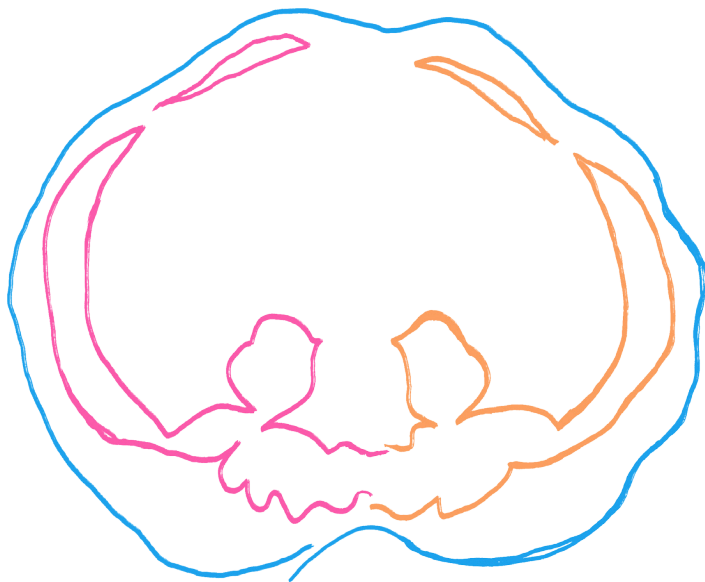# IMAGING
ALL THE PEOPLE

Associations of computed tomography-based
body composition with mortality and fatigue in
stage I-III colorectal cancer patients

Harm van Baar

Propositions

1. BMI is an outdated measure in the clinical setting.
   (this thesis)
2. Skeletal muscle quality goes above quantity for health.
   (this thesis)
3. All scientists are biased when interpreting study results.
4. The maximum number of (co-)authors allowed on scientific papers should be limited to eight.
5. Using p-value alone to conclude whether there is an association, correlation or effect is bad scientific practice.
6. Celiac disease is unrecognized in too many children, therefore universal early childhood screening is critical.
7. Adolescents should not be allowed on an e-bike before adulthood.

Propositions belonging to the thesis, entitled

Imaging all the people

Associations of computed tomography-based body composition with mortality and fatigue in stage I-III colorectal cancer patients

Harm van Baar

Wageningen, 21 October 2021

Imaging all the people

Associations of computed tomography-based body composition with mortality and fatigue in stage I-III colorectal cancer patients

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Imaging all the people

Associations of computed tomography-based body composition with
mortality and fatigue in stage I-III colorectal cancer patients

Harm van Baar

Thesis
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Table of contents

Chapter 1

General introduction

Colorectal cancer (CRC) is the third most common cancer worldwide (1). Mainly due to the implementation of screening practices and improved treatment, CRC survival rates have increased for several decades (2). For stage I-III (non-metastatic) CRC patients, 5-year survival is currently >70% (2). Identifying modifiable factors associated with worse prognosis could contribute to further improvement of cancer outcomes such as mortality and recurrence in patients with localized CRC. One potential modifiable factor might be body composition. Because overweight and obesity are major risk factors for CRC development, many CRC patients are overweight or obese (3), which could lead to worse prognosis. Furthermore, various, mostly small-scale, studies predating this PhD project observed an association of low skeletal muscle mass with increased mortality risk in various (mixed) cancer populations (4-10).

With survival rates increasing, the importance of improving quality of life (QoL) of CRC survivors by reducing (long-term) side-effects of the cancer and the anticancer treatment is being recognized. One of the most common adverse effects of cancer and cancer treatment is fatigue (11). Up to 85% of the CRC patients experience fatigue during and shortly after treatment (12, 13) and up to 40% of CRC patients experience fatigue in the first 5 years after diagnosis (14). Fatigue has a negative impact on work, social relationships, mood and overall QoL (15). The fatigue experienced during and post-treatment is thought to have a multi-factorial etiology, with inflammation probably playing a key role in the underlying mechanism (15). Contributing factors for fatigue that have been found so far include: treatment (radio- and chemotherapy), stage of disease, presence of (multiple) comorbidities, specific medications with sedating side-effects, psychological factors (e.g. depression), decreased physical activity, and malnutrition (15-18). To improve non-pharmacological (e.g. behavioral) treatment strategies for fatigue, identifying modifiable contributing factors is key. Body composition may be one of such factors, since obesity has been linked to fatigue in non-cancer populations (19) and Kilgour *et al* (20) observed an association between low skeletal muscle mass and increased fatigue levels among 84 stage III–IV gastrointestinal or non-small cell lung cancer patients. Up till now, the association between body composition and fatigue experienced by cancer patients, including CRC, has hardly been studied.

Over the last decade, the relationship of skeletal muscle and adipose tissue mass, estimated from computed tomography (CT) images (**see Text Box 1**) with cancer outcomes has received much attention. Because many cancer patients have CT images in their medical files from diagnostic workup, studies started using this technique to investigate the association of skeletal muscle mass with various outcomes including mortality, complications after surgery, length of hospital stay, QoL and fatigue (4, 5, 7, 8, 10, 21-24).

Additionally, various studies started investigating the association of the size of adipose tissue depots and the distribution of adipose tissue into subcutaneous and visceral depots with mortality using CT images (25-30). These measures have many advantages over body mass index (BMI, weight divided by height squared), the most commonly used method to assess body fatness. First, BMI does not differentiate between fat and muscle tissue, even though two persons from the same sex have the same BMI, muscle and fat mass assessed by CT images can vary largely (see **Figure 1**). Second, BMI does not give information about distribution of adipose tissue into different adipose tissue depots: the amount of visceral adipose tissue (VAT, i.e. belly fat) and subcutaneous adipose tissue (SAT, i.e. fat stored just beneath the skin), while CT images do. The distribution of VAT and SAT can be different between two persons with a similar BMI and from the same sex, as illustrated in **Figure 2**. The distinction between VAT and SAT is relevant, because VAT and SAT have different physiological roles in the body. VAT is more metabolically active than SAT and is a risk factor for cardiovascular diseases and the development of insulin resistance (31), while SAT is mainly considered to be a buffer for circulating fatty acids and triglycerides (32). Because of this, VAT and SAT should be considered separately when studying the role of adipose tissue.

