the first year, followed by the 14 years maintenance and follow-up period make the design of this intervention unique.

Data on role of diet on disease outcomes and survival in CRC survivors is limited. To date, several cohort studies, but no RCTs have investigated the effects of food-based dietary interventions on these outcomes.
Data from a US cohort study with stage III colon cancer patients suggest that high intakes of red and processed meat, fat, refined grains and dessert, i.e. a Western dietary pattern, after diagnosis are associated with a significantly reduced disease-free and OS [13]. Similar findings are reported in a Canadian cohort study with CRC patients staged I-III, where patients with the highest intake of processed meat the previous year before diagnosis had an 82% increased risk of recurrence or death compared with patients with the lowest intake [15].

Prospective cohort studies have consistently reported that physical activity after colorectal cancer diagnosis reduces risk of mortality. In a meta-analysis of six prospective cohort studies, including 7522 CRC survivors, the authors observed that the most physical active survivors had a 42% lower risk of total mortality compared to those who were least active. The risk reduction of cancer-specific mortality was 39% [94]. No RCT has so far confirmed that physical activity impacts mortality in CRC survivors.

Interventions designed to investigate the effect of diet separated from other lifestyle factors (smoking, physical activity, weight regulation) are needed in order to investigate whether there is a causal relationship between diet and survival as well as disease-related outcomes. Our intervention is intended to change the dietary habits towards a diet in agreement with the NFBDG. These dietary guidelines are developed to prevent chronic diseases, including cancers, in the general population. Several large cohort studies have shown that there is a consistent inverse association between adherence to cancer prevention guidelines and cancer-specific and all-cause mortality [89, 95]. Among cancer survivors, reduction in total mortality between highest versus lowest score in adherence to diet recommendations has been documented in five different cohort studies, ranging from 24% to 36% (follow-up period from 3.7 to 13.6 years) [88–93]. Association of adherence to American Cancer Society guidelines and reduction in death attributed to cancer has been shown to be 25 and 26% in men and women, respectively [89]. Hastert et al documented an association of adherence to the WCRF/AICR guidelines and reduction in cancer-specific mortality of 61% in respondents with the highest compared to the lowest WCRF/AICR score (follow-up time of 7.7 years) [95].

Furthermore, NFBDG include advice regarding red and processed meat, dietary fibre, dairy products and garlic, all of which are related to risk of CRC. Whether these dietary factors also may have effect on survival and disease outcomes, remain unclear. With improvement in cancer survival, these perspectives are increasingly important. The main objective of the present study is to test if diet will improve survival and cancer-related outcomes, mediated through reduced inflammation and oxidative stress. To strengthen this assumption, we have chosen to select specific foods within the NFBDG which have been identified as anti-inflammatory or antioxidant-rich in previous preclinical and clinical studies [23–29, 37–39, 41–48, 53, 55, 96].

Four aspects that may be of importance for achieving lifestyle changes and facilitate compliance to the intervention: 1) timing of intervention, 2) choice of motivational approach to achieve lifestyle changes, 3) duration of intervention, and 4) use of incentives and methods to achieve sustainable changes. Previous studies have shown that cancer patients in general are particularly motivated to change dietary habits at the time of diagnosis, often reported as the teachable moment [97–99]. Interventions designed to include this teachable moment have shown to be successful [97–99]. In our trial, we introduce the dietary intervention within a few months from surgery, thereby expecting we reach the patients within the time frame of this teachable moment. Furthermore, principles from MI [61] are implemented in each of the dietary counselling sessions by trained registered clinical dietitians. Previously published trials that have succeeded in changing lifestyle behaviours in cancer patients are based on theoretical frameworks and theories, including social cognitive behaviour therapy and use of MI. It is emphasized that the patient defines her/his own goals to increase the chances that he or she will succeed in changing dietary habits. We suggest that it is important to focus on a few realistic goals at the time, instead of aiming at changing the whole diet immediately. In addition, follow-up by the same registered clinical dietitian during the entire intensive intervention period may be of importance for the commitment to the intervention goals.

In order to increase the chances of sustainable lifestyle changes and compliance to the intervention, our intervention consists of a one year intensive period and a subsequent maintenance period which lasts for until 14 years. Taking into account that the teachable moment may vary among the patients and it may take time to establish new sustainable dietary habits, the inclusion of a maintenance period will probably be beneficial with regard to an increased long-term adherence to the intervention. Previous published trials that have failed in compliance from the patients may have had too short time frame of diet intervention.

Different strategies are reported to be effective in promoting lifestyle changes in cancer survivors [14, 97, 98, 100, 101]. Interventions focusing on individual counseling [14, 98, 100], and also interventions with a mixed strategy of individual in-person counselling, telephone counselling and mailed materials [97, 101] have been shown to be effective in health behaviour change among CRC survivors. Thus, during the first year, the CRC-
NORDIET study offers individualized counselling, free foods, a discount card on healthy foods, access to a login-restricted web page, printed materials, cooking courses and inspiration day, which may all be effective incentives to follow the NFBDG.

In placebo-controlled RCTs, an intervention is tested by comparing one group of individuals who receive the intervention with a control group who receives a placebo. This type of placebo-controlled RCT is most often not possible when studying food-, or exercise-based interventions, since placebo-foods or placebo-exercise do not exist. In addition, no food based intervention can be analysed thoroughly without considerations regarding energy intake and energy expenditure. We have therefore selected to give the intervention group and the control group the same advice on physical activity. We include careful monitoring of physical activity to control for any confounding effects of physical activity. Of ethical reasons, we also include standard dietary advice (i.e. standard clinical care) in the control group, as well invitations to group meetings and feed-back reports on health status. Thus, while the control group in the present study is not identical to a placebo group, this particular study design was used in order to isolate the effect of diet intervention on CRC patients, and to reduce drop-outs from the control group, which is a common concern in long-term intervention trials.

Sample size estimation is not straightforward in RCTs with complex diet intervention and long term hard outcomes. This is especially the case when no similar trials have been previously published. By using the best available information from scientific literature and Norway Cancer Registry on survival rates in the control group and expected reduction in mortality rates in the intervention group, we have performed power calculations on the two primary outcomes after 5, 10 and 15 years after baseline. We conclude that 250 patients in each group would give us a reasonable chance (at 80% power) to detect any significant effects (see Methods and Design section for details) after 5, 10 and 15 years.

In a similar study, testing the effects of two different 6 months adjuvant cytostatic protocols (i.e. the MOSAIC study [102]), 2246 patients who had undergone curative resection for stage II and III colon cancer, were recruited. After a median follow-up for 38 months, fewer cancer-related events was observed in the alternative treatment group compared to the standard treatment group (HR 0.77, 0.002). The main reason for the lower number of patients required in our CRC-NORDIET study compared to the MOSAIC study is due to an older population with more expected events (50–80 years versus 19–75 years) and a longer follow-up time (10 and 15 years versus 3 years).

We have also performed a number of power estimations on secondary outcomes (data not shown). In general, the CRC-NORDIET study is expected to have enough statistical power to detect significant effects in a majority of these intermediate outcomes (to be published in relevant reports). These power calculations on primary and secondary outcomes are also supported from the RCTs with physical activity intervention in CRC and breast cancer patients; Friedenreich and Courneya detected significant effects on intermediate outcomes (e.g. inflammation biomarkers) as well as disease outcomes in RCTs with 200–250 patients per group [103–105].

Conclusion and perspectives
The CRC-NORDIET study investigates whether a diet aimed at dampening inflammation and oxidative stress and in full accordance with the NFBDG will improve survival and disease outcomes in CRC patients. This RCT is unique in several aspects related to the interventions as well as outcomes. Since previous research on the role of diet for CRC survivors is limited, the study is important in order to improve health outcomes and survival in this population.

Additional files

- **Additional file 1:** The CRC-NORDIET intervention program (DOC 42 kb)
- **Additional file 2:** Summary of the 13 recommendations of the Norwegian food-based dietary guidelines (NFBDG) (directly translated from the NFBDG) (DOCX 23 kb)
- **Additional file 3:** Detailed list of foods and drinks with high contents of redox-active compounds and/or antioxidative effects (DOCX 22 kb)
- **Additional file 4:** Questionnaires, biological samplings and measurements (DOC 43 kb)

Abbreviations

- 6MW: 6-min walking test; ACS: American Cancer Society; AICR: American Institute of Cancer Research; BIA: Bioelectrical impedance analysis; BMI: Body mass index; BP: Blood pressure; CIMP: CpG island methylator phenotype; CRC: Colorectal cancer; CRP: C-reactive protein; CT: Computed tomography; CTCAE: Common terminology criteria for adverse events; CVD: Cardiovascular diseases; DBS: Dried blood spots; DFS: Disease-free survival; DNA: Deoxyribonucleic acid; DXA: Dual-energy x-ray absorptiometry; EDTA: Ethylene diamine tetraacetic acid; FFQ: Food frequency questionnaire; FQ: Fatigue questionnaire; HbA1c: Glycated hemoglobin A1c; HR: Hazard ratio; HQL: Health-related quality of life; HUNT: Helseundersøkelsen i Nord-Trøndelag; ICD: International classification of diseases and related health problems; IL: Interleukin; L3: Third lumbar vertebra; LDL: Low density lipoprotein; METs: Metabolic equivalents; MI: Motivational interview; NFBDG: Norwegian food-based dietary guidelines; OS: Overall survival; PBMC: Peripheral blood mononuclear cell; PG-SGA: Patient-Generated Subjective Global Assessment; RCT: Randomized controlled trial; RT-PCR: Real-time quantitative reverse transcription polymerase chain reaction; SF-36: Short form-36; TNFα: Tumor necrosis factor alpha; TNM: Tumor Node Metastases; WCRF: World Cancer Research Fund; WHR: Waist hip-ratio

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Availability of data and materials
Anonymized data resulting from this study may be available upon request by corresponding author.

Authors’ contributions
SHi and RB had a main responsibility for writing the manuscript. SHi, JP, ASK, SÅB, MTE, GW, IE, AF, MBV, MZ, SS and RB contributed to the study design and protocol. RB is the principal investigator. All authors contributed to the writing and approval of the final manuscript.

Competing interests
RB is a shareholder in the company Vitas AS. The authors declare that they have no competing interests.

Consent for publication
Not applicable.

Ethics approval and consent to participate
This study is approved by the Regional Committees for Medical and Health Research Ethics (REC Protocol Approval 2011/836) and by the data protection officials in Oslo University Hospital and Akershus University Hospital. All biological materials are stored in a biobank at University of Oslo. The biobank will expire in 2040 (according to the REC approval). The study is registered on the National Institutes of Health Clinical Trials (www.ClinicalTrials.gov; Identifier: NCT01570010). Written informed consent to participate has been obtained from the patients enrolled in the CRC-NORDIET study.

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References
### Additional file 1. The CRC-NORDIET intervention program

<table>
<thead>
<tr>
<th></th>
<th><strong>Intervention group</strong></th>
<th><strong>Control group</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Dietary counselling by a registered clinical dietician</td>
<td>Individual counseling at all visits at the study center.</td>
<td>No dietary intervention, only general dietary advice as part of the standard care</td>
</tr>
<tr>
<td></td>
<td>Telephone dietary counseling between visits during the first year of intervention.</td>
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<tr>
<td></td>
<td>Telephone dietary counseling once a year during the maintenance period of intervention</td>
<td></td>
</tr>
<tr>
<td>Discount-card</td>
<td>A discount card with 25% discount on all fresh vegetables, fruit, berries and fish and several healthy foods available during the first year of intervention</td>
<td></td>
</tr>
<tr>
<td>Delivery of free foods items</td>
<td>Delivery of free healthy food items at every visit at study center during the first 12 months of intervention</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Delivery of a box with free food items to their homes two times during the first 12 months of intervention</td>
<td></td>
</tr>
<tr>
<td>Cooking course</td>
<td>A one-day cooking course arranged by registered dietitians following a protocol in accordance with the NFBDG</td>
<td></td>
</tr>
<tr>
<td>CRC-NORDIET webpage</td>
<td>A log-in restricted dynamic webpage containing extensive information regarding the NFBDG, dietary advice, recipes, week menus and portion sizes.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>The webpage is available to the end of study and it is continuously updated with new recipes in accordance to the NFBDG</td>
<td></td>
</tr>
<tr>
<td>Inspiration days</td>
<td>The first inspiration day will contain:</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Lectures about the NORDIET study</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Diet: practical demonstrations of foods (fruits, vegetables, whole-grain products) and portion sizes according to the NFBDG performed by registered clinical dietitians</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Physical activity: Lecture and practical demonstration by the physical therapists of home-based exercises to incorporate in daily life</td>
<td></td>
</tr>
<tr>
<td></td>
<td>The patients are invited to inspiration group meetings every year after the first year of intervention. The group meetings will particularly focus on foods dampening oxidative stress and inflammation, and to encourage the patients to continue following the NFBDG. The physical activity section will consist of different topics at each meeting</td>
<td></td>
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<tr>
<td></td>
<td>The first inspiration day will contain:</td>
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<tr>
<td></td>
<td>- Lectures about the NORDIET study</td>
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<tr>
<td></td>
<td>- Physical activity: Lecture and practical demonstration by the physical therapists of home-based exercises to incorporate in daily life</td>
<td></td>
</tr>
<tr>
<td>Physical exercise</td>
<td>All the participants are offered free access to exercise facilities at “Pusterommet” (<a href="http://pusterommene.no/">http://pusterommene.no/</a>) during the first year of intervention. They also get individual counseling by a physiotherapist</td>
<td></td>
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</table>