Text Box 1: Assessing body composition with Computed Tomography (CT)
Together with magnetic resonance imaging (MRI), CT is considered the gold standard for assessing body composition (33). With CT imaging, it is possible to identify the various tissues using the radiodensity/attenuation properties of those tissues. These radiodensity properties correspond to a specific level of grey values within the image and are measured as Hounsfield Units (HU) (34). Air has a radiodensity of -1000 HU, water as 0 HU and bone tissue as +1000 HU. Although different HU ranges have been used for skeletal muscle and adipose tissue, the most common ranges used at the moment are: -29 to +150 HU for muscle; -150 to -30 HU for visceral adipose tissue (VAT); -190 to -30 HU for subcutaneous adipose tissue (SAT) and intermuscular adipose tissue (IMAT, accumulated fat between muscle groups) (33). Additionally, the skeletal muscle radiodensity (SMR) can be measured by calculating the mean density (in HU) of the total skeletal muscle cross-sectional area. In 2000, a study observed a high correlation between the mean radiodensity of skeletal muscle and fat infiltration within the skeletal muscle, where lower levels of radiodensity indicate more fat infiltration (35).
Because total body quantification of muscle and adipose tissue with CT and MRI is laborious, earlier studies have investigated which single image is most representative for muscle and adipose tissue content of the total body. Already in 1986, Kvist *et al* (36) found a high correlation (r = 0.99) between adipose tissue from a single image at the level of the fourth-fifth lumbar vertebrae (L4-L5) and 22-image-based adipose tissue volume. In 2004 Shen *et al* (37) found high correlations (r=0.97) between skeletal muscle and adipose tissue surface from a single image assessed 5 cm above L4-5 and total body skeletal muscle and adipose tissue volumes. In 2008 Mourtzakis *et al* (33) found high correlations (r = 0.83) between skeletal muscle and adipose tissue surface from a single image assessed at L3 and total body skeletal muscle and adipose tissue volumes. In the last decade the level of the third lumbar vertebrae (L3) has become the most commonly used landmark in the abdomen to quantify skeletal muscle or adipose tissue and skeletal muscle radiodensity (38, 39).
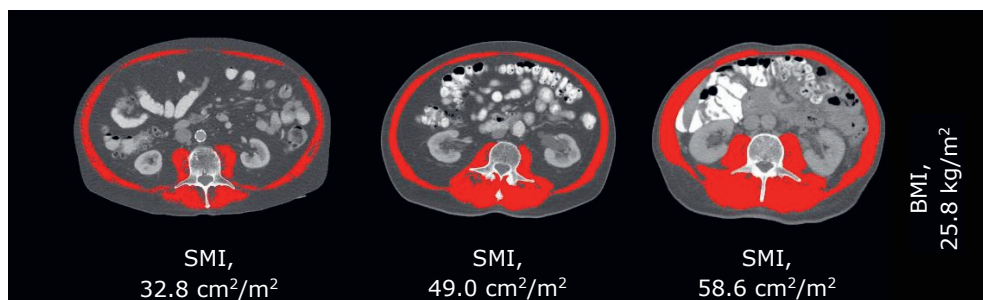
Figure 1. Differences in skeletal muscle mass index (SMI, skeletal muscle cross-sectional surface area divided by height squared in meters) at the level of third lumbar vertebrae among three male colorectal cancer patients with a BMI of 25.8 kg/m^2. (Pictures from own database)
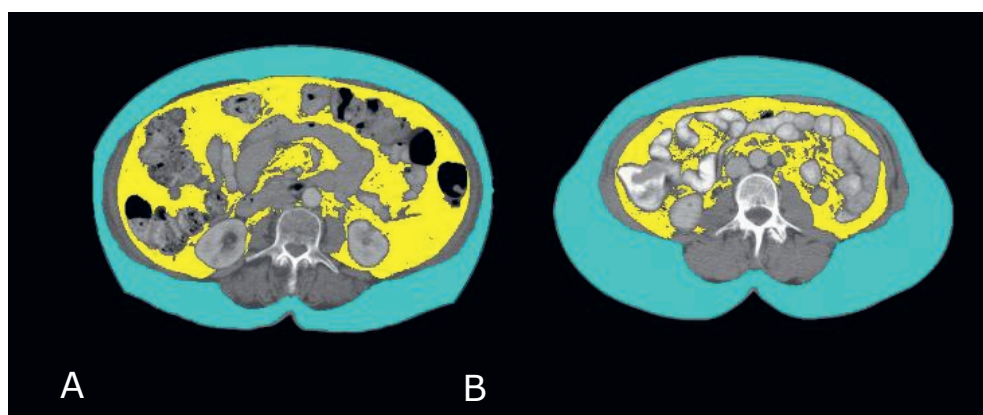
Figure 2. Differences in distribution of visceral and subcutaneous adipose tissue at the level of third lumbar vertebrae among two female colorectal cancer patients with an identical BMI of 28.7 kg/m^2. Yellow is visceral adipose tissue, blue subcutaneous adipose tissue. For patient A the visceral and subcutaneous adipose tissue cross-sectional surface area are 215 cm^2 and 235 cm^2 respectively, for patient B 82 cm^2 and 299 cm^2. (Pictures from own database)

Low skeletal muscle index, mortality and fatigue in stage I-III colorectal cancer

Low skeletal muscle mass, often assessed by means of the skeletal muscle index (SMI, skeletal muscle cross-sectional surface area divided by height squared in meters), can be caused by various factors like: ageing, prolonged bed rest or a sedentary lifestyle, chronic diseases and certain drug treatments (40). In 2015, when this PhD project started, several papers had been published on the relationship between low SMI and mortality in cancer patients. Several (4, 5, 7, 8, 10, 21), but not all (6, 9) studies found a statistically significant association of low SMI with increased mortality risk (range of hazard ratios [HRs]: 1.15-

4.20). The focus of those studies was mainly metastatic cancer patients with low survival rates (4-10). Severe weight loss and muscle wasting (cachexia) are highly prevalent among stage IV CRC patients, due to metabolic alterations induced by the tumor (41). The association between low muscle mass and increased mortality might therefore be more likely the consequence of tumor progression than of the muscle mass loss itself. Among patients with stage I–III CRC cancer, cachexia is less common; low skeletal muscle mass is more likely to be related to other factors such as ageing and/or a sedentary lifestyle. Because there is also a large difference in survival rates between stages I-III and stage IV, the association between low SMI and mortality might also be different between stage I-III and stage IV CRC. Of the previously mentioned studies, only one was performed exclusively in stage I-III CRC patients (21). That study observed an association between low SMI and higher mortality risk in 220 stage I-III CRC patients (low vs normal SMI: HR 2.18; 95% CI, 1.20-3.94). However, larger studies among stage I-III CRC patients exclusively are needed to confirm these results.

Measures of body composition may not only be associated with survival, but also with fatigue. Already in 2010, an association between lower levels of SMI (measured by dual-energy X-ray absorptiometry (DEXA)) and higher levels of fatigue was observed among 84 stage III–IV gastrointestinal or non-small cell lung cancer patients (per 1 kg/m^2 increase, β -7.5; 95% CI -13.0 to -2.0) (20). Before the start of this PhD trajectory in 2015, no other study had been performed to confirm this finding in a larger study.

Low skeletal muscle radiodensity, mortality and fatigue in stage I-III colorectal cancer

Besides skeletal muscle quantity, there has been an increasing interest in the relation of skeletal muscle radiodensity (SMR, see **Text box 1**) with mortality. Martin *et al* (5) published the first study investigating the association between low SMR and mortality among cancer patients, and observed low SMR to be strongly associated with increased mortality risk (low vs normal SMI: HR 1.36; 95% CI, 1.19-1.55) in a mixed group of cancer patients of various diagnoses, mostly with very poor prognosis. At the start of this PhD project, however, no study had been performed exclusively among stage I-III CRC patients.

The association between low SMR and fatigue had also not been investigated before the start of this PhD project. Lower levels of SMR are usually seen in patients with an inferior overall condition (i.e. multiple comorbidities, higher American Society of Anesthesiologists (ASA) physical status score) (42, 43). Because of this, patients with low SMR may experience more (long-term) side-effects from treatment, including fatigue.

High adipose tissue, mortality and fatigue in stage I-III colorectal cancer

In 2015, when this PhD project started, only a few papers had been published on associations of (CT-based) adipose tissue levels with mortality in CRC patients (25-30). These studies focused only on VAT; study populations were relatively small (n between 66 and 273); and used different methods to quantify VAT (i.e. different HU ranges to identify VAT, the use of a single or multiple CT images) and different cut-off values to identify high VAT. Likely because of those inconsistencies between studies, results about the associations of VAT and mortality were inconsistent. Larger studies, using a standardized protocol to assess VAT and SAT with CT, were therefore needed to gain more knowledge about the relationship of different adipose tissue depots with mortality.

Furthermore, the relationship of VAT and SAT with fatigue, needed to be investigated. Earlier studies suggested a possible association between BMI and fatigue in cancer patients, but results were inconclusive (44, 45). These inconclusive findings might (partly) be explained because BMI cannot distinguish between VAT and SAT. With visceral obesity being associated with low-grade inflammation (46) and the hypothesis that low-grade inflammation is playing an important role in the underlying mechanism of fatigue (15), VAT could be more strongly related to fatigue than SAT.

Lifestyle and fatigue in stage I-III colorectal cancer

In 2018, the World Cancer Research Fund / American Institute for Cancer Research (WCRF/AICR) published their updated lifestyle recommendations for cancer prevention and cancer survivors (47). The WCRF/AICR recommendations include guidelines on body weight, physical activity, and a healthy diet. Two studies among stage I-III CRC survivors had observed an association between higher adherence to the 2007 WCRF/AICR lifestyle recommendations for cancer survivors and less fatigue 5 and 8 years post-diagnosis (48, 49). None of these studies investigated the association between adherence to the WCRF/AICR recommendations and fatigue experienced during or shortly after treatment, when fatigue prevalence is highest (12). As mentioned before, inflammation is thought to play an important role in the underlying mechanism of fatigue in cancer patients (15). Better adherence to the WCRF/AICR lifestyle recommendations for cancer prevention on physical activity, body weight and healthy diet may potentially impact fatigue by attenuating systemic inflammatory processes (50-52). However, the potential mediating role of inflammation needs to be confirmed at present.

Aims of the thesis

The overall objectives of this thesis are 1) to increase knowledge about the association of body composition in stage I-III CRC patients with both mortality and fatigue by using CT images to quantify skeletal muscle mass, skeletal muscle radiodensity and adipose tissue, and 2) to increase knowledge about the association of adherence to the WCRF/AICR lifestyle recommendations with fatigue among stage I-III CRC and to elucidate the underlying mechanism.

This resulted in the following aims for this thesis:

1. To investigate the association of skeletal muscle mass, skeletal muscle radiodensity, and adipose tissue with mortality among a large population of stage I-III CRC patients.
2. To investigate the association of skeletal muscle mass, skeletal muscle radiodensity and adipose tissue with fatigue among stage I-III CRC patients.
3. To investigate the association between adherence to the WCRF/AICR lifestyle recommendations and fatigue experienced during or shortly after treatment among stage I-III CRC patients and to determine if inflammation is (partly) mediating this association.

Thesis outline

- In **Chapter 2** the association between skeletal muscle radiodensity and overall mortality, CRC-specific mortality and disease-free survival is investigated in stage I-III CRC patients. *(aim 1)*
- **Chapter 3** aims to investigate the associations of abdominal skeletal muscle index, visceral adipose tissue, and subcutaneous adipose tissue with overall mortality among men and women with stage I–III CRC. *(aim 1)*
- In **Chapter 4** it is described how skeletal muscle index, skeletal muscle radiodensity, visceral adipose tissue, and subcutaneous adipose tissue at diagnosis are associated with fatigue up to 24 months post-diagnosis in stage I-III CRC patients. *(aim 2)*
- **Chapter 5** examines if inflammation mediates a potential association between adherence to the WCRF/AICR lifestyle recommendations, and fatigue during and shortly after cancer treatment. *(aim 3)*
- **Chapter 6** is the final chapter and includes the general discussion. The main findings of the studies are summarized, methodological considerations are discussed, and recommendations for clinical practice and future research opportunities are provided.

References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: a cancer journal for clinicians. 2018;68(6):394-424.
2. Siegel RL, Miller KD, Fedewa SA, Ahnen DJ, Meester RG, Barzi A, et al. Colorectal cancer statistics, 2017. CA: a cancer journal for clinicians. 2017;67(3):177-93.
3. Abar L, Vieira AR, Aune D, Sobiecki JG, Vingeliene S, Polemiti E, et al. Height and body fatness and colorectal cancer risk: an update of the WCRF–AICR systematic review of published prospective studies. European journal of nutrition. 2018;57(5):1701-20.
4. Fujiwara N, Nakagawa H, Kudo Y, Tateishi R, Taguri M, Watadani T, et al. Sarcopenia, intramuscular fat deposition, and visceral adiposity independently predict the outcomes of hepatocellular carcinoma. Journal of hepatology. 2015;63(1):131-40.
5. Martin L, Birdsell L, MacDonald N, Reiman T, Clandinin MT, McCargar LJ, et al. Cancer cachexia in the age of obesity: skeletal muscle depletion is a powerful prognostic factor, independent of body mass index. Journal of Clinical Oncology. 2013:JCO. 2012.45. 722.
6. Lodewick TM, Nijnatten TJ, Dam RM, Mierlo K, Dello SA, Neumann UP, et al. Are sarcopenia, obesity and sarcopenic obesity predictive of outcome in patients with colorectal liver metastases? HPB. 2015;17(5):438-46.
7. Prado CM, Lieffers JR, McCargar LJ, Reiman T, Sawyer MB, Martin L, et al. Prevalence and clinical implications of sarcopenic obesity in patients with solid tumours of the respiratory and gastrointestinal tracts: a population-based study. Lancet Oncol. 2008;9(7):629-35.
8. Sharma P, Zargar-Shoshtari K, Caracciolo JT, Fishman M, Poch MA, Pow-Sang J, et al., editors. Sarcopenia as a predictor of overall survival after cytoreductive nephrectomy for metastatic renal cell carcinoma. Urologic Oncology: Seminars and Original Investigations; 2015: Elsevier.
9. Thoresen L, Frykholm G, Lydersen S, Ulveland H, Baracos V, Prado CMM, et al. Nutritional status, cachexia and survival in patients with advanced colorectal carcinoma. Different assessment criteria for nutritional status provide unequal results. Clinical Nutrition. 2013;32(1):65-72.
10. Van Vledder MG, Levolger S, Ayez N, Verhoef C, Tran TCK, Ijzermans JNM. Body composition and outcome in patients undergoing resection of colorectal liver metastases. British Journal of Surgery. 2012;99(4):550-7.
11. Berger AM, Mooney K, Alvarez-Perez A, Breitbart WS, Carpenter KM, Cella D, et al. Cancer-related fatigue, version 2.2015. Journal of the National Comprehensive Cancer Network. 2015;13(8):1012-39.
12. Bower JE. Cancer-related fatigue—mechanisms, risk factors, and treatments. Nature reviews Clinical oncology. 2014;11(10):597.
13. Hofman M, Ryan JL, Figueroa-Moseley CD, Jean-Pierre P, Morrow GR. Cancer-related fatigue: the scale of the problem. The oncologist. 2007;12(Supplement 1):4-10.
14. Thong MS, Mols F, Wang XS, Lemmens VE, Smilde TJ, van de Poll-Franse LV. Quantifying fatigue in (long-term) colorectal cancer survivors: a study from the population-based patient reported