*NFBDG: Norwegian food-based dietary guidelines*
Additional file 2. Summary of the 13 recommendations of the Norwegian food-based dietary guidelines (NFBGDG) (translated from the NFBGDG)

<table>
<thead>
<tr>
<th>Recommendation</th>
<th>Explanations to the recommendations</th>
</tr>
</thead>
</table>
| 1 A primarily plant-based diet is recommended, including plenty of vegetables, fruit, berries, wholegrain and fish, and limited quantities of red and processed meat, salt, added sugar and energy-rich foods | - A varied diet is the best way to achieve favourable health effects and an optimum intake of nutrients.  
- Choose mainly foods that contain limited quantities of fat, sugar and salt.  
- Choose foods that help to ensure an adequate intake of nutrients. |
| 2 It is recommended to maintain a balance between energy intake and energy expenditure | - Energy intake from foods and drinks and energy consumption through physical activity, should be balanced, so that weight is maintained within the normal range.  
- Regular physical activity helps to maintain the energy balance. A large proportion of the population is overweight. For the overweight, weight loss should combine more physical activity with an energy-reduced diet.  
- The consumption of foods with high energy content should be limited.  
- The consumption of drinks with added sugar, such as carbonated drinks should be limited.  
- Within each food group, it is recommended to choose products that have the Keyhole label |
| 3 Eat at least five portions of vegetables, fruit and berries every day | - It is recommended to eat at least five portions, corresponding to at least 500 grams altogether, of vegetables, fruit and berries every day.  
- About half of this intake should be in the form of vegetables and about half fruit and berries.  
- A portion corresponds to about 100 grams, for example as mixed salad, carrot, broccoli or cauliflower as an accompaniment to a main meal, a medium sized piece of fruit (apple, pear or orange) or a small bowl of berries. As a maximum, one glass of juice can be included as one portion.  
- The recommendation is to eat a variety of vegetables, fruit and berries of different colours, and include tomatoes and vegetables in the onion family in the diet.  
- Fresh, tinned, frozen, raw and cooked vegetables, fruit and berries can all be included. Dried fruit can also be included, but the portion size should be adjusted downward, and products with no added sugar should be chosen.  
- It is recommended to consume a moderate amount of nuts (about 140 grams per week). The nuts should be unsalted. The nuts are in addition to the recommended five portions of vegetables, fruit and berries. Nuts have high energy content and a high intake can promote weight increase.  
- Potatoes are not included in the recommended five portions of vegetables, fruit and berries. Potatoes are however an important food in the Norwegian diet and can certainly be included in a varied diet. Potatoes have a higher content of dietary fibre and more vitamins and minerals per energy unit that ordinary rice or pasta. Choose boiled or baked potatoes rather than chips, crisps and other potato products with added fat and sugar.  
- Pulses, seeds, spices and herbs are not included in the recommended five portions of vegetables, fruit and berries. These foods do however often have a high nutrient content and can certainly be included in a varied diet. |
| 4  | Eat at least four portions of wholegrain products every day | • Four portions of wholegrain products corresponds to about 70-90 grams of wholegrain per day (75 g of wholegrain per 10 MJ (2.400 kcal)).  
• Three slices of wholemeal bread or a large portion of wholemeal pasta or brown rice all correspond to about 75 grams of wholegrain. Breakfast cereals, porridge and crisp bread made with wholegrain are also good wholegrain sources.  
• At least half of the total consumption of grain products should be in the form of wholegrain.  
• Preferably, choose grain products with a high fibre content and low content of sugar, fat and salt, such as Keyhole-labelled products and wholemeal breads.  
• Limit the consumption of grain products with a high content of fat, salt and sugar, such as a number of types of cakes, cereals, pizza and snacks. |
| 5  | Eat the equivalent of two or three portions of fish per week | • Weekly consumption of about 300-450 grams of fish is recommended. This corresponds to two or three main meal portions per week.  
• Alternatively, fish as a main meal can be replaced with fish as a sandwich topping. Six sandwich topping portions of fish approximately correspond to one main meal portion.  
• Both fatty and lean fish can be included, but it is recommended that at least 200 grams of the intake should be of fatty fish.  
• Preferably, choose Keyhole-labelled fish products. |
| 6  | Low-fat dairy products should be included in your daily diet | • The daily consumption of low-fat dairy products is important for most people in order to ensure an adequate intake of certain nutrients, including calcium and iodine. Low-fat dairy products should therefore be included in the diet.  
• The consumption of dairy products that contain high levels of saturated fat and/or a high energy content (i.e. more than 950-1,150 kJ or 225-275 kcal per 100 grams), such as full-cream milk, cream, fatty cheese and butter, should be limited. This advice must be seen in context with the other dietary recommendations, so as to ensure a good fat quality in the complete diet.  
• Preferably, choose Keyhole-labelled dairy products. |
| 7  | It is recommended to eat lean meat and lean meat products, and limit the intake of red meat and processed meat | • Lean meat products are important for most people in order to ensure an adequate consumption of a number of nutrients. Moderate consumption of lean meat products can therefore be included in the diet.  
• This advice must be seen in context with the other dietary recommendations, so as to ensure a good fat quality in the complete diet.  
• Choose meat and meat products with a low fat and salt content. Preference should be given to the consumption of unprocessed meat.  
• Limit the consumption of red meat (beef, pork, lamb and goat) to 500 grams per week. This corresponds to two main meals with red meat and a limited amount as sandwich topping a week. When reducing the consumption of red meat, preference should be given to cutting the consumption of processed red meat.  
• Those with a high consumption of red meat could preferably replace some of this with white meat and fish.  
• Consumption of processed meat products (smoked, salted or preserved with nitrate or nitrite) should be limited.  
• Choosing Keyhole-labelled meat and meat products is recommended. |
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| **8** | **It is recommended to use cooking oil, liquid margarine or soft margarine** | - Cooking oils and margarine with a low content of saturated fatty acids and a high content of unsaturated fatty acids, such as plant oils (e.g. rapeseed, sunflower, olive and soya oils) and liquid or soft margarine, should be used in preference to similar products with a great proportion of saturated fatty acids (such as those that contain a high percentage of palm oil) and low proportion of unsaturated fatty acid.  
- Limit the use of butter and butter-margarine blends because these have a high content of saturated fatty acids and a low content of polyunsaturated fatty acids. Butter and animal fats also may contain trans fatty acids and cholesterol.  
- The consumption of foods with high energy content should be limited. Cooking oils and soft or liquid margarine have high energy content, but also contribute with polyunsaturated fatty acids and fat soluble vitamins, and should therefore be included in the diet.  
- This advice must be seen in context with the other dietary recommendations, so as to ensure a good fat quality in the complete diet. |
| **9** | **Water is recommended as the primary choice of drinks** | - It is recommended that water makes up the major part of the fluid requirement. This includes tap water and bottled and mineral water (only normal mineral water not sweetened and carbonated soft drinks).  
- Tap water and most types of mineral water contain insignificant amounts of sodium (salt), but some types of mineral water can contain significant amounts of sodium (see Recommendation 11).  
- Skimmed milk and extra low-fat milk can certainly be included as a drink in the diet, so as to ensure an adequate intake of calcium and iodine (see Recommendation 6).  
- Consumption of alcohol is not recommended.  
- The consumption of drinks with added sugar, such as carbonated drinks should be limited (see Recommendations 2 and 10).  
- Fruit juice may be included as part of the recommendation for fruit, berries and vegetables (see Recommendation 3). High consumption of fruit juice should however be avoided.  
- Consumption of acidic (low pH) drinks, such as carbonated drinks with sugar or artificial sweeteners, and juice should be limited outside from mealtimes. |
| **10** | **Limit your intake of added sugar** | - It is recommended that the intake of added sugar should be limited to less than 10% of the total energy intake.  
- It is recommended to reduce the consumption of carbonated drinks, soft drinks, nectar, sweet biscuits, cakes and sweets.  
- The consumption of drinks with added sugar should be limited (see Recommendations 2 and 9). |
| **11** | **Limit your intake of salt** | - It is recommended to limit the intake of salt (sodium chloride) to a maximum of 6 grams per day (which corresponds to 2.4 grams of sodium).  
- Preferably, choose food with a low salt content. If food products state salt content, choose products with a low salt content or those with the Keyhole label.  
- Limit the consumption of food products with a high salt content. Industrial and processed food products contribute 70-80% of salt consumption for most people. Non-processed food contains far less salt than most processed food products.  
- Limit the use of table salt and salt in the preparation of food. Use other flavourings such as herbs and salt-free spices instead of salt.  
- Limit the consumption of mineral water with high levels of sodium or a high salt content. Tap water contains insignificant amounts, while mineral water may contain a considerable amount (1 gram of salt per litre, i.e. 0.4 grams of sodium per litre). |
12 Dietary supplements may be necessary to ensure an adequate intake of nutrients for some groups in the population

- Dietary supplements are unnecessary for most people if they have a varied and healthy diet.
- If a deficiency of a nutrient is clinically documented, a dietary supplement may be a good alternative if a corresponding intake from foods is difficult. This applies for example to iron deficiency, which is not uncommon among women. Iron supplements are not recommended as a general preventive measure, only if iron deficiency anaemia or low iron status has been documented.
- Persons who do not eat fatty fish or who have an intake lower than the recommended lower limit (i.e. 200 grams per week) should take a daily supplement of cod liver oil or other omega-3 supplements, so as to ensure an adequate intake of long-chain polyunsaturated omega-3 fatty acids (EPA, DHA). The primary advice however is to eat fatty fish (see Recommendation 5).
- Persons who do not have a sufficient intake of vitamin D should take cod liver oil or another vitamin D supplement daily during the period of the year with little exposure to the sun. Elderly people who spend little time out in the sunlight should take cod liver oil or another supplement with 10 micrograms of vitamin D per day in addition to regular dietary consumption. This also applies to persons with dark skin and others with too low exposure to sunlight.
- Persons with a low energy intake (6.5-8 MJ/day or 1.550-1.900 kcal/d), should consider taking a multivitamin and mineral supplement in addition to their regular diet.
- Persons with a very low energy intake (less than 6.5 MJ/d or 1.550 kcal/d) should always take a multivitamin and mineral supplement in addition to the regular diet. This applies especially to elderly people with low dietary intakes.
- Women of childbearing age are recommended to take a supplement containing 400 micrograms of folate every day for a month before anticipated conception and for the first two or three months of pregnancy.
- Care is advised when taking several supplements that contain the same nutrient, since a high intake could have a damaging effect.

13 It is recommended that everyone participates in at least 30 minutes of physical activity per day

- Spend at least 30 minutes a day in moderate physical activity, corresponding to at least a brisk walk. If your general condition allows, this can be increased to an hour or more every day. Generally speaking, any form of physical exercise is better than none.
- The time spent in physical activity can be divided into periods during the course of the day.
- Physical activity is favourable for weight reduction and for prevention of weight increase after weight reduction. To maintain a large weight loss, 60 to 90 minutes of moderate physical activity most days a week is recommended.
Additional file 3. Detailed list of foods and drinks with high content of redox-active compounds and/or antioxidative effects

The following foods and drinks have high content of redox-active compounds and/or may have antioxidative effects individually or in combination in vitro models, animal models, clinical trials and/or epidemiological studies: coffee [1-8], green tea [2-4, 6, 9-11], black tea [2-4, 6], onion [1, 12], broccoli [1, 6, 9-11], tomatoes [6, 9-11], red cabbage [2-4, 9-11], kale [9-11], Brussel sprouts [9-11], artichoke [2-4], curly kale [2-4], peppers/paprika [2-4], chili peppers [2-4], carrots [9-11], pomegranates [2-4, 9-11], garlic [6], kiwifruit [13, 14], apples [6, 9-11], orange [6, 9-11], grapes [2-4, 9-11], plums [2-4], cherries [9-11], walnuts [2-4, 6, 9-11], chestnuts [2-4], peanuts [2-4], hazel nuts [2-4], almonds [2-4], thyme [1-4, 9-11, 15], oregano [1-4, 9-11, 15], lemon balm [15], clove [2-4, 15], allspice [2-4, 15], peppermint [2-4, 15], sage [2-4, 15], turmeric [1], rosemary [1-4, 9-11, 15], saffron [2-4], estragon [2-4], elderberries [16], dog rose [1-4, 9-11], cinnamon [2-4, 6, 15], chokeberries [9-11], blueberries/bilberries [2-4, 6, 9-11, 16, 17], blackberries [2-4, 9-11, 16], cranberries [2-4, 9-11], strawberries [2-4, 9-11], raspberries [2-4, 9-11], crowberries [2-4], black currants [2-4], dark chocolate [1-4, 9-11], pecan nuts [2-4, 9-11], olive [2-4, 9-11] and barley [2-4].

Furthermore, we have also identified that the following foods and drinks may have anti-inflammatory effects individually or in combination in cell cultures, animal models, clinical trials and/or epidemiological studies: coffee [5, 18-22], tomatoes [18, 21], carrots [21], pomegranates [21], walnuts [21, 22], nuts [23], strawberries [21], blueberries/bilberries [24, 25], crowberries [21], blackberries [21], dog rose [14], whole grains [26], thyme [21, 22], oregano [21, 22], turmeric [21], clove [21], allspice [21] and rosemary [21].
List of references for additional file 3:


### Additional file 4. Questionnaires, biological samplings and measurements

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*CT images are routinely taken for clinical purposes pre-surgery and 5 and 10 years after surgery.