outcomes following initial treatment and long term evaluation of survivorship registry. European journal of cancer. 2013;49(8):1957-66.

15. Bower JE. Cancer-related fatigue—mechanisms, risk factors, and treatments. Nature Reviews Clinical Oncology. 2014;11:597.

16. Mitchell SA. Cancer-related fatigue: state of the science. PM&R. 2010;2(5):364-83.

17. Aapro M, Scotte F, Bouillet T, Currow D, Vigano A. A Practical Approach to Fatigue Management in Colorectal Cancer. Clinical colorectal cancer. 2017;16(4):275-85.

18. Vissers PAJ, Thong MSY, Pouwer F, Zanders MMJ, Coebergh JWW, van de Poll-Franse LV. The impact of comorbidity on Health-Related Quality of Life among cancer survivors: analyses of data from the PROFILES registry. Journal of Cancer Survivorship. 2013;7(4):602-13.

19. Vgontzas AN, Bixler EO, Chrousos GP. Obesity-related sleepiness and fatigue: the role of the stress system and cytokines. Annals of the New York Academy of Sciences. 2006;1083(1):329-44.

20. Kilgour RD, Vigano A, Trutschnigg B, Hornby L, Lucar E, Bacon SL, et al. Cancer-related fatigue: the impact of skeletal muscle mass and strength in patients with advanced cancer. Journal of Cachexia, Sarcopenia and Muscle. 2010;1(2):177-85.

21. Miyamoto Y, Baba Y, Sakamoto Y, Ohuchi M, Tokunaga R, Kurashige J, et al. Sarcopenia is a negative prognostic factor after curative resection of colorectal cancer. Annals of surgical oncology. 2015;22(8):2663-8.

22. van Vugt JL, van den Braak RRC, Lalmahomed ZS, Vrijland WW, Dekker JW, Zimmerman DD, et al. Impact of low skeletal muscle mass and density on short and long-term outcome after resection of stage I-III colorectal cancer. European Journal of Surgical Oncology. 2018;44(9):1354-60.

23. Aro R, Mäkäräinen-Uhlbäck E, Ämmälä N, Rautio T, Ohtonen P, Saarnio J, et al. The impact of sarcopenia and myosteatosis on postoperative outcomes and 5-year survival in curatively operated colorectal cancer patients–A retrospective register study. European Journal of Surgical Oncology. 2020.

24. van Roekel EH, Bours MJL, te Molder MEM, Breedveld-Peters JJL, Olde Damink SWM, Schouten LJ, et al. Associations of adipose and muscle tissue parameters at colorectal cancer diagnosis with long-term health-related quality of life. Quality of Life Research. 2017;26(7):1745-59.

25. Guiu B, Petit JM, Bonnetain F, Ladoire S, Guiu S, Cercueil JP, et al. Visceral fat area is an independent predictive biomarker of outcome after first-line bevacizumab-based treatment in metastatic colorectal cancer. Gut. 2010;59(3):341-7.

26. Lee CS, Murphy DJ, McMahon C, Nolan B, Cullen G, Mulcahy H, et al. Visceral Adiposity is a Risk Factor for Poor Prognosis in Colorectal Cancer Patients Receiving Adjuvant Chemotherapy. Journal of Gastrointestinal Cancer. 2015;46(3):243-50.

27. Kang J, Baek S-E, Kim T, Hur H, Min BS, Lim JS, et al. Impact of fat obesity on laparoscopic total mesorectal excision: more reliable indicator than body mass index. International journal of colorectal disease. 2012;27(4):497-505.

28. Yamamoto N, Fujii S, Sato T, Oshima T, Rino Y, Kunisaki C, et al. Impact of body mass index and visceral adiposity on outcomes in colorectal cancer. Asia-Pacific J Clin Oncol. 2012;8(4):337-45.

29. Clark W, Siegel EM, Chen YA, Zhao X, Parsons CM, Hernandez JM, et al. Quantitative measures of visceral adiposity and body mass index in predicting rectal cancer outcomes after neoadjuvant chemoradiation. Journal of the American College of Surgeons. 2013;216(6):1070-81.

30. Rickles AS, Iannuzzi JC, Mironov O, Deeb AP, Sharma A, Fleming FJ, et al. Visceral Obesity and Colorectal Cancer: Are We Missing the Boat with BMI? Journal of Gastrointestinal Surgery. 2013;17(1):133-43.

31. Hamdy O, Porramatikul S, Al-Ozairi E. Metabolic obesity: the paradox between visceral and subcutaneous fat. Current diabetes reviews. 2006;2(4):367-73.

32. Freedland ES. Role of a critical visceral adipose tissue threshold (CVATT) in metabolic syndrome: implications for controlling dietary carbohydrates: a review. Nutrition & metabolism. 2004;1(1):1-24.

33. Mourtzakis M, Prado CM, Lieffers JR, Reiman T, McCargar LJ, Baracos VE. A practical and precise approach to quantification of body composition in cancer patients using computed tomography images acquired during routine care. Applied Physiology, Nutrition, and Metabolism. 2008;33(5):997-1006.

34. Goodpaster BH, Thaete FL, Kelley DE. Composition of Skeletal Muscle Evaluated with Computed Tomography. Annals of the New York Academy of Sciences. 2000;904(1):18-24.

35. Goodpaster BH, Kelley DE, Thaete FL, He J, Ross R. Skeletal muscle attenuation determined by computed tomography is associated with skeletal muscle lipid content. Journal of Applied Physiology. 2000;89(1):104-10.

36. Kvist H, Sjostrom L, Tylen U. Adipose tissue volume determinations in women by computed tomography: technical considerations. International journal of obesity. 1986;10(1):53-67.

37. Shen W, Punyanitya M, Wang Z, Gallagher D, Onge M-PS-, Albu J, et al. Total body skeletal muscle and adipose tissue volumes: estimation from a single abdominal cross-sectional image. Journal of Applied Physiology. 2004;97(6):2333-8.