**Tumor tissue will be collected at surgery

FFQ: Food frequency questionnaire; 6MWT: 6 minutes walking test; BIA: bioelectrical impedance analysis; CT: computerized tomography; DXA: dual-energy x-ray absorptiometry; EDTA: ethylenediaminetetraacetic acid; PG-SGA: Patient-Generated Subjective Global Assessment; PA: physical activity
The ability of PG-SGA to detect low fat-free mass in colorectal cancer patients

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\textbf{Shortened version of the title:} The ability of PG-SGA to detect low FFM

\textbf{Keywords:} Patient-Generated Subjective Global Assessment, fat-free mass, fat-free mass index, colorectal cancer, sarcopenia
Abstract

Low fat-free mass (FFM) is associated with adverse outcomes in colorectal cancer (CRC) patients. Patient-generated subjective global assessment (PG-SGA) is a widely used assessment tool developed to detect patients with malnutrition or patients at risk of malnutrition. The aim of this study was to investigate the ability of PG-SGA to detect low FFM in patients with non-metastatic CRC. Ninety-seven patients were included and categorized as well nourished (PG-SGA A, n=67) or malnourished (PG-SGA B, n=30). Bioelectrical impedance analysis (BIA) was used to assess FFM. Low FFM was defined as low fat-free mass index (FFMI) according to cut-off values recently proposed by The European Society for Clinical Nutrition and Metabolism (ESPEN). Twenty-nine percent of the patients were identified with low FFMI. The proportion with low FFMI was significantly higher among patients classified as malnourished by PG-SGA compared to well nourished (p=0.015). The sensitivity was however low, as the PG-SGA categorization classified only 50.0 % of the patients with low FFMI as malnourished (PG-SGA B). Using the PG-SGA scores (cut-off point > 4), the sensitivity increased to 60.7 %. Physical examination in the PG-SGA identified only 64.3 % of the patients with low FFMI as muscle depleted. In conclusion, our results indicate that the PG-SGA does not identify with sufficient sensitivity patients with low FFMI among patients with non-metastatic CRC. In clinical practice, PG-SGA should be accompanied by muscle mass assessments by BIA or other methods in order to detect low FFM in this patient group.
### Introduction

Malnutrition and weight loss in cancer occurs due to a negative energy balance caused by a reduced food intake in combination with metabolic alterations induced by the tumor, such as elevated resting metabolic rate, lipolysis, and proteolysis driven by systemic inflammation and catabolic factors.

It is now recognized that in particular the loss of fat-free mass (FFM) is linked to adverse outcomes in cancer patients. Progressive loss of FFM and skeletal muscle, the major constituent of FFM, is shown to be an independent predictor of chemotherapy toxicity, post-operative complications and mortality in cancer patients. Depletion of FFM may occur with or without loss of fat mass, and may therefore be masked by a stable body weight. Furthermore, weight gain during recovery may be characterized by an increase in fat mass rather than FFM.

Loss of skeletal muscle mass may subsequently lead to sarcopenia, defined by the European Working Group on Sarcopenia in Older People (EWGSOP) as “a syndrome characterized by progressive and generalized loss of skeletal muscle mass and strength, which is associated with adverse outcomes such as physical disability, poorer quality of life and death.”

We have recently shown that low FFM is common in patients with non-metastatic CRC. Low FFM is shown to be associated with reduced survival in patients with non-metastatic primary CRC. For CRC patients, identification of low FFM and sarcopenia is therefore of clinical importance since appropriate interventions may improve prognosis. Interventions focusing on optimizing food intake and reducing nutritional impact symptoms (i.e. symptoms affecting food intake) may decrease weight loss or facilitate weight gain in cancer patients. Ravasco and coworkers demonstrated that early individualized nutritional counselling reduced radiotherapy toxicity and improved nutritional status, quality of life and survival in colorectal cancer (CRC) patients receiving radiotherapy. According to the European Society for Clinical Nutrition and
Metabolism (ESPEN) guidelines on nutrition in cancer patients, nutritional therapy should be combined with physical therapy, i.e. counseling regarding physical activities of daily life, resistance and aerobic exercise training, to maintain or increase muscle mass. ESPEN recently defined low FFM as FFMI below 15 kg/m² and 17 kg/m², in females and males, respectively. FFMI can be estimated by the use of different modalities, including air displacement plethysmography, labeled water-isotope dilution techniques, dual energy x-ray absorptiometry (DXA) and bioelectrical impedance analysis (BIA). In clinical practice, access to these methods is limited. The Scored Patient-generated subjective global assessment (PG-SGA) is one of few comprehensive nutritional assessment tools used to identify malnutrition in cancer patients and the method also includes an evaluation of body composition changes, i.e. loss of subcutaneous fat and muscle mass. The examination consists of visual inspection and palpation of muscles, subcutaneous fat and edema. The PG-SGA includes four patient-generated components (weight history, food intake, nutritional impact symptoms and activities and function) and three professional components (age and diagnosis, metabolic stress and physical examination). Based on an evaluation of these components, patients are categorized as well-nourished (PG-SGA A), moderately malnourished (PG-SGA B) or severely malnourished (PG-SGA C). The scored version also includes numerical scores for each of the components as well as a total numerical score. PG-SGA is recommended by the Academy of Nutrition and Dietetics as one of the nutritional assessment tools to use in clinical oncology practice. However, although PG-SGA includes an evaluation of muscle and fat depletion, it is not known whether PG-SGA is suitable to detect low FFM in cancer patients. The aim of this study was therefore to investigate the ability of PG-SGA to detect low FFM in patients with non-metastatic CRC.


**Subjects and methods**

**Patients**

Patients were enrolled between August 2013 and March 2015. Eligible patients were women and men aged 50 to 80 years with a confirmed primary CRC (ICD-10 18-20), and staged I-III according to the tumor node staging (TNM) system\(^{19}\). Patients with distant metastases were not included. All patients had undergone surgery at two Hospitals in Norway.

The patients included in this cross-sectional study were recruited from the ongoing randomized clinical trial (RCT), The Norwegian Dietary Guidelines and Colorectal Cancer Survival (CRC-NORDIET) study\(^{20}\). All measurements were performed prior to the diet intervention. The CRC-NORDIET study was carried out in accordance to the Helsinki Declaration and informed consent was obtained from all participants. The study was approved by the Regional Committees for Medical and Health Research Ethics (REC Protocol Approval 2011/836) and by the data protection officials in the hospitals, and registered on the National Institutes of Health Clinical Trials (www.ClinicalTrials.gov; Identifier: NCT01570010).

**Measurements**

All measurements were conducted at start of the clinical trial (2-9 months post-surgery) and were performed by trained personnel. The patients were instructed to fast overnight and until all measurements were completed.

**Nutritional assessment by the scored PG-SGA**

The Norwegian version of the scored PG-SGA (15-004 v10.13.16) was used in the present study, and permission for use was given by the copyright holder of the instrument. The assessment was carried out by trained registered clinical dietitians, and the scoring was controlled by one researcher.
Patients were classified as well-nourished (PG-SGA A), moderately malnourished (PG-SGA B) or severely malnourished (PG-SGA C). In order to classify patients, emphasis was being placed on weight loss, reduced food intake and loss of muscle mass detected by the physical examination. If there was uncertainty regarding which category the patient should be classified within, the least severe category was chosen. Each section of the PG-SGA was scored according to the manual and a total PG-SGA score was calculated for each patient.

Total PG-SGA score in the range of 4–8 indicates need of an intervention supervised by a dietitian targeting the reported symptoms, and total PG-SGA score ≥ 9 indicates a critical need of a nutritional intervention. The number of patients with scores in the range 4–8 and the number of patients with scores ≥ 9 were therefore identified. The PG-SGA includes registration of current body weight as well as body weight one month and six months prior to assessment. According to the PG-SGA manual, scoring of weight loss should preferably be based on weight history the last month instead of the last six months. Weight loss was therefore calculated by subtracting the current weight from the one-month weight.

The physical examination was performed according to the manual. Muscle wasting was investigated by visual inspection and palpation of muscles with loss of bulk and tone in temporal areas, deltoids and quadriceps indicating muscle depletion. The triceps and midaxillary line at the level of the lower ribs were investigated with regard to depletion of subcutaneous fat. Ankles were examined for the presence of edema. The degree of muscle and fat depletion was evaluated and rated as “normal”, mild to moderate” or “severe deficit”.

Questions within the PG-SGA with several categories were merged into two or three categories. The three food intake categories “normal”, “increased” and “reduced” were re-categorized into two categories “normal/increased” and “reduced”. The 14 nutrition impact symptoms, were grouped into “no symptom”, “1 symptom” and “2 or more symptoms”. The five categories within “Activity
and function” were collapsed into two categories, “normal” and “reduced”, with “normal” corresponding to the first category; “normal with no limitations” and “reduced” corresponding to the remaining four categories.

**Body weight, height and body mass index (BMI)**

Body weight was measured by the use of a non-slip Marsden M-420 Digital Portable Floor Scale (Marshden, Rotherham, South Yorkshire, United Kingdom) or a digital wireless measuring station for height and weight, Seca 285 (Seca, Birmingham, United Kingdom). Measurements were performed with patients wearing light clothes and no shoes. Body weight was subtracted by 0.5 kg to adjust for clothing. Height (cm) was measured post-surgery by the use of either a mechanical height rod (Kern MSF- 200) or a digital wireless stadiometer (Seca 285). BMI was calculated based on recorded weight and height.

**BIA**

To obtain FFM estimates, a single frequency whole-body BIA, BIA 101 (SMT Medical, Würzburg, Germany) was used. BIA measures body composition indirectly by measuring the impedance (i.e. the resistance and reactance) of a low-voltage current passing through the body. FFM is then calculated by the BIA software, which utilizes the impedance data in empiric regression equations incorporated in the software. We have previously validated BIA against DXA in a subgroup of CRC patients included in the CRC-NORDIET study.

BIA was performed under standardized conditions according to the manufacturer’s protocol. Measurements were performed by placing two skin electrodes on the right hand and two electrodes
on the right foot of the patient when lying in supine position. The device applies current of 400 μA at a constant frequency of 50 kHz.

**Determination of low FFMI and sarcopenia**

FFM values from BIA were used to calculate FFMI (FFM (kg)/height (m²)). FFMI was grouped into “low FFMI” (<15 kg/m² for women and < 17 kg/m² for men) and “normal FFMI” (≥ 15 kg/m² for women and ≥ 17 kg/m² for men) according to cut-off values for FFMI proposed as part of the new diagnostic criteria for malnutrition by the ESPEN¹⁴.

Patients with sarcopenia were identified by the use of the diagnostic criteria for age-related sarcopenia as proposed by EWGSOP⁵; presence of low muscle mass (criterion 1) and low muscle function (strength (criterion 2) or performance (criterion 3)). Criterion 1, and either 2 or 3 must be present to diagnose sarcopenia. In the current study, we defined low muscle mass as low FFMI. Low muscle strength was defined as low hand grip strength according to the cut-off values published by Fried²¹. Grip strength was assessed with a hand grip dynamometer (KERN & SOHN GmbH, Balingen, Germany) as described in the manufacturer’s protocol. We defined low physical performance as low gait speed and/or low number of sit to stands. Gait speed was measured with a 6-min walk test according to the guidelines from the American Thoracic Society²² and gait speed < 1 m/s was defined as “low”²³. The sit-to-stand test was performed by instructing the participants to sit on a chair with arms folded across their chest, and then to stand up and sit down as frequently as possible within 30 s, keeping both arms folded across the chest. The number of full stands was counted, and stands < 18 and < 22, i.e. the lower cut-off values for the 95 % CI for a reference population of healthy Norwegian women and men in the age of 60 years, were defined as “low”²⁴.
Statistical analyses

Determination of sample size was performed in accordance to a guide for sample size for sensitivity and specificity analysis published by Bujang and Adnan in 2016. According to this guide, the sensitivity for a screening study must be pre-determined to be at least 0.50. We estimated the prevalence of low FFM in CRC patients to be 33%, based on our previous findings. Hence, a minimum sample size of 67 patients would be needed to achieve a minimum power of 80% in order to detect a change in sensitivity from 0.50 to 0.80, based on a significance level of 0.05. Data were checked for normality using the Kolmogorov-Smirnov test and visual inspection of the histograms. Normally distributed data were presented as means and standard deviations, and non-normally distributed data as medians and range (minimum-maximum). Pearson chi-square test for independence or Fisher’s exact test was performed to investigate differences in proportions between groups. Mann-Whitney test was used to test differences in medians for non-normally distributed continuous variables. Independent samples t-test was used to explore differences in means for normally distributed variables. P-values (2-sided) ≤ 0.05 were considered significant. Sensitivity and specificity were calculated to evaluate PG-SGA as a screening tool with FFMI assessed by BIA as reference method. All statistical analyses were performed using SPSS (IBM SPSS Statistic 22).
Results

Subject characteristics

One hundred and six patients were included in the study and assessed with the PG-SGA tool. Of these, nine patients were excluded from the analyses due to lack of data needed to determine FFMI. Of the 97 eligible patients, 28 patients (29 %) were identified with low FFMI. Subject characteristics are shown for patients with low and normal FFMI, respectively (Table 1).