38. Aleixo G, Shachar S, Nyrop K, Muss H, Malpica L, Williams G. Myosteatosis and prognosis in cancer: Systematic review and meta-analysis. Critical Reviews in Oncology/Hematology. 2020;145:102839.

39. Hopkins JJ, Skubleny D, Bigam DL, Baracos VE, Eurich DT, Sawyer MB. Barriers to the Interpretation of Body Composition in Colorectal Cancer: A Review of the Methodological Inconsistency and Complexity of the CT-Defined Body Habitus. Annals of surgical oncology. 2018;25(5):1381-94.

40. Cruz-Jentoft AJ, Baeyens JP, Bauer JM, Boirie Y, Cederholm T, Landi F, et al. Sarcopenia: European consensus on definition and diagnosis Report of the European Working Group on Sarcopenia in Older People. Age and ageing. 2010;39(4):412-23.

41. Wie G-A, Cho Y-A, Kim S-Y, Kim S-M, Bae J-M, Joung H. Prevalence and risk factors of malnutrition among cancer patients according to tumor location and stage in the National Cancer Center in Korea. Nutrition. 2010;26(3):263-8.

42. Margadant C, Bruns E, Sloothaak D, van Duijvendijk P, van Raamt A, van der Zaag H, et al. Lower muscle density is associated with major postoperative complications in older patients after

1

surgery for colorectal cancer. European Journal of Surgical Oncology (EJSO). 2016;42(11):1654-9.

43. Xiao J, Caan BJ, Weltzien E, Cespedes Feliciano EM, Kroenke CH, Meyerhardt JA, et al. Associations of pre-existing co-morbidities with skeletal muscle mass and radiodensity in patients with non-metastatic colorectal cancer. Journal of cachexia, sarcopenia and muscle. 2018.

44. Huang X, Zhang Q, Kang X, Song Y, Zhao W. Factors associated with cancer-related fatigue in breast cancer patients undergoing endocrine therapy in an urban setting: a cross-sectional study. BMC Cancer. 2010;10(1):453.

45. Schmidt ME, Chang-Claude J, Seibold P, Vrieling A, Heinz J, Flesch-Janys D, et al. Determinants of long-term fatigue in breast cancer survivors: results of a prospective patient cohort study. Psycho-Oncology. 2015;24(1):40-6.

46. Park HS, Park JY, Yu R. Relationship of obesity and visceral adiposity with serum concentrations of CRP, TNF-α and IL-6. Diabetes research and clinical practice. 2005;69(1):29-35.

47. World Cancer Research Fund/American Institute for Cancer Research. Diet, Nutrition, Physical Activity and Cancer: A Global Perspective. Continuous Update Project Expert Report 2018. 2018.

48. Breedveld-Peters JJ, Koole JL, Müller-Schulte E, van der Linden BW, Windhausen C, Bours MJ, et al. Colorectal cancers survivors' adherence to lifestyle recommendations and cross-sectional associations with health-related quality of life. British Journal of Nutrition. 2018;120(2):188-97.

49. van Veen MR, Mols F, Bours MJ, Weijenberg MP, Kampman E, Beijer S. Adherence to the World Cancer Research Fund/American Institute for Cancer Research recommendations for cancer prevention is associated with better health–related quality of life among long-term colorectal cancer survivors: results of the PROFILES registry. Supportive Care in Cancer. 2019;27(12):4565-74.

50. Baguley JB, Bolam AK, Wright RLO, Skinner LT. The Effect of Nutrition Therapy and Exercise on Cancer-Related Fatigue and Quality of Life in Men with Prostate Cancer: A Systematic Review. Nutrients. 2017;9(9).

51. Meneses-Echávez JF, Correa-Bautista, J.E., González-Jiménez, E., Schmidt Río-Valle, J., Elkins, M.R., Lobelo, F., Ramírez-Vélez, R. The Effect of Exercise Training on Mediators of Inflammation in Breast Cancer Survivors: A Systematic Review with Meta-analysis. Cancer Epidemiology Biomarkers & Prevention. 2016;25(7):1009.

52. Himbert C, Ose J, Lin T, Warby CA, Gigic B, Steindorf K, et al. Inflammation-and angiogenesis-related biomarkers are correlated with cancer-related fatigue in colorectal cancer patients: Results from the ColoCare Study. European journal of cancer care. 2019;28(4):e13055.

Chapter 2

Low radiographic muscle density is associated with lower overall and disease-specific survival in early-stage colorectal cancer patients

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Background: In cancer patients with a poor prognosis low skeletal muscle radiographic density is associated with higher mortality. Whether this association also holds for early-stage cancer is less clear. We aimed to study the association between skeletal muscle density and overall mortality among early-stage (stage I-III) colorectal cancer (CRC) patients. Furthermore we investigated the association between skeletal muscle density and both CRC-specific mortality and disease-free survival in a subset of the study population

Methods: Skeletal muscle density was assessed in 1,681 early-stage CRC patients, diagnosed between 2006-2015, using pre-operative computed tomography (CT) images. Adjusted Cox proportional hazard models were used to evaluate the association between muscle density and overall mortality, CRC-specific mortality and disease-free survival.

Results: The median follow-up time was 48 months (range, 0 – 119 months). Low muscle density was detected in 39% of CRC patients. Low muscle density was significantly associated with higher mortality (low vs normal: adjusted HR 1.91, 95% CI 1.53 - 2.38). After stratification for comorbidities, the association was highest in patients with ≥ 2 comorbidities (HR 2.11, 95% CI 1.55 - 2.87). Furthermore, low skeletal muscle density was significantly associated with poorer disease-free survival (HR 1.68, 95% CI 1.14 – 2.47), but not with CRC-specific mortality (HR 1.68, 95% CI 0.89 – 3.17) in a subset of the study population.

Conclusion: In early-stage CRC patients low muscle density was significantly associated with higher overall mortality, and worse disease-free survival.

ABSTRACT

Introduction

In recent years there has been a growing interest in the influence of skeletal muscle (radio-) density on cancer prognosis. Skeletal muscle density, measured by computed tomography (CT) and quantified in Hounsfield Units (HU), reflects the lipid content of the muscle cells (1); higher muscle lipid content in the muscle cells presents as lower skeletal muscle density (1). Lower skeletal muscle density has been found to be associated with higher total adiposity (2), obesity (3) and increasing age (4). In a recent study an association between low skeletal muscle density and pre-existing comorbidities was also reported suggesting a potential shared mechanism between fat infiltration in the muscle and comorbidities (5).