Fifty-nine percent of the patients had colon cancer, 35 % had rectum cancer and 7 % patients had rectosigmoid cancer. The median time from CRC surgery to assessments was 4 months.

Patients with normal and low FFMI were compared with regard to clinical characteristics. In general, there were few differences between the groups. There were no significant differences in gender, cancer localization, TNM stage or proportions receiving neoadjuvant or adjuvant treatment between the groups. Mean BMI was found to be significantly lower in patients with low FFMI, and the proportion of underweight patients was significantly higher among patients with low FFMI compared to patients with normal FFMI (p<0.001). Patients with low FFMI were significantly older than patients with normal FFMI (p=0.027). This finding was expected since loss of FFM is associated with increased age.
The ability of PG-SGA to detect patients with low FFMI

Based on the PG-SGA global assessment, 67 (69.1 %) and 30 (30.9 %) of 97 eligible patients were categorized as well-nourished (PG-SGA A) and moderately malnourished (PG-SGA B), respectively (Table 2). No patients were categorized as severely malnourished (PG-SGA C). The proportion of patients with low FFMI estimated by BIA was significantly higher among patients classified by PG-SGA as malnourished compared to well nourished (46.7 vs 20.9 %, \( p=0.015 \)) (Table 2). Furthermore, median PG-SGA total score was found to be significantly higher among patients with low FFMI compared to patients with normal FFMI (5 vs 3, \( p=0.036 \)). However, the sensitivity, i.e. the proportion of patients with low FFMI classified as malnourished by PG-SGA categories, was calculated to only 50.0 %. The specificity, i.e. the proportion of patients with normal FFMI classified as well nourished by PG-SGA, was found to be 76.8 %. Using the PG-SGA numerical score, 60.7 % of the patients with low FFMI were identified with score > 4, i.e. the lowest cut-off for a nutritional intervention. These results indicate that the PG-SGA global rating does not have sufficient sensitivity and specificity to detect low FFMI, however, using the PG-SGA score increase the sensitivity.

The individual components of the PG-SGA in patients with low FFMI

In order to elucidate why a significant proportion of the patients with low FFMI (estimated by BIA) was evaluated as well nourished by the PG-SGA, we investigated the individual components of the assessment tool (Table 3). Regarding all patients with low FFMI independently of PG-SGA categorization, 66.7 % of the patients reported weight loss within the last 6 months, whereas only 16.7 % reported weight loss the last month, indicating that the patients experienced their weight loss earlier in the trajectory of the disease, and that the majority of the patients were weight increasing at the time of assessment. Furthermore, 60.7 % reported a normal food intake (i.e. unchanged or
increased) the last month and 28.6 % had symptoms affecting food intake. Furthermore, 53.6 % of the patients reported having reduced activity and function level (Table 3). The sensitivity of PG-SGA examination to detect muscle mass depletion (i.e. visual inspection and palpation of muscles in temporal areas, deltoids and quadriceps) was calculated. Only 64.3 % of the patients assessed with low FFMI by BIA were evaluated as muscle depleted by PG-SGA. The specificity (i.e. proportion of patients with normal FFMI correctly classified with “no deficit”) was 78 %. Taken together, these findings suggest that when investigating the various components of the PG-SGA in patients with low FFMI, the majority of these patients experienced weight increase at the time of assessments, accompanied by a normal food intake (i.e. stable or increased) and no symptoms affecting food intake. Furthermore, the results indicate that the physical examination does not have sufficient sensitivity and specificity to detect low FFMI.

Comparison of well nourished (PG-SGA A) and malnourished (PG-SGA B) patients among patients with low FFMI

To further elucidate why a significant proportion of the patients with low FFMI was evaluated as well nourished by the PG-SGA, we selected the patients with low FFMI and compared well nourished and malnourished patients with regard to the individual components of the PG-SGA. Patients categorized as PG-SGA A had significantly lower median total PG-SGA score compared to patients categorized as PG-SGA B (3 vs 6, p<0.001) (Table 3). This finding was expected since PG-SGA category is related to the PG-SGA score. Furthermore, none of the patients with PG-SGA A reported a reduced food intake, whereas the majority of the patients categorized as PG-SGA B reported reduced food intake (p<0.001). We found no differences between the groups with regard to weight loss the last 6 months, weight loss the last month, presence of anorexia, presence of nutritional impact symptoms or physical function and activity. Among patients identified with
“mild to moderate deficit” by the PG-SGA physical examination, a significantly lower proportion of the patients were classified as PG-SGA A compared to PG-SGA B (27.8 vs 72.2 %, p=0.004). As PG-SGA category was set mainly based on the three components weight loss the last month, reduced food intake the last month and muscle mass depletion, these findings were quite expected, except for weight loss that did not differ between the groups. The groups did not differ with regard to BMI.

**BMI according to physical examination status among patients with low FFMI**

In order to investigate why a high proportion (i.e. 36 %) of the patients with low FFMI were not detected by the physical exam in the PG-SGA, we investigated if there was a difference in BMI between patients detected and patients not detected by the PG-SGA within patients with low FFMI (Table 4). Mean BMI was significantly higher in patients not detected by the PG-SGA (24.6 vs 21.5, p=0.006). Furthermore, we found a significantly higher proportion of patients with overweight among these patients compared to those that were found to be muscle depleted (66.7 vs 33.3 %, p=0.025). A possible explanation for this finding may be that high BMI camouflages low muscle mass in patients with low FFMI.

**The ability of PG-SGA to detect patients with sarcopenia**

In the current study, we also investigated the ability of PG-SGA to detect patients with sarcopenia. Of 97 patients included in this study, 95 patients were eligible for the diagnosis of sarcopenia. About twenty-two % (n= 21) of the patients were diagnosed with sarcopenia (Table 5). The proportion of patients with sarcopenia did not significantly differ between patients classified by PG-SGA as well nourished and malnourished, respectively. PG-SGA classified 42.9 % of the patients...
with sarcopenia as malnourished. With regard to the PG-SGA numerical score, we found no difference in median total score when we compared sarcopenic patients with non-sarcopenic patients. Furthermore, 61.9 % of the patients with sarcopenia were identified with total PG-SGA score > 4, i.e. the lowest cut-off for a nutritional intervention. These results were similar to the results from the analysis of patients with low and normal FFMI. The sensitivity of PG-SGA to detect patients with sarcopenia was low, however, we observed increased sensitivity by the use of the PG-SGA scoring.
Discussion

In this study we investigated the ability of PG-SGA to identify patients with low FFM among patients with non-metastatic CRC. About twenty-nine percent of the patients had low FFMI according to the cut-off values proposed by ESPEN. The PG-SGA categorization classified only 50% of these patients as malnourished (PG-SGA B). Use of the PG-SGA total scores improved sensitivity (61%). However, only 64% of the patients with low FFMI assessed by BIA were evaluated as muscle depleted in the physical examination in the PG-SGA. Our results indicate that the PG-SGA do not have sufficient sensitivity to detect low FFM.

Few previous studies have examined the ability of PG-SGA to detect low FFM, and to the best of our knowledge, no studies are performed in non-metastatic patients with CRC. The clinical implications of muscle depletion and sarcopenia is mainly studied in patients with metastatic cancer, however, the high percentage of patients with low FFMI in our population suggests that it may have a broader relevance. Vigano and coworkers examined associations between PG-SGA scores and features of cancer cachexia in a mixed population of patients with advanced lung and gastrointestinal cancers. Although they observed that the PG-SGA score was able to predict several features of cancer cachexia, including decrease of muscle strength and loss of fat mass, PG-SGA was not able to detect differences in lean body mass, in agreement with our results. In patients with gynecologic cancers, FFM was not found to differ between PG-SGA categories. In the study performed by Guerra and colleges, FFMI was significantly lower among malnourished patients according to the PG-SGA in a sample consisting of 455 inpatients with a broad spectrum of diagnoses.

Our study demonstrated poor specificity and sensitivity for the PG-SGA categories to detect low FFMI. Only half of the patients with low FFMI were classified as malnourished. Consequently, half
of the patients were missed by the use of these categories. The literature on sensitivity and specificity of PG-SGA to detect low FFM or muscle mass is scarce, however, our findings are in line with the results reported by Elkan et al, who observed that SGA, the earlier version of PG-SGA, showed poor sensitivity (46 %) in detection of low FFMI in patients with rheumatoid arthritis assessed with DXA. Similar to our data, they observed a higher specificity than sensitivity for SGA.

In order to investigate why a significant proportion of the patients with low FFMI was categorized as well nourished, we investigated the individual components of the PG-SGA. We observed that the majority of the patients with low FFMI were anabolic at the time of assessments, reporting a normal food intake and no symptoms affecting food intake. Since PG-SGA is developed to detect patients with malnutrition or patients at risk of malnutrition with main focus on recent weight loss, nutritional impact symptoms and reduction in food intake, the implication of this is that patients with prior muscle mass depletion, but a stable or increasing body weight may be categorized as PG-SGA A.

Moreover, when we analyzed differences between those who were categorized as well nourished (PG-SGA A) and those who were categorized as malnourished (PG-SGA B) among patients with low FFMI, we observed differences with regard to 1) food intake and 2) proportions detected by the physical examination. A significantly higher proportion of the patients categorized as PG-SGA B reported reduced food intake, and a significantly higher proportion of these patients were detected with muscle mass depletion, compared to the patients categorized as PG-SGA A. Since reduced food intake and muscle mass depletion constitute two of the three components that were emphasized in the PG-SGA categorization, it provides a plausible explanation for why these patients were categorized as PG-SGA B.
We observed that use of the PG-SGA total score improved sensitivity compared to PG-SGA categories, suggesting that the scoring is better at capturing patients with low FFMI. It should, however, be mentioned that the physical examination only in minor extent contributes to the total PG-SGA score, with the maximum score of 3 points indicating severe adipose and muscle deficit. Hence, patients with low FFMI may hypothetically have a low total score, i.e. a total score below the lowest cut-off for an intervention.

Although a significantly higher proportion of the patients categorized as malnourished by PG-SGA were detected as muscle depleted by the physical examination (i.e. visual inspection and palpation of muscles in temporal areas, deltoids and quadriceps) compared to well nourished patients, the sensitivity and specificity was found to be low. In order to elucidate why many patients were missed by the physical examination in the PG-SGA, we hypothesized that muscle mass depletion could be more difficult to detect in patients with high BMI. In the current study, BMI was found to be significantly higher in those patients who were not identified as muscle depleted (as indicated by loss of bulk and tone in selected muscles examined by visual inspection and palpation) by the PG-SGA physical examination. Furthermore, we observed a higher proportion of overweight patients among these patients compared to those who were captured as depleted. Based on these findings, we conclude that PG-SGA is not sensitive enough to detect muscle mass depletion, particularly in overweight and obese patients. Studies utilizing imaging analyses have confirmed that excessive muscle wasting can be obscured in patients with excessive fat mass\(^5,30\), with computed tomography (CT) images demonstrating equal low total muscle amounts in obese and underweight patients. With a growing prevalence of overweight and obesity in several patient populations, including cancer populations, it is important to be aware of this limitation in the application of PG-SGA.
Monitoring weight loss, nutritional impact symptoms and reduction in food intake are important aspects of the nutritional assessments of cancer patients. However, since an ongoing loss of muscle mass may be masked by a stable or increased body weight, particularly in overweight and obese patients, assessing and monitoring body weight and food intake is not sufficient. Increase of body weight in terms of body fat rather than FFM may lead to sarcopenic obesity, a syndrome that entails the combined health risks of both sarcopenia and obesity. This highlights the importance of including appropriate tools to identify low FFM as part of the nutritional evaluation. As PG-SGA seems not be sensitive enough to detect muscle mass depletion, we suggest that the tool should be accompanied by muscle mass assessments by BIA or other methods.

In the current study we chose to use the FFMI cut-off values recently proposed by ESPEN, to determine low FFM. Since these cut-offs were published in 2015, validation studies have confirmed the prognostic impact of the malnutrition criteria on clinical outcomes and survival.

Our estimates of FFM were generated from BIA. Compared to imaging techniques such as DXA, CT and magnetic resonance imaging (MRI) that measure lean body mass and muscle mass with high precision, BIA measures these compartments indirectly by measuring the impedance of the current applied to the body. The impedance data (i.e. resistance and reactance) is utilized in empiric equations to calculate FFM. One of the main limitations with BIA is that these empirical equations are developed in healthy euvoletic adults with a normal body composition, and may therefore provide less reliable estimates in individuals with disturbances in fluids and alterations in body composition, such as cancer patients. However, the BIA used in the current study was previously validated against DXA in a subgroup of CRC patients included in the CRC-NORDIET study, and use of the equation incorporated in the BIA software for calculation of FFM showed good agreement with DXA estimates of FFM.
Similar to the results from the analysis of patients with low FFMI, we observed low sensitivity of the PG-SGA categories in detection of patients diagnosed with sarcopenia, and furthermore, increased sensitivity by the use of the PG-SGA scores. Although PG-SGA is primarily developed to identify patients with malnutrition and increased risk of malnutrition and not sarcopenia, our study demonstrates that a high proportion of patients diagnosed with sarcopenia who need to be further evaluated for nutritional therapy, are considered “no need for nutritional intervention” by the PG-SGA.