Various studies have reported an association between low skeletal muscle density and higher mortality (6-12) in cancer patients. Until now the majority of the studies have been performed in late-stage (stage IV) cancer patients (6, 8, 10) and/or in cancer types with poor prognosis (7, 9, 11). Three studies (13-15) investigated the association between skeletal muscle density and mortality exclusively in early-stage (stage I-III) colorectal (CRC) patients, who have a much better prognosis than stage V patients (i.e. five year survival rate of >70% vs <15% in late-stage CRC (16)), and results were not consistent. The first two studies were performed in European populations and did not show significant associations between skeletal muscle density and overall mortality (13, 14), CRC-specific mortality (14) or disease free survival (13) in early-stage CRC patients after adjusting for confounding factors. A caveat is that both studies used cut-off points for low muscle density, which were defined in a mixed group of cancer patients of various diagnosis, mostly with very poor prognosis (17). Those cut-off points may not be appropriate in an early-stage European CRC patient population. The third study, performed in the US (15) reported that lower skeletal muscle density was associated with higher overall and CRC-specific mortality. Given the inconsistency of findings so far and the challenges in the interpretation in a European population, we decided to study the association between skeletal muscle density and overall mortality in a large cohort of European early-stage CRC patients. Furthermore we investigated the association between skeletal muscle density and CRC-specific mortality and disease-free survival in a subset of the study population.

Materials and Methods

Subjects

Data from two ongoing prospective cohort studies (n=1,111) were combined with registry-based data from three hospitals (n=1,537). The prospective cohort studies, i.e., the COLON (18) and EnCoRe (19) studies, started in 2010 and 2012, respectively. Both

investigate the role of lifestyle in CRC patients; details have been described earlier (18, 19). CRC patients included in the COLON study participants were diagnosed between August 2010 and November 2015; CRC patients included in the EnCoRe study were diagnosed between April 2012 and November 2014. For the registry-based data the Netherlands Cancer Registry was used to select all stage I-III CRC patients diagnosed between January 2007 and December 2010 in two hospitals and January 2007 and January 2013 in a third hospital.

For all cohorts, exclusion criteria were: missing data on height, weight, stage of disease or comorbidities; stage IV CRC; missing or unusable CT images (i.e. CT images of poor quality or scans where muscle tissue was partly cut-off). Furthermore, only pre-operative CT images assessed within 3 months of diagnosis were considered representative for skeletal muscle status at diagnosis.

The COLON study was approved by the Committee on Research involving Human Subjects, region Arnhem-Nijmegen, the Netherlands. The EnCoRe study was approved by the Medical Ethics Committee of the University Hospital Maastricht and Maastricht University, the Netherlands. All participants of the COLON and EnCoRe study provided written informed consent. According to the Central Committee on Research involving Human Subjects (CCMO), studies using the data of the Netherlands Cancer Registry do not require approval from an ethics committee in the Netherlands. The study was approved by the Privacy Review Board of the Netherlands Cancer Registry. In addition, the ethical committees of the three participating hospitals gave permission to use additional data from CT images.

Body composition and anthropometry

Skeletal muscle cross-sectional area was assessed using pre-operative CT images at the level of the 3rd lumbar vertebrae. Skeletal muscle was quantified using Slice-O-Matic 5.0 (Tomovision, Montreal, Canada). Standard density thresholds were used to define skeletal muscle: -29 to +150 HU (20). Standardized procedures were followed by trained researchers to correctly identify and quantify the psoas, erector spinae, quadratus lumborum, transversus abdominus, external and internal obliques, and rectus abdominus. Skeletal muscle density was measured as the mean density (in HU) of the total skeletal muscle cross-sectional area. Furthermore, as potential confounders, visceral adipose tissue, subcutaneous adipose tissue and intermuscular adipose tissue were quantified using the following standard density thresholds: -150 and -50 HU for visceral adipose tissue; -190 and -30 HU for both subcutaneous and intermuscular adipose tissue. Total adipose tissue was calculated as the sum of visceral-, subcutaneous- and intermuscular adipose tissue.

The association between skeletal muscle density and overall mortality, CRC-specific mortality and disease-free survival were assessed using continuous and dichotomized (low vs normal skeletal muscle density) variables. For the identification of patients with low skeletal muscle density gender- and body mass index (BMI)-specific cut-off values were determined using optimal stratification (21), which is a statistical method previously used in comparable studies (17, 22, 23) using a macro in SAS (version 9.3; SAS Institute, Cary, NC). We decided to create specific cut-off values for our population, as established cut-off values to identify and low skeletal muscle density had been determined within cancer populations with a much higher mortality rate (17) or in other continents (North-America) (15) which might not be representative for a European cohort. As acknowledged by others (24, 25), reference cut-off values for an European population are important because of possible differences in body composition and prevalence of obesity between European, US and other populations. This optimal stratification procedure, resulted in the following cut-off levels for skeletal muscle density: for men with a BMI < 25 kg/m^2 the cut-off value for low skeletal muscle density was 36.4 HU, for men with a BMI ≥ 25 kg/m^2 31.6 HU; for women with a BMI < 25 kg/m^2 31.1 HU; for women with a BMI ≥ 25 kg/m^2 29.3 HU.

Height (m) and weight (kg) at diagnosis were self-reported within the COLON study; measured by trained research assistants within the EnCoRe study; and collected from medical records by the Netherlands Cancer Registry for the registry-based data.

Medical history and mortality

Data on age, gender, type of cancer, stage of disease, comorbidities, and date of surgery were collected from medical records for the prospective cohort studies and from the Netherlands Cancer Registry for the registry-based data. Mortality data were retrieved from the Municipal Personal Records Database. Overall mortality was measured as number of months alive after the assessment date of the CT image until time of death or January 31, 2017. Patients who were alive on this date were censored. For participants of the COLON study (n= 715) recurrence data was retrieved in collaboration with the Netherlands Cancer Registry and cause of death was obtained by linkage with Statistics Netherlands (CBS). The International Classification of Diseases 10th Revision (ICD-10) was used to identify CRC specific mortality (ICD-10 codes C18 - C20). DFS was calculated as number of months between the assessment date of the CT image and either a recurrence, metastasis of disease, or death from any cause.

Statistical analyses

 Hazard ratios (HR) and corresponding 95% confidence intervals (CI) for overall mortality, CRC-specific mortality and disease-free survival were obtained using Cox proportional hazard analyses. Proportional hazard assumptions were tested by log–log curves, with no violations noted.

The model for overall mortality was tested for effect modification by gender, age and number of comorbidities (0, 1, ≥ 2), by calculating the p for interaction. Of these variables, number of comorbidities was the only variable that was identified as an effect modifier for skeletal muscle density. Thus the models for skeletal muscle density and overall mortality were stratified for number of comorbidities. Since no difference was found between zero and one comorbidity, these two categories were combined into one category: the final model was stratified into two groups (i.e. 0-1 comorbidity vs ≥ 2 comorbidities). Data on cause of death, recurrence and metastasis, were only available for the COLON study. Due to the limited sample size of the COLON study population (n=715), statistical power precluded the possibility to assess effect modification in the analyses for CRC-specific mortality and disease-free survival.

Based on existing literature, age, stage of disease and gender were selected as potential confounding variables and were included in the final multivariable Cox proportional hazard models. Other variables, i.e., BMI (continuous and categorical), study type (prospective or registry-based), tumor location (colon or rectal), treatment (chemotherapy yes or no; radiotherapy yes or no), number of comorbidities (0-1 or ≥ 2), skeletal muscle index (i.e. skeletal muscle mass corrected for height in meters squared), visceral adipose tissue, subcutaneous adipose tissue and total adipose tissue were included in the final model if they changed the HR for mortality with 10% or more when the variable was individually added to the model. Based on these criteria, only age, gender and stage were included in the final adjusted models.