Patients identified with low FFMI who have not fully developed sarcopenia, is particularly interesting as target for nutritional intervention. According to the European Working Group on Sarcopenia in Older People (EWGSOP), low muscle mass without the presence of reduced strength or physical performance, corresponds to the stage “presarcopenia”. Identifying these patients and selecting appropriate treatment, may prevent further loss of muscle mass and inhibit progressive functional impairment.

Although PG-SGA does not perform sufficient sensitivity to detect low FFM, it covers several important aspects of malnutrition and sarcopenia. Hence, PG-SGA may be useful to characterize nutritional problems in patients where low FFM has been documented by the use of BIA or other methodology. It rapidly provides a detailed overview of the patient’s nutritional status as the assessment takes only approximately 5 minutes. Furthermore, the PG-SGA scoring may be useful in the follow up of these patients, by using the scores to monitor changes during and after nutritional therapy. In addition, PG-SGA score has been shown to predict clinical outcomes$^{26}$, quality of life$^{33}$ and survival$^{26,34}$ in cancer patients.

To our knowledge, this is the first study that has evaluated the ability of the PG-SGA to identify low FFM in colorectal cancer patients. Since PG-SGA is widely used and accepted as an assessment tool in oncology it is important to be aware of its strengths and limitations.
Conclusion

The nutritional assessment tool PG-SGA classified only half of the patients with low FFMI as malnourished. Use of the total PG-SGA score increased the sensitivity. However, only 64.3% of the patients with low FFMI were detected by the physical examination which is part of the PG-SGA. In clinical practice, PG-SGA scores should be accompanied by muscle mass assessments by BIA or other methods in order to more accurately identify low FFM in this patient group.

List of abbreviations

BIA: Bioelectrical impedance analysis; BMI: Body mass index; CRC: Colorectal cancer; CT: Computed tomography; DXA: Dual energy x-ray absorptiometry; ESPEN: European Society for Clinical Nutrition and Metabolism; EWGSOP: European Working Group on Sarcopenia in Older People; FFM: Fat-free mass; FFMI: Fat-free mass index; ICD: International classification of diseases and related health problems; MRI: Magnetic resonance imaging; PG-SGA: Patient-generated subjective global assessment; RCT: Randomized clinical trial; TNM: Tumor node metastasis.

Acknowledgements

We would like to thank the patients for their valuable contribution to this study. We would also like to thank Magnhild Håskjold and Siv Åshild Billington for their contribution in data collection. Finally, we acknowledge Kristine Lillebø Holm for assistance in the Norwegian translation of the PG-SGA version used in this study.

Conflict of interest statement

R.B is a shareholder in the company Vitas AS. All other authors declare that they have no competing interests.

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References


### Table 1. Characteristics of the population

<table>
<thead>
<tr>
<th></th>
<th>Normal FFMI (n=69)</th>
<th>Low FFMI* (n=28)</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (years) (std)</td>
<td>64.9 (8.2)</td>
<td>68.1 (5.3)</td>
<td>0.027</td>
</tr>
<tr>
<td><strong>Gender, n (%)</strong></td>
<td></td>
<td></td>
<td>0.058</td>
</tr>
<tr>
<td>Women</td>
<td>46</td>
<td>18 (39.1)</td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>51</td>
<td>10 (19.6)</td>
<td></td>
</tr>
<tr>
<td><strong>Cancer localization, n (%)</strong></td>
<td></td>
<td></td>
<td>0.264</td>
</tr>
<tr>
<td>Colon cancer</td>
<td>54</td>
<td>36 (66.7)</td>
<td></td>
</tr>
<tr>
<td>Rectosigmoid cancer</td>
<td>6</td>
<td>6 (100)</td>
<td></td>
</tr>
<tr>
<td>Rectum cancer</td>
<td>32</td>
<td>23 (71.9)</td>
<td></td>
</tr>
<tr>
<td><strong>TNM stage, n (%)</strong></td>
<td></td>
<td></td>
<td>0.199</td>
</tr>
<tr>
<td>Stage 1</td>
<td>10</td>
<td>8 (80.0)</td>
<td></td>
</tr>
<tr>
<td>Stage 2</td>
<td>46</td>
<td>36 (78.3)</td>
<td></td>
</tr>
<tr>
<td>Stage 3</td>
<td>33</td>
<td>20 (60.6)</td>
<td></td>
</tr>
<tr>
<td><strong>Neoadjuvant treatment, n (%)</strong></td>
<td></td>
<td></td>
<td>0.531</td>
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<tr>
<td>No</td>
<td>81</td>
<td>59 (72.8)</td>
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<tr>
<td>Yes</td>
<td>14</td>
<td>9 (64.3)</td>
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<tr>
<td><strong>Adjuvant treatment, n (%)</strong></td>
<td></td>
<td></td>
<td>0.512</td>
</tr>
<tr>
<td>None</td>
<td>74</td>
<td>55 (74.3)</td>
<td></td>
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<tr>
<td>Ongoing</td>
<td>16</td>
<td>10 (62.5)</td>
<td></td>
</tr>
<tr>
<td>Completed</td>
<td>5</td>
<td>3 (60.0)</td>
<td></td>
</tr>
<tr>
<td><strong>BMI</strong></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mean (kg/m²) (std)</td>
<td>27.2 (4.3)</td>
<td>22.6 (3.0)</td>
<td></td>
</tr>
<tr>
<td><strong>BMI categories, n (%)</strong></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Underweight (BMI &lt; 20)</td>
<td>8</td>
<td>1 (12.5)</td>
<td></td>
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<tr>
<td>Normal range (BMI 20-24.9)</td>
<td>32</td>
<td>20 (62.5)</td>
<td></td>
</tr>
<tr>
<td>Overweight (BMI 25-29.9)</td>
<td>43</td>
<td>34 (79.1)</td>
<td></td>
</tr>
<tr>
<td>Obese (BMI &gt;30)</td>
<td>14</td>
<td>14 (100)</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: FFMI, Fat-free mass index; TNM, Tumor node metastasis; BMI, Body mass index

*Independent samples t-test, chi-square test for independence or Fisher’s exact test, significance level $p \leq 0.05$

*Low FFMI defined as FFMI $< 17$ kg/m² for men and $< 15$ kg/m² for women.*
**Table 2.** Global rating and PG-SGA scoring according to normal and low FFMI

<table>
<thead>
<tr>
<th></th>
<th>Normal FFMI (n=69)</th>
<th>Low FFMI* (n=28)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Global rating, n (%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Well nourished (A)</td>
<td>67</td>
<td>53 (79.1)</td>
<td>14 (20.9)</td>
</tr>
<tr>
<td>Moderately malnourished (B)</td>
<td>30</td>
<td>16 (53.3)</td>
<td>14 (46.7)</td>
</tr>
<tr>
<td>Severely malnourished (C)</td>
<td>0</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td><strong>Total PG-SGA score</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PG-SGA score &lt; 4, n (%)</td>
<td>53</td>
<td>42 (79.2)</td>
<td>11 (20.8)</td>
</tr>
<tr>
<td>PG-SGA score 4-8, n (%)</td>
<td>35</td>
<td>22 (62.9)</td>
<td>13 (37.1)</td>
</tr>
<tr>
<td>PG-SGA score ≥ 9, (%)</td>
<td>9</td>
<td>5 (55.6)</td>
<td>4 (44.4)</td>
</tr>
<tr>
<td>Median (range)</td>
<td>97</td>
<td>3 (1-17)</td>
<td>5 (1-20)</td>
</tr>
</tbody>
</table>

Abbreviations: PG-SGA, Patient-generated subjective global assessment; FFMI, Fat-free mass index

*Mann-Whitney test, chi-square test for independence or Fisher’s exact test, significance level p ≤ 0.05

*Low FFMI defined as FFMI < 17 kg/m² for men and < 15 kg/m² for women."
Table 3. Comparison of PG-SGA A and PG-SGA B among patients with low FFM with regard to the various components of the PG-SGA and BMI

<table>
<thead>
<tr>
<th>Patients with low FFMI*</th>
<th>N</th>
<th>Patients with PG-SGA A (n=14)</th>
<th>Patients with PG-SGA B (n=14)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>PG-SGA score, median (range)</td>
<td>28</td>
<td>3 (1-8)</td>
<td>6 (3-20)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Weight loss last 6 months, n (%)</td>
<td>18</td>
<td>8 (44.4)</td>
<td>10 (55.6)</td>
<td>0.420</td>
</tr>
<tr>
<td>Yes</td>
<td>9</td>
<td>6 (66.7)</td>
<td>3 (33.3)</td>
<td>0.596</td>
</tr>
<tr>
<td>No</td>
<td>4</td>
<td>1 (25.0)</td>
<td>3 (75.0)</td>
<td>0.481</td>
</tr>
<tr>
<td>Presence of anorexia, n (%)</td>
<td>2</td>
<td>0 (0)</td>
<td>2 (100)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Yes</td>
<td>26</td>
<td>14 (53.8)</td>
<td>12 (46.2)</td>
<td>0.209</td>
</tr>
<tr>
<td>No</td>
<td>8</td>
<td>2 (25.0)</td>
<td>6 (75.0)</td>
<td>0.058</td>
</tr>
<tr>
<td>Symptom, n (%)</td>
<td>17</td>
<td>14 (82.4)</td>
<td>3 (17.6)</td>
<td>0.004</td>
</tr>
<tr>
<td>Normal</td>
<td>11</td>
<td>10 (90.0)</td>
<td>5 (45.5)</td>
<td>0.046</td>
</tr>
<tr>
<td>Reduced</td>
<td>8</td>
<td>2 (25.0)</td>
<td>6 (75.0)</td>
<td>0.018</td>
</tr>
<tr>
<td>Physical function and activity, n (%)</td>
<td>12</td>
<td>9 (69.2)</td>
<td>4 (30.8)</td>
<td>0.003</td>
</tr>
<tr>
<td>Normal</td>
<td>15</td>
<td>5 (33.3)</td>
<td>10 (66.7)</td>
<td>0.004</td>
</tr>
<tr>
<td>Reduced</td>
<td>10</td>
<td>9 (90.0)</td>
<td>1 (10.0)</td>
<td>0.047</td>
</tr>
<tr>
<td>Physical examination, n (%)</td>
<td>12</td>
<td>5 (41.7)</td>
<td>7 (58.3)</td>
<td>0.623</td>
</tr>
<tr>
<td>Underweight (BMI &lt; 20)</td>
<td>9</td>
<td>6 (66.7)</td>
<td>3 (33.3)</td>
<td>0.059</td>
</tr>
<tr>
<td>Normal range (BMI 20-24.9)</td>
<td>0</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Abbreviations: FFMI, Fat-free mass index; PG-SGA: Patient-generated subjective global assessment; BMI: Body mass index.

Mann-Whitney test (PG-SGA score), independent samples t-test (BMI), chi-square test for independence or Fisher’s exact test significance level p ≤ 0.05

*Low FFMI defined as FFMI < 17 kg/m² for men and < 15 kg/m² for women.14
**Table 4.** BMI according to physical examination status in the PG-SGA among patients with low FFMI.

<table>
<thead>
<tr>
<th>N</th>
<th>No deficit (n=10) by the PG-SGA</th>
<th>Mild to moderate deficit (n=18) by the PG-SGA</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI, mean (std)</td>
<td>28</td>
<td>24.6 (2.7)</td>
<td>21.5 (2.7)</td>
</tr>
<tr>
<td>BMI categories, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI &lt; 20</td>
<td>7</td>
<td>0 (0)</td>
<td>7 (100)</td>
</tr>
<tr>
<td>BMI 20-24.9</td>
<td>12</td>
<td>4 (33.3)</td>
<td>8 (66.7)</td>
</tr>
<tr>
<td>BMI 25-29.9</td>
<td>9</td>
<td>6 (66.7)</td>
<td>3 (33.3)</td>
</tr>
<tr>
<td>BMI &gt;30</td>
<td>0</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

Abbreviations: FFMI, Fat-free mass index; PG-SGA: Patient-generated subjective global assessment; BMI, Body mass index.

*Independent-samples t-test, chi-square test for independence or Fisher’s exact test, significance level p ≤ 0.05

*Low FFMI defined as FFMI <17 kg/m² for men and < 15 kg/m² for women.

**Table 5.** Global rating and PG-SGA scoring according to sarcopenia and no sarcopenia

<table>
<thead>
<tr>
<th>N</th>
<th>No sarcopenia (n=74)</th>
<th>Sarcopenia* (n=21)</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Global rating, n (%)</td>
<td></td>
<td></td>
<td>0.127</td>
</tr>
<tr>
<td>Well nourished (A)</td>
<td>67</td>
<td>55 (82.1)</td>
<td>12 (17.9)</td>
</tr>
<tr>
<td>Moderately malnourished (B)</td>
<td>28</td>
<td>19 (67.9)</td>
<td>9 (32.1)</td>
</tr>
<tr>
<td>Severely malnourished (C)</td>
<td>0</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Total PG-SGA score</td>
<td></td>
<td></td>
<td>0.162</td>
</tr>
<tr>
<td>PG-SGA score &lt; 4, n (%)</td>
<td>52</td>
<td>44 (84.6)</td>
<td>8 (15.4)</td>
</tr>
<tr>
<td>PG-SGA score 4-8, n (%)</td>
<td>34</td>
<td>23 (67.6)</td>
<td>11 (32.4)</td>
</tr>
<tr>
<td>PG-SGA score ≥ 9, n (%)</td>
<td>9</td>
<td>7 (77.8)</td>
<td>2 (22.2)</td>
</tr>
<tr>
<td>Median (range)</td>
<td>95</td>
<td>3 (1-17)</td>
<td>4 (1-20)</td>
</tr>
</tbody>
</table>

Abbreviations: PG-SGA: Patient-generated subjective global assessment.

*Mann-Whitney test, chi-square test for independence or Fisher’s exact test, significance level p ≤ 0.05

*Sarcopenia was diagnosed based on EWGSOP diagnostic criteria.
Original article

Validity of bioelectrical impedance analysis in estimation of fat-free mass in colorectal cancer patients

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SUMMARY

Background & aims: Bioelectrical impedance analysis (BIA) is an accessible and cheap method to measure fat-free mass (FFM). However, BIA estimates are subject to uncertainty in patient populations with altered body composition and hydration. The aim of the current study was to validate a whole-body and a segmental BIA device against dual-energy X-ray absorptiometry (DXA) in colorectal cancer (CRC) patients, and to investigate the ability of different empiric equations for BIA to predict DXA FFM (FFMDXA).

Methods: Forty-three non-metastatic CRC patients (aged 50–80 years) were enrolled in this study. Whole-body and segmental BIA FFM estimates (FFMwhole-bodyBIA, FFMsegmentalBIA) were calculated using 14 empiric equations, including the equations from the manufacturers, before comparison to FFMDXA estimates.

Results: Strong linear relationships were observed between FFMBIA and FFMDXA estimates for all equations ($R^2=0.94–0.98$ for both devices). However, there were large discrepancies in FFM estimates depending on the equations used with mean differences in the ranges $-6.5–6.8$ kg and $-11.0–3.4$ kg for whole-body and segmental BIA, respectively. For whole-body BIA, $77\%$ of BIA derived FFM estimates were significantly different from FFMDXA, whereas for segmental BIA, $85\%$ were significantly different. For whole-body BIA, the Schols* equation gave the highest agreement with FFMDXA with mean difference $\pm SD$ of $0.16 \pm 1.94$ kg ($p=0.582$). The manufacturer’s equation gave a small overestimation of FFM with $1.46 \pm 2.16$ kg ($p<0.001$) with a tendency towards proportional bias ($r=0.28, p=0.066$). For segmental BIA, the Heitmann* equation gave the highest agreement with FFMDXA ($0.17 \pm 1.83$ kg ($p=0.546$)). Using the manufacturer’s equation, no difference in FFM estimates was observed ($-0.34 \pm 2.06$ kg ($p=0.292$)), however, a clear proportional bias was detected ($r=0.69, p<0.001$). Both devices demonstrated acceptable ability to detect low FFM compared to DXA using the optimal equation.

Conclusion: In a population of non-metastatic CRC patients, mostly consisting of Caucasian adults and with a wide range of body composition measures, both the whole-body BIA and segmental BIA device provide FFM estimates that are comparable to FFMDXA on a group level when the appropriate equations are applied. At the individual level (i.e. in clinical practice) BIA may be a valuable tool to identify patients with low FFM as part of a malnutrition diagnosis.

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1. Introduction

Rapid loss of fat-free mass (FFM) and skeletal muscle, the major constituent of FFM, has been shown to be an independent predictor of severe toxicity following cancer treatment [1], and to negatively affect efficacy of treatment [2] and survival [3,4]. Depletion of FFM may be masked by a stable body weight or weight gain [5].
cancer patients experiencing treatment-related weight loss, weight gain during or after recovery may be characterized by an increase in fat mass (FM) rather than FFM [6]. Easy available instruments that can be used in the clinic to monitor loss of FFM are therefore needed.

Dual-energy X-ray absorptiometry (DXA) is an instrument that allows for precise whole-body and regional determination of FFM by a low X-ray exposure [7]. Therefore DXA is considered one of the reference methods for measurement of body composition. Access to DXA may, however be limited in clinical practice. Bioelectrical impedance analysis (BIA) is a more easily available method for body composition analysis as it is relatively cheap, provides rapid results and requires minimal operator training. BIA may therefore be a useful tool in clinical practice to identify patients with low FFM as a part of the diagnostic criteria for malnutrition [8]. BIA estimates body composition indirectly. A low-voltage current is passed through the body, whereby impedance (i.e. tissue resistance and reactance) is measured. Impedance data is then utilized in empiric equations to estimate body composition. Such equations have been developed for different populations and incorporate impedance data with variables such as height, weight, age and gender to calculate FFM [9].

There are several types of BIA devices commercially available. Single-frequency BIA measures impedance at one frequency, usually 50 kHz, whereas multi-frequency BIA measures impedance at several frequencies. Moreover, BIA devices can be based on a whole-body or a segmental approach. With the whole-body approach the body is viewed as a cylindrical conductor with a uniform cross-sectional area. This model is demonstrated to be valid in healthy individuals with BMI within the range 16.0–34 kg/m², provided that hydration is normal and the BIA equation used is applicable to the population studied [10]. However, since it does not take into account the differences in impedance represented by the various body segments, e.g. the trunk consisting of ~50% of the body weight and only contributing to 5–12% of the whole-body resistance, it has limited validity in populations with abnormal body composition [11]. Segmental BIA has more recently been developed to overcome the inconsistencies between the resistance and body mass of the trunk. Additional research is however required to determine whether this model is better adapted at measuring body composition under these circumstances.

There are a large number of equations available in the literature for estimation of FFM. These equations have mostly been developed in healthy euvoletic adults with a normal body composition. Therefore, the equations may yield less reliable estimates in individuals where these conditions are not met [10]. Patients with colorectal cancer (CRC) are particularly interesting as they are vulnerable to fluid imbalance and alterations in body composition post-operatively and during chemotherapy and/or radiotherapy. CRC patients often experience symptoms such as anorexia, vomiting, diarrhoea and obstipation as a result of treatment. This may adversely affect weight status as well as influence water and electrolyte balance. Furthermore, obesity and abdominal obesity are main risk factors for CRC [12] and thus, many CRC patients will have excess body weight at the time of diagnosis.

Few studies have tested the ability of BIA to estimate FFM in cancer patients using DXA as a reference method, none of which has simultaneously compared two different BIA devices with DXA. Hence, the aim of this study was to validate two different BIA devices, a whole body BIA and a segmental BIA device, against DXA in CRC patients, and to investigate the ability of 14 different empiric equations, including the equations from the manufacturers, to predict DXA FFM (FFMDXA).

2. Methods

2.1. Patients

All patients in this validation study were recruited from an ongoing randomized clinical trial, the Norwegian Dietary Guidelines and Colorectal Cancer Survival (CRC-NORDIET) study. The CRC-NORDIET study is carried out in accordance to the Helsinki Declaration and informed consent was obtained from all participants. The study was approved by the Regional Committees for Medical and Health Research Ethics (REC Protocol Approval 2011/836) and by the data protection officials at Oslo University Hospital and Akershus University Hospital. The study is registered on the National Institutes of Health Clinical Trials (www.ClinicalTrials.gov; Identifier: NCT01570010).

Eligible patients were women and men aged 50–80, with a confirmed CRC (ICD-10 18–20), and staged I-III according to the TNM staging system [13] when they entered the CRC-NORDIET study. Patients with pacemakers were excluded since current from the BIA device could possibly alter the pacemaker activity. To increase generalizability, we chose to include patients with abnormalities in body shape (for example amputations), obesity, orthopedic prosthesis/implants, chronic diseases and fluid disturbances (presence of oedema). All patients had undergone surgery for CRC within the last 4 years.

2.2. Measurements

All measurements took place between December 1st 2015 and February 1st 2016 at the Department of Nutrition, University of Oslo. The patients were instructed to fast overnight and until all measurements were completed. They were also encouraged to void their bladders before measurements. For each patient, all measurements were conducted in the morning in a sequential manner within a timeframe of 2 h.

2.3. BIA

BIA measures body composition indirectly by measuring the impedance of a low-voltage current passing through the body. The impedance consists of two components, resistance (R), the opposition of an ionic solution in both intra- and extracellular spaces and reactance (Xc), representing the capacitance from cell membranes [9]. Estimates of various body compartments, including FFM, are calculated from R or R and Xc values, based on equations, either incorporated in the software or reported in the literature.

Two different BIA devices were used, one whole-body single-frequency (50 KHz) BIA, BIA-101 (SMT Medical, Würzburg, Germany), hereby referred to as whole-body BIA, and a multifrequency segmental BIA, Seca mBCA515 (Seca, Birmingham, United Kingdom), hereby referred to as segmental BIA. For both instruments, BIA was performed under standardized conditions according to the manufacturer’s protocol. All measurements were performed with light clothing and with metal objects (e.g. jewelry, keys) being removed.

The whole-body BIA measurements were performed by placing two adhesive single-use skin electrodes (purchased from Maltron International Ltd, UK) on the right hand and foot, respectively, on the patient when lying in supine position. The device applies a current of 400 μA at constant frequency of 50 kHz.

The segmental BIA measurements were performed on patients standing barefoot on the instrument platform. The device has an integrated scale and uses four pairs of electrodes of stainless steel that are positioned at each hand and foot, through which the
current enters the limbs. The device enables segmental impedance measurements of the right arm, left arm, trunk, right leg, left leg and the right and left body side. A current of 100 µA is applied at frequencies of 1, 1.5, 2, 3, 5, 7.5, 10, 15, 20, 30, 50, 75, 100, 200, 300, 500, 750 and 1000 kHz. In the current study, measurements at 50 kHz were utilized.

For estimation of FFM, a selection of equations was used (Tables 3A and B). These included the equations incorporated into the manufacturers software, The Kyle (“Geneva”) equation [14] recommended by the European Society for Clinical Nutrition and Metabolism (ESPEN) and equations previously tested in cancer populations, see Appendix 1 [14–23]. Of the 14 equations used, 11 were originally developed in healthy volunteers [14–18,20–22,24]. The equations varied with regards to the predictor variables included (e.g. R, Xc, weight, height, age and gender) and whether they were gender-specific (indicated by “♂♂”) or not in addition to the empirically obtained parameter estimates (intercept and regression coefficients).

2.4. DXA

Total body DXA scans were performed using GE Lunar iDXA enCORE version 16 (GE Healthcare). All scans were performed using automated mode by a trained GE Lunar iDXA operator. Patients were instructed to wear a hospital gown and all jewelry was removed. Regions of interest were automatically detected by the software and verified by the operator according to instructions provided by the Lunar enCORE operator manual.

2.5. Body weight, height, body mass index (BMI) and fat-free mass index (FFMI)

Body weight was measured with light clothes and without shoes by the use of the incorporated scale in the body composition analyzer Seca mBCA515. This measured body weight was applied in both BIA devices. DXA assessment was based on the weight recorded by the DXA software according to standard procedure in the manufacturer’s manual. Height was measured by the use of a digital wireless stadiometer, Seca 285 (Seca, Birmingham, United Kingdom), and recorded in cm.

BMI (kg/m²) was calculated based on the recorded weight and height. FFM adjusted for height, FFMI (kg/m²), was calculated both for BIA (FFMBIA) and DXA (FFMDXA). To detect low FFMI, the following cut-off values were used: 15 kg/m² for women and 17 kg/m² for men [8].

2.6. Nutritional status

Nutritional status was assessed with the Patient-Generated Subjective Global Assessment (PG-SGA). This is a nutritional assessment tool, specifically designed to identify malnutrition or risk of malnutrition in cancer patients [25]. The assessment was carried out by trained registered clinical dietitians, and the scoring was controlled by one researcher (H.R). Patients were classified as either well-nourished, moderately malnourished or severely malnourished [26].

The PG-SGA form also provides information on status with regards to problems related to ascites, ankle oedema, vomiting and diarrhoea, all of which can affect the BIA raw data. Differences in raw data (R, Xc and phase angle (PhA)) and FFMI estimates between the various devices were tested for normality by the Shapiro–Wilk normality test and visual inspection of histograms.

To compare raw data assessed by the two BIA devices, paired sample t-tests and bivariate (Pearson’s) correlation analysis was performed.

FFM estimates derived from equations in the BIA softwares (hereby referred to as manufacturer’s equations) as well as selected previously published equations (see Appendix 1) were compared with DXA estimates using paired sample t-test and linear regression. To determine the most suitable equations, the following aspects of validity were considered: 1) Equality of means, determined by non-significant difference between mean FFMBIA and FFMDXA using paired sample t-tests, and 2) Ability of FFMBIA to predict FFMDXA determined by high coefficients of determination (R²) and low prediction error (SEE) using linear regression models. These equations, as well as the manufacturer’s equations were tested further for validity by constructing scatter plots, Bland–Altman plots and by calculating Lin’s concordance correlation coefficient (CCC). Bland–Altman plots were constructed to diagnose eventual bias (estimated by mean differences), limits of agreement (mean difference ± 1.96 SD) and presence of outliers in the data. Proportional bias (e.g. variation in the vertical spread of scatter points with increasing value of FFM) was assessed by analysing the Pearson’s correlation coefficient between mean of FFMBIA and FFMDXA and differences in FFMBIA and FFMDXA. CCC, which takes both equality of means and strength of linear relationship into consideration, was calculated using the following formula: \[ CCC = \frac{2(m_x \cdot m_y)}{s_x^2 + s_y^2 + C_0} \]

where \(\rho\) is the correlation coefficient \(r\) between the two methods, \(m_x\) and \(m_y\) are the means for the two methods and \(s_x^2\) and \(s_y^2\) are the corresponding variances.