As a sensitivity analyses, we repeated all analyses, excluding patients who died or had a recurrence or death within one year of follow-up.

Level of significance was set at 0.05. Analyses were performed using IBM SPSS 23.0 (SPSS Inc., Chicago, IL, USA).

Results

Out of 2,648 eligible patients, data from 580 patients had to be excluded because either no CT images were available, no pre-surgical CT image within 3 months of diagnosis was available, or the CT image was unusable due to low quality. An additional 68 patients were excluded because the stage of disease was unknown, for 282 patients data on height and/or weight were missing and for 37 patients comorbidity data were missing (**Figure 1**). The final dataset consisted of 1681 patients. The average age of the 967 excluded patients was 68.5 ± 12.4 years; 43 % were women; 61 % had a tumor located in the colon; and of the

excluded patients with stage of disease data available (79%), 26% had stage I disease, 38% stage II and 36% stage III CRC.

The average age of the study population was 67.7 ± 10.3 years and 41% were women; 67% of the patients had a tumor located in the colon (**Table 1**). The majority of these patients had a BMI between 25 - 29.9 kg/m^2 (43%) or 20 - 24.9 kg/m^2 (36%), while 17% had a BMI $\geq$ 30 kg/m^2 and 4% a BMI < 20 kg/m^2. In total, 414 patients (25%) died before January 31, 2017 and the median follow-up time was 48 months (range, 0 - 119 months).
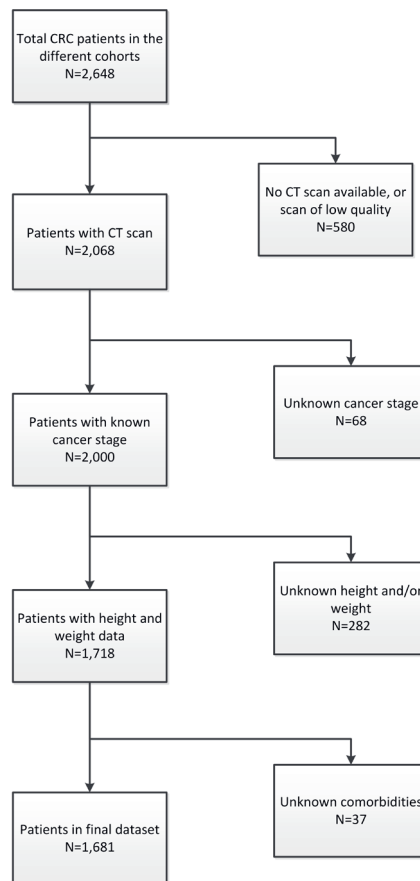
Figure 1: Flowchart of the study

Thirty-nine percent of the patients had low skeletal muscle density (Table 1). Mean age and the percentage of women were higher in the low skeletal density group compared to the normal skeletal density group (73.2 ± 8.8 vs 64.3 ± 9.7 years; 43% vs 39%, respectively). Furthermore, the percentage of patients with a BMI $\geq$ 30 kg/m^2 was higher in the low skeletal density group compared to the normal skeletal density group (24% vs 13%,

respectively), while the percentage of patients with a BMI 25 - 29.9 kg/m^2 was lower in the low skeletal muscle density group vs the normal skeletal density group (37% vs 47%).

Table 1: Baseline characteristics in early-stage CRC patients with or without low skeletal muscle density

	Total population	Skeletal muscle density	
	(n=1681)	Low (n=648) (39%)	Normal (n=1033) (61%)
Demographic factors			
Age [yrs, mean (SD)]			
	67.7 (10.3)	73.2 (8.8)	64.3 (9.7)
Gender [n (%)]			
Men	999 (59)	372 (57)	627 (61)
Women	682 (41)	276 (43)	406 (39)
BMI [kg/m^2, mean (SD)]			
BMI [kg/m^2, n (%)]			
<20	65 (4)	17 (3)	48 (5)
20-24.9	609 (36)	238 (37)	371 (36)
25-29.9	718 (43)	238 (37)	480 (47)
≥30	289 (17)	155 (24)	134 (13)
Clinical factors			
Cancer stage [n (%)]			
I	393 (23)	127 (20)	266 (26)
II	547 (33)	251 (39)	296 (29)
III	741 (44)	270 (42)	471 (46)
Tumor location [n (%)][a]			
Colon	1123 (67)	465 (72)	658 (64)
Rectal	552 (33)	180 (28)	372 (36)
Number of comorbidities [n (%)]			
0-1	966 (58)	283 (44)	683 (66)
≥ 2	715 (43)	365 (56)	350 (34)
Treatment			
Radiotherapy [n (%)][b]			
No	1136 (73)	462 (77)	674 (71)
Yes	419 (27)	140 (23)	279 (29)
Chemotherapy [n (%)][c]			
No	1117 (72)	471 (78)	646 (68)
Yes	440 (28)	130 (22)	310 (32)

CT image analysis			
Skeletal muscle index [cm²/m², mean (SD)]			
Men	51 (8)	48 (8)	53 (7)
Women	40 (6)	39 (6)	41 (6)
Muscle attenuation [HU, mean (SD)]			
Men	36 (8)	27 (5)	41 (5)
Women	32 (10)	23 (5)	39 (7)
Visceral adipose tissue [cm², median (range)]	147 (0-535)	168 (1-535)	133 (0-495)
Subcutaneous adipose tissue [cm², median (range)]	159 (0-713)	166 (0-713)	154 (1-596)
Total adipose tissue [cm², median (range)]	341 (5-1070)	383 (35-1070)	316 (5-924)
Study [n (%)]			
Prospective	852 (51)	242 (37)	610 (59)
Registry-based	829 (49)	406 (63)	423 (41)
Follow-up time **[months, median (range)]**	48 (0-119)	48 (0-119)	48 (0-119)
Deceased patients [n (%)]	414 (25)	261 (40)	153 (15)

[a]= Data of 6 patients were missing. [b]=Data of 126 patients were missing. [c]=Data of 124 patients were missing. Abbreviations: BMI, body mass index; SD, standard deviation; HU, Hounsfield unit.

Data for CRC-specific mortality and disease-free survival were only available for participants of the COLON study. The average age of these patients was slightly lower than in the total study population: 65.6 ± 9.1 years vs 67.7 ± 10.3 years (**Table 2**). Furthermore, the follow-up time was shorter (i.e. 37 months vs 48 months) and the percentage of deceased patients was lower (i.e. 11% vs 25%). Other variables were comparable with the total population. Twenty-seven percent of the patients had low skeletal muscle density.