For the most suitable BIA equations, as well as the manufacturer’s equations, sensitivity and specificity for identification of low and normal FFMI was calculated using DXA as reference method [27]. Low FFMI was defined as <17 kg/m² for men and <15 kg/m² for women.

SPSS 21.0 for Windows (SPSS, Chicago, IL, USA) was used for all statistical analyses. Statistical significance was defined as \(p<0.05\).

3. Results

3.1. Characteristics of the study population

All the 45 eligible patients completed the DXA scan, while 42 and 41 completed the whole-body BIA and segmental BIA assessment, respectively. Two participants were excluded from BIA measurements due to pacemakers. Furthermore, 2 patients were not able to perform the segmental BIA measurement due to upper extremity amputation and technical issues during the assessment, respectively.

Subject characteristics are shown in Table 1. Median (interquartile range (Q1–Q3)) time from surgery to assessment was 293 (190, 500) days. The participants had a wide range of BMI (18–43) kg/m², FFMI (33–72) kg, FM (11–63) kg and visceral adipose tissue (72–2734) g assessed by DXA. Thirty-nine percent of the women and 41% of the men were classified as abdominally obese (>88 cm for women and >102 cm for men). Seventeen percent were classified as moderately malnourished according to the PG-SGA assessment tool. None of the patients were classified as severely malnourished. Regarding factors possibly contributing to altered water- and electrolyte balance, 12% had clinically visible ankle oedema, 18% had an ileostomy or a colostomy and 29% had diarrhoea. None of the patients had ascites.
Mean difference ± SD between body weight assessed by DXA and by the weight scale used for BIA assessments (BIA—DXA) were 0.3 kg ± 1.0 (p = 0.046), indicating a slight overestimation of body weight using the body composition analyser Seca mBCA515 compared to DXA.

3.2. Comparison of raw data between the two BIA devices

As raw data, i.e. the resistance and the reactance values (R and Xc, respectively), are important determinants of FFM estimates, we compared R and Xc values generated by the two BIA-devices at 50 kHz. We observed significant differences in both R and Xc values between whole-body BIA and segmental BIA. Whole-body BIA gave lower mean R values compared to segmental BIA, 547 and 622 Ohm, respectively, and higher mean Xc values, 61 and 49 Ohm, respectively (Table 2). Phase angle ((PhA), i.e. (Xc/R) × (180°/π)), an indicator of functional and nutritional status, was significantly higher for whole-body BIA compared to segmental BIA, with mean PhA values of 6.4 and 4.6, respectively.

3.3. Validity of whole-body BIA to assess FFM

Whole-body BIA FFM estimates (FFM_{BIA-whole-body}) were calculated using different empiric equations including the equation derived from the manufacturer’s software. These estimates were then compared with DXA estimates.

There was a high degree of linear relationship between BIA and DXA estimates for all equations tested with R2 ranging from 0.94 to 0.97 (Table 3A). However, large discrepancies in FFM estimates were observed depending on the equations used, with mean differences ranging from −6.5 to 6.8 kg (Table 3A, Fig. 1A). Only three equations produced similar mean FFM values compared to DXA (i.e. not significantly different by paired t-tests). Those were the Gray*, Schols* and Segal* equations (Table 3A). Of these equations, the Schols* and Gray* equations demonstrated lower SEE (2.0 and 1.9 kg, respectively) than the Segal* equation (2.4 kg), and were hence considered more suitable.

The manufacturer, Gray* and Schols* equations were tested further for validity by constructing scatter plots (Fig. 2A–C), Bland–Altman plots (Fig. 2D–F) and by calculating CCC. Visual inspection of the scatter plots clearly revealed how FFM estimates varied with the corresponding DXA estimates for all equations. However, the Bland–Altman plots showed that only the Schols* equation gave satisfactory agreement with FFMDXA, indicated by no significant differences in both R and Xc values between whole-body BIA and segmental BIA. Whole-body BIA gave lower mean R values compared to segmental BIA, 547 and 622 Ohm, respectively, and higher mean Xc values, 61 and 49 Ohm, respectively (Table 2). Phase angle ((PhA), i.e. (Xc/R) × (180°/π)), an indicator of functional and nutritional status, was significantly higher for whole-body BIA compared to segmental BIA, with mean PhA values of 6.4 and 4.6, respectively.

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### Table 1

**Characteristics of the study population. Numbers are median (interquartile range (Q1–Q3)).**

<table>
<thead>
<tr>
<th>Variable</th>
<th>All subjects (n = 43)</th>
<th>Women (n = 26)</th>
<th>Men (n = 17)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>67.0 (62.0, 71.5)</td>
<td>63.5 (58.0, 71.0)</td>
<td>69.0 (65.0, 72.0)</td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White, n (%)</td>
<td>41 (97.6)</td>
<td>25 (100.0)</td>
<td>16 (94.1)</td>
</tr>
<tr>
<td>Others, n (%)</td>
<td>1 (2.4)</td>
<td>0 (0.0)</td>
<td>1 (5.9)</td>
</tr>
<tr>
<td>Ostomy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes, n (%)</td>
<td>7 (18.4)</td>
<td>2 (8.7)</td>
<td>5 (33.3)</td>
</tr>
<tr>
<td>Time since surgery, days</td>
<td>293 (189.5, 499.5)</td>
<td>316.0 (229.0, 550.0)</td>
<td>278.0 (152.0, 441.0)</td>
</tr>
</tbody>
</table>

### Table 2

**Comparison of resistance (R), reactance (Xc) and phase angle (PA) assessed by whole-body BIA and segmental BIA.**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Whole-body BIA</th>
<th>Segmental BIA</th>
<th>Correlations</th>
</tr>
</thead>
<tbody>
<tr>
<td>R, Ohm</td>
<td>546.9 (91.3)</td>
<td>622.2 (102.1)</td>
<td>&lt;0.001 0.99 &lt;0.001</td>
</tr>
<tr>
<td>Xc, Ohm</td>
<td>60.6 (13.3)</td>
<td>49.3 (6.9)</td>
<td>&lt;0.001 0.58 &lt;0.001</td>
</tr>
<tr>
<td>PhA</td>
<td>6.4 (1.3)</td>
<td>4.6 (0.6)</td>
<td>&lt;0.001 0.57 &lt;0.001</td>
</tr>
</tbody>
</table>

Abbreviations: SD, Standard deviation; r, Correlation coefficient (Spearman’s); R, Resistance; Xc, Reactance; PhA, Phase angle.

P-value: p^2; Paired sample t-tests, p^2; test for bivariate correlations (Pearson’s).
constant bias (i.e. fixed bias), no proportional bias and small limits of agreement (−3.96, 3.64 kg). For the manufacturer’s equation, there was a small fixed bias of 1.46 (−2.77, 5.69 kg) with a tendency towards proportional bias (r = 0.28, p = 0.066). For the Gray* equation, wide limits of agreement was observed (−5.50, 6.22 kg), as well as a clear proportional bias (r = 0.73, p < 0.001), with underestimation and overestimation for low and high values of FFM, respectively. CCC values (i.e. a combined measure of equality of means and strength of linear relationship), were within the range 0.95–0.99 for all three equations (data not shown), which is considered substantial. The CCC value was higher for the Schols* equation (CCC = 0.98) than the manufacturer’s and Gray* equation (CCC of 0.97 for both). Taken together, these results suggest that the Schols* equation may be superior to the other equations for estimation of FFM using whole-body BIA.

3.4. Validity of segmental BIA to assess FFM

Similar to whole-body BIA, segmental BIA FFM estimates (FFM_{BIA-segmental}) were calculated using the different empiric equations including the equation derived from the manufacturer’s software, and compared with DXA estimates.

As for whole-body BIA, all equations showed strong linear agreement with DXA estimates with R² ranging from 0.94 to 0.98 (Table 3B) but large variations in FFM estimates depending on the equation used with mean differences ranging from −11.0 to 3.4 kg (Table 3B, Fig. 1B). Only two equations gave similar FFM estimates as FFM_{DXA}, the manufacturer’s and the Heitmann* equations (Table 3B).

The manufacturer and Heitmann* equations were tested further for validity by constructing scatter plots (Fig. 3A–B), Bland–Altman plots (Fig. 3C–D) and by calculating CCC. The scatter plots demonstrated how FFM estimates co-varied with the corresponding FFM_{DXA} estimates for both equations. Furthermore, inspection of the Bland–Altman plots showed no fixed bias for any of the equations with limits of agreement ranging from −4.38 to 3.70 kg and −3.42 to 3.76 kg for the manufacturer’s and Heitmann* equations, respectively. Whereas no proportional bias was seen for the Heitmann* equation, a clear positive association was observed for the manufacturer’s equation (r = 0.69, p < 0.001) with underestimation and overestimation for low and high values of FFM, respectively. CCC values were 0.98 for both equations (data not shown). Taken together, these results suggest that the Heitmann* equation may be the superior equation for estimation of FFM using segmental BIA.

3.5. Use of BIA-derived FFM estimates to diagnose malnutrition

The type of BIA device and equation used to assess FFM may have clinical implication for the diagnosis of malnutrition. According to the new consensus statement from ESPEN [8], malnutrition can be diagnosed by either low BMI alone or a combination of unintentional weight loss (mandatory) and low BMI or low FFMI. Using the cut-off values for FFMI, 33% of our patients were identified with low FFMI based on FFM_{BIA} (Table 4). Whole-body BIA with use of the manufacturer’s equation resulted in the lowest proportion of patients with low FFMI (26%) compared to segmental BIA using the manufacturer’s equation resulting in the highest
proportion (44%). Considering DXA as a reference method to assess FFMI, the highest sensitivity (i.e. proportion with low FFMI correctly identified as such) was seen for whole-body BIA using the Schols* equation (93%), followed by segmental BIA using the manufacturer’s equation (86%). Specificity (i.e. proportion with normal FFMI correctly identified as such) was highest for whole-body BIA using the manufacturer’s equation (100%), followed by segmental BIA using the Heitmann* equation (96%) and whole-body BIA using the Schols* equation (93%). The results demonstrate that type of BIA device and equation used have implications for the proportion of patients categorized with low FFMI, and consequently the ability to correctly classify patients as malnourished.

4. Discussion

To our knowledge, this is the first study to test the validity of two different BIA devices for estimation of FFMI by using DXA as a reference method in CRC patients. Furthermore, our study is the first to evaluate various existing equations for estimation of FFMI in order to find the most appropriate BIA equation(s) for estimating FFMI in a cohort consisting solely of CRC patients.

The results of the present study show that estimating body composition from impedance data is dependent on type of BIA device and equation used. The two BIA devices tested in the current study, a whole-body BIA and a segmental BIA, resulted in significantly different R and Xc values, and hence FFM estimates, despite...
using the same equation. This suggests that the two BIA-devices should not be used interchangeably; the accuracy of the equation will relate to the type of BIA device used in addition to population-specific factors such as age, gender, ethnicity and body composition characteristics.

We observed a strong linear relationship between BIA and DXA estimates for all equations tested. However, there was a discrepancy in FFM estimates depending on the equations used. For whole-body BIA, mean difference in FFM estimates were in the range –6.5 and 6.8 kg, whereas for segmental BIA, estimates varied from –11.0 to 3.4 kg. Our results are in accordance with observations reported in a newly published review article by Haverkort et al. [28], including surgical and oncological patients, and underscore that selection of BIA equation has significant implications for the accuracy of the estimates.

For whole-body BIA, the manufacturer’s equation resulted in a small overestimation of FFM by 1.5 kg whereas for segmental BIA, no differences were detected. Despite small or no differences at the

Table 4
Performance of whole-body and segmental BIA to detect low FFMI (FFMI < 15 kg/m² for women and <17 kg/m² for men) using selected equations against DXA as reference method.

<table>
<thead>
<tr>
<th></th>
<th>DXA</th>
<th>Whole-body BIA</th>
<th>Segmental BIA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Manufacturer</td>
<td>Gray*</td>
</tr>
<tr>
<td>Low FFMI, %</td>
<td>32.6</td>
<td>25.6</td>
<td>34.9</td>
</tr>
<tr>
<td>Sensitivity, %</td>
<td>–</td>
<td>78.6</td>
<td>78.6</td>
</tr>
<tr>
<td>Specificity, %</td>
<td>–</td>
<td>100.0</td>
<td>86.2</td>
</tr>
<tr>
<td>PPV, %</td>
<td>–</td>
<td>100.0</td>
<td>73.3</td>
</tr>
<tr>
<td>NPV, %</td>
<td>–</td>
<td>90.6</td>
<td>89.3</td>
</tr>
</tbody>
</table>

Reference values: FFMI derived from DXA measurements (n = 43).
Abbreviations: BIA, Bioelectrical impedance analysis; DXA, Dual energy X-ray absorptiometry; FFMI, Fat-free mass index; PPV, Positive predictive values; NPV, Negative predictive value.
group level, we observed that both devices demonstrated limitations with regards to proportional bias when using the manufacturer’s equations. We cannot explore this bias further, as the equations incorporated into the BIA devices software are unknown, a phenomena referred to as black-box equations in the literature.

Most of the BIA equations available are developed in healthy individuals and consequently may not provide valid estimates in patient populations due to differences across populations, for instance with regard to body shape, fat fraction and hydration of FFM. It is therefore recommended not to use general equations without prior validation in the population of interest [9]. In absence of a CRC-specific equation, we chose to test the validity of BIA by use of the Geneva equation recommended by ESPEN and a selection of equations previously tested in cancer patients. Using a broad range of validity measures, our results suggest that the Schols* and Heitmann* equations are the most suitable equations for estimation of FFM using whole-body and segmental BIA, respectively. The Schols* equation was developed in patients with chronic obstructive pulmonary disease using deuterium dilution as the reference method [23], while the Heitmann* equation was developed in a healthy population using densitometry as the reference method [18]. The validity of the Schols* equation was tested in 51 patients with locally advanced or metastatic cancer of the lung or colorectum [29]. In contrast to our results, this study reported poor ability of BIA to estimate FFM in cancer patients. The discrepancy between our results and the results of Mourtzakis et al. [29] may be due to the older foot-to-foot BIA-device (Tanita Body Composition Analyzer TBF-300A) used by Mourtzakis et al. The validity of the Heitmann* equation was tested in a group of 26 patients who had undergone major abdominal surgery. Similar to our results, Jensen et al. found good agreement between FFM estimates derived from BIA and DXA [30].

In clinical practice, the manufacturer’s equations will most often be utilized. On a group level, the use of these equations resulted in small differences in FFM for the whole-body BIA device and no difference for the segmental BIA device. On the individual level, FFM estimates were within \(-2.8 \pm 5.7\) and \(-4.4 \pm 3.7\) kg, using whole-body and segmental BIA, respectively, for approximately 95% of the patients. Furthermore, both equations gave proportional bias. Taken together, these findings suggest that both BIA devices may be appropriate to determine body composition of groups, however, considering the variation in measurement accuracy at the individual level, single measurements should be interpreted with care. Furthermore, one must be aware that the direction of bias may be affected by the size of the FFM compartment.

To investigate the clinical implications of using two different BIA devices, we used FFM estimates from both devices, based on the manufacturer’s equation as well as the most suitable equations (i.e. the Schols* and Gray* equation for whole-body BIA and the Heitmann* equation for segmental BIA) to identify the proportion of patients with low FMMI according to the new consensus definition for malnutrition proposed by ESPEN [8]. We observed that the proportion of patients identified with low FMMI varied from 26 to 44% depending on device and equation used, with sensitivity ranging from 79 to 93%. Thus, some patients will not be correctly identified with low FMMI if a suboptimal equation is used. On the other hand, by using the optimal equation, whole-body BIA as well as segmental BIA (to a lower degree) may have acceptable ability to detect low FFM from a single measurement.

In the current study, including a high proportion of patients with obesity and abdominal obesity, we compared two different BIA devices relying on different approaches to estimate FFM: a whole-body approach and a segmental approach. For the whole-body approach, the various segments of the body will contribute differently to resistance values based on conductive mass. Hence, it has been suggested that segmental BIA may provide more accurate estimates of FFM in patients at extremes of BMI ranges [10]. However, this has yet to be confirmed in clinical studies [31]. Our study demonstrated that both BIA devices showed good agreement with DXA when using the appropriate equation. This was demonstrated in the group as a whole, and when looking at the obese subjects only (data not shown). Hence, we could not confirm the superiority of segmental BIA over whole-body BIA in estimation of FFM in this CRC population.

A strength of our study is the unselected inclusion of patients within the study patient cohort, e.g. inclusion of patients with abnormal body shapes, obesity, presence of chronic diseases and orthopedic prosthesis/implants. In previous validation studies, patients with these conditions have often been excluded due to possible interference with the BIA measurements, resulting in highly selected patient populations. Despite our broad inclusion, we observed high agreement between FFM estimates from both BIA devices and DXA using the appropriate equation, increasing the generalizability of our results in this patient population.

5. Conclusion

In a population of non-metastatic CRC patients, mostly consisting of Caucasian adults and with a wide range of body composition measures, FFM estimates from both whole-body and segmental BIA shows good agreement with DXA when using the appropriate equation. For whole-body BIA, the highest agreement was observed for the Schols’ equation, whereas for segmental BIA, the Heitmann* equation was the superior choice. At the individual level, both BIA-devices show acceptable ability to detect low FFM when using the optimal equation. We recommend using one of these combinations of device and equation for measuring FFM in this population.

Statement of authorship

HR and ASK had the main responsibility for data analysis and writing the manuscript. HR, ASK, CH, GF, HBH, SKB, IP, SS and RB contributed to the conception and the design of the study, analysis and interpretation of the data and drafting of the manuscript. ASK, HR, CH, GF, HBH, SKB and IP contributed to acquisition of data. All authors contributed to the writing and final approval of the manuscript.

Conflict of interest statement

All other authors declare that they have no competing interests.

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Acknowledgements

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List of abbreviations

BIA  Bioelectrical impedance analysis
BMI  Body mass index
CCC  Concordance correlation coefficient
CRC  Colorectal cancer
DXA  Dual energy X-ray absorptiometry
ESR  European Society for Clinical Nutrition and Metabolism
FFM  Fat-free mass
FFMI  Fat-free mass index
FM  Fat mass
ICD  International classification of diseases and related health problems
NPV  Negative predictive value
N  Number
PG-SGA  Patient-generated subjective global assessment
PhA  Phase angle
PPV  Positive predictive value
R  Resistance
SD  Standard deviation
SEE  Standard error of the estimate
TNM  Tumor node metastasis
Xc  Reactance

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.clnu.2016.12.028.

References

Appendix 1.
Existing empiric equations applied in the current study. The table provides information about the population in which the equations are
developed, type of BIA instrument and reference method used as well as components included in the various equations.

<table>
<thead>
<tr>
<th>Author (year)</th>
<th>Population developed</th>
<th>BIA instrument</th>
<th>Phase-sensitive</th>
<th>Reference method</th>
<th>Equation</th>
<th>Sex</th>
<th>Age</th>
<th>Weight</th>
<th>Height</th>
<th>R</th>
<th>Xc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bosy-Westphal (2013) (“Manufacturer’s equation”, segmental BIA) (1)</td>
<td>Healthy adults (18-65 years) with BMI; 18.5-35.0 kg/m² (n=124)</td>
<td>Segmental MF-BIA (Seca mBCA515, Seca, Birmingham, United Kingdom)</td>
<td>x</td>
<td>Four-compartment model (ADP, D₂O, DXA)</td>
<td>Unknown</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td></td>
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<tr>
<td>Unknown (“Manufacturer’s equation”, whole-body BIA)</td>
<td>Unknown</td>
<td>Whole-body SF-BIA (BIA-model 101, SMT Medical, Würzburg, Germany)</td>
<td>x</td>
<td>Unknown</td>
<td>Unknown</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
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<tr>
<td>Kyle (2001) (“The Geneva equation”) (2)</td>
<td>Healthy adults (20-94 years) with BMI; 17.0-33.8 kg/m² (n=343)</td>
<td>Whole-body SF-BIA (Xitron 4000B, Xitron Technologies, San Diego, CA, USA)</td>
<td>x</td>
<td>DXA (Hologic QDR-4500)</td>
<td>FFM = -4.104 + (0.518 x Ht²/R₅₀) + (0.231 x weight) + (0.130 x Xc₅₀) + (4.229 x sex)</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
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<tr>
<td>Lukaski (1986) (3, 4)²</td>
<td>Healthy adults (18-50 years) with FFM; 34.4-96.3 kg (n=114)</td>
<td>Whole-body SF-BIA (RJL-model 101, RJL Systems, Detroit, MI, USA)</td>
<td>x</td>
<td>Densitometry</td>
<td>FFM = (0.810 x Ht²/R₅₀) + 6.39</td>
<td>x</td>
<td>x</td>
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<tr>
<td>Lukaski* (1986) (3)²</td>
<td>Healthy adults (18-50 years) with FFM; 34.4-96.3 kg (n=114)</td>
<td>Whole-body SF-BIA (RJL-model 101, RJL Systems, Detroit, MI, USA)</td>
<td>x</td>
<td>Densitometry</td>
<td>FFM for men = (0.827 x Ht²/R₅₀) + 5.214</td>
<td>x</td>
<td>x</td>
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<tr>
<td>Method</td>
<td>Sample Description</td>
<td>FFM Calculation</td>
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<tr>
<td>Whole-body SF-BIA</td>
<td>Healthy adults (17-62 years) with FM; 3.0-56.0% (n=1567)</td>
<td>$\text{FFM for men} = (0.00132 \times \text{Ht}^2) - (0.04394 \times \text{R}_{50}) + (0.30520 \times \text{weight}) - (0.16760 \times \text{age}) + 22.66827$</td>
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<tr>
<td>Whole-body SF-BIA</td>
<td>Healthy adults (35-65 years) (n=139)</td>
<td>$\text{FFM for women} = (0.00108 \times \text{Ht}^2) - (0.02090 \times \text{R}_{50}) + (0.23199 \times \text{weight}) - (0.06777 \times \text{age}) + 14.59453$</td>
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<tr>
<td>Whole-body SF-BIA</td>
<td>Healthy adults (35-65 years) (n=139)</td>
<td>$\text{FFM} = (0.295 \times \text{Ht}^2/R_{50}) + (0.204 \times \text{weight}) + (5.009 \times \text{sex}) - (0.076 \times \text{age}) + (0.227 \times \text{Ht}) - 17.04$</td>
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<td>Four-compartment model (Body potassium (whole body counting) and total body water (dilutometry))</td>
<td></td>
<td>$\text{FFM for men} = (0.244 \times \text{Ht}^2/R_{50}) + (0.270 \times \text{weight}) + (0.284 \times \text{Ht}) - 28.02$</td>
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<tr>
<td>Deurenberg (1990) (7)</td>
<td>Healthy adults (60-83 years) (n=72)</td>
<td>Whole-body SF-BIA (RJL-model 101, RJL Systems, Detroit, MI, USA)</td>
<td>x</td>
<td>Densitometry</td>
<td>FFM for women = ((0.411 \times Ht^2/R50) + (0.141 \times \text{weight}) + (0.267 \times Ht) - 28.61)</td>
<td>x</td>
<td>x</td>
<td>x</td>
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<tr>
<td>Deurenberg (1991) (8, 9)⁵</td>
<td>Healthy adults (&gt;16 years) (n=661)</td>
<td>Whole-body SF-BIA (RJL-model 101, RJL Systems, Detroit, MI, USA)</td>
<td>x</td>
<td>Densitometry</td>
<td>FFM = ((0.671 \times Ht^2/R50) + (3.1 \times \text{sex}) + 3.9)</td>
<td>x</td>
<td>x</td>
<td>x</td>
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<tr>
<td>Lohman* (1988) (10, 11)⁶</td>
<td>Healthy adults (18-29 years) (n=153)</td>
<td>Whole-body SF-BIA (Unknown model, Valhalla Scientific, San Diego, CA, USA)</td>
<td>Unknown</td>
<td>Densitometry</td>
<td>FFM for men = ((0.485 \times Ht^2/R50) + (0.338 \times \text{weight}) + 5.32) FFM for women = ((0.475 \times Ht^2/R50) + (0.295 \times \text{weight}) + 5.49)</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Schols* (12)⁷</td>
<td>COPD patients (n=117)</td>
<td>Whole-body SF-BIA (Unknown manufacturer and model)</td>
<td>Unknown</td>
<td>Dilutometry</td>
<td>FFM for men: (8.383 + (0.465 \times Ht^2/R50) + (0.213 \times \text{weight}) - 12.44)</td>
<td>x</td>
<td>x</td>
<td>x</td>
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</tbody>
</table>
Indicates gender-specific equations; women = 0, men = 1. None of the equations tested included anthropometric measures (e.g., thigh or ankle circumference).

Both the equations incorporated into the whole-body BIA and segmental BIA were unknown. However, for segmental BIA the components were known.

The equation tested was developed in 47 male volunteers and cross-validated against a previous linear regression equation developed in 37 male volunteers (15). The results of the cross-validation showed no significant difference between the slopes or the intercepts of the individual regression lines. Furthermore, no significant difference was found between the slopes or the intercepts of the individual regression lines for males (n=84) and females (n=67).

The equation tested is the best-fitting regression line from 4 laboratories pooled but with separate equations for men and women. A quadruple cross-validation was performed to determine the reproducibility across laboratories of the relationship between densitometrically determined FFM and FFM predicted from BIA and other variables.

The equation is taken from the supplementary material of the review article by Haverkort et al (2015) (4). The original article by Heitmann et al (1990) (6) is not available at www.pubmed.gov (abstract only).

The original article by Deurenberg et al (8) is not available at www.pubmed.gov (abstract only). The equation used in the present study is taken from the review article by Kyle et al (2004) (9).
The equation tested (“The Lohman equation”) is taken from an article by Graves et al (1989) (11) referring to unpublished results from the 1986 Valhalla inter-laboratory investigation of BIA (10).

The equation tested (“The Schols* equation”) is taken from an article by Steiner et al (2002) (12) referring to sex-specific regression equations derived from Schols et al (University of Maastricht, Maastricht, the Netherlands) (personal communication).

Abbreviations: BIA; Bioelectrical impedance analysis, DXA; Dual energy x-ray absorptiometry, ADP; Air displacement plethysmography, D2O; Deuterium oxide, SF; Single-frequency, MF; Multi-frequency, BMI; Body mass index, FFM; Fat free mass, FM; Fat mass, COPD; Chronic obstructive pulmonary disease, Ht; Height, R50; Resistance at 50 kHz, Xc; reactance at 50 kHz.
References