Low skeletal muscle density was significantly associated with higher mortality (low vs normal skeletal muscle density: adjusted HR 1.91, 95% CI 1.53 - 2.38), **Table 3**. Number of comorbidities was identified as an effect modifier (p for interaction = 0.02). In the stratified analysis low skeletal muscle density was significantly associated with higher mortality in patients with zero or one comorbidities (low vs normal skeletal muscle density: adjusted HR 1.67, 95% CI 1.20 - 2.31), yet this association was even stronger in the group of patients with two or more comorbidities (low vs normal skeletal muscle density: adjusted HR 2.11, 95% CI 1.55 - 2.87).

Within the sub-population of the COLON study (n=715) low skeletal muscle density was associated with higher overall mortality (low vs normal skeletal muscle density: adjusted HR 2.15, 95% CI 1.32 - 3.50) and worse disease-free survival (low vs normal skeletal muscle

density: adjusted HR 1.68, 95% CI 1.14 - 2.47), but not significantly with colorectal cancer-specific disease (low vs normal: adjusted HR 1.68, 95% CI 0.89 - 3.17) (**Table 4**).

Table 2: Baseline characteristics of the participants from the COLON study

	COLON study	Skeletal muscle density	
	(n=715)	Low (n=196) (27%)	Normal (n=519) (73%)
Demographic factors			
Age [yrs, mean (SD)]	65.6 (9.1)	70.8 (7.8)	63.8 (8.9)
Gender [n (%)]			
Men	440 (62)	122 (62)	318 (61)
Women	275 (39)	71 (38)	201 (39)
BMI [kg/m², n (%)]			
<20	25 (4)	6 (3)	19 (4)
20-24.9	255 (36)	75 (38)	180 (35)
25-29.9	316 (44)	64 (33)	252 (49)
≥30	119 (17)	51 (26)	68 (13)
Clinical factors			
Cancer stage [n (%)]			
I	210 (29)	56 (29)	154 (30)
II	204 (29)	63 (32)	141 (27)
III	301 (42)	77 (39)	224 (43)
Tumor location [n (%)]			
Colon	483 (68)	136 (69)	247 (67)
Rectal	232 (32)	60 (31)	172 (33)
Number of comorbidities [n (%)]			
0-1	444 (62)	103 (53)	341 (66)
≥ 2	271 (38)	93 (47)	178 (34)
Follow-up time [months, median (range)]	37 (1-77)	38 (3-77)	36 (1-77)
Deceased patients [n (%)]	76 (11)	40 (24)	36 (7)

[a]= data of 6 patients missing. [b]= data of 4 patients missing

Table 3: Association between skeletal muscle density and overall mortality.

	No. of patients	No. of deaths *n* (%)	HR (95% CI)*
Skeletal muscle density, total population			
Normal	1033	153 (15)	REF
Low	648	261 (40)	1.91 (1.53-2.38)
Skeletal muscle density, 0-1 comorbidities			
Normal	683	93 (14)	REF
Low	283	88 (31)	1.67 (1.20-2.31)
Skeletal muscle density, ≥ 2 comorbidities			
Normal	350	60 (17)	REF
Low	365	173 (47)	2.11 (1.55-2.87)
Skeletal muscle density, HU (continuous)	1681	414 (25)	0.98 (0.96-0.99)

*=adjusted for age, stage of disease, gender. Abbreviations: HR, hazard ratio; 95% CI, 95% confidence interval; REF, reference value; HU, Hounsfield unit.

Table 4: Association between skeletal muscle density and overall mortality, CRC-specific mortality and disease-free survival in a subpopulation (n = 715).

		No. of patients	No. of deaths *n* (%)	HR (95% CI)*
	Skeletal muscle density			
Overall mortality	Normal	519	36 (7)	REF
	Low	196	40 (20)	2.15 (1.32-3.50)
	Skeletal muscle density			
CRC-specific mortality	Normal	519	26 (5)	REF
	Low	196	19 (10)	1.68 (0.89-3.17)
	Skeletal muscle density			
Disease-free survival	Normal	519	72 (14)	REF
	Low	196	52 (27)	1.68 (1.14-2.47)

*=adjusted for age, stage of disease, gender. Abbreviations: HR, hazard ratio; 95% CI, 95% confidence interval; REF, reference value.

Discussion

In our cohort of early-stage CRC patients, low skeletal muscle density was significantly associated with higher mortality. This association was strongest in patients with ≥ 2 comorbidities. Within a subset of the total study population were data on disease-free

survival and cause of death were available, low skeletal muscle density was associated with worse disease-free survival, but not statistically significantly with CRC-specific survival.

Our findings support the findings from Kroenke *et al* (15), who also found that low skeletal muscle density was associated with worse survival. Although speculative, our findings also underline the importance of using cut-off points for low skeletal muscle density that are defined in an appropriate population, as two other studies did not find associations between muscle density and survival (13, 14). The cut-off points we defined in our study population, are very comparable to the cut-offs defined in the study by Kroenke *et al* (15). The two studies that did not find associations, used cut-off levels for low skeletal muscle density that were defined in mixed group of cancer patients of various diagnoses, mostly with very poor prognosis (17).

In the present study, we did not find a statistically significant association between low skeletal muscle density and CRC-specific mortality. This might just be a matter of limited statistical power, as data on CRC-specific mortality were only available for the sub-population of participants enrolled in the COLON study. In the large study by Kroenke *et al* (15), an association was found with higher CRC-specific mortality. Further studies are needed to understand the mechanisms behind the association of skeletal muscle density and survival.

In the present study, the associations between low muscle density and both higher overall mortality and lower disease-free survival were independent of age, adipose tissue and BMI, suggesting that the patients with higher risk of dying were not just the older, obese CRC patients. It could be possible that patients with a high skeletal muscle density were more physically active in daily life. Studies investigating the effect of training and detraining (i.e. the effect of reduced physical training after a training program), and of strength and endurance training showed that those training programs increased skeletal muscle density (3, 26, 27), whereas detraining reduced skeletal muscle density (26). Very limited data are available on other determinants of skeletal muscle density, thus, whether other lifestyle factors like smoking and drinking are associated with decreased skeletal muscle density is still unknown.

We showed that the association of low muscle density and higher mortality was strongest among patients with multiple comorbidities. In a recent study (5) an association between low skeletal muscle density and comorbidities was reported. Yet, our results suggests that lower skeletal muscle density is not simple a measure of worse health status, as among those patients with more co-morbidities (thus with worst health status), the association was strongest. Potentially, the health status of those patients makes them extra vulnerable

for the detrimental effects of fat infiltration in the muscle, but mechanistic studies are needed to explore this further.

This study has some limitations. First, data from patients without a CT image within 3 months of diagnosis had to be excluded. Since CT-diagnostic imaging was not common practice in clinical care of CRC in the Netherlands until 2008, a lot of scans were missing for patients diagnosed before 2008. Mean age, percentage of women, stage of disease and percentage of colon tumors were comparable between the included and excluded patient group. Therefore, we do not expect that excluding these patients affected our results. Second, due to the availability of cause of death and recurrence data, we could only perform the analyses for CRC-specific mortality and disease-free survival in the participants of the COLON study (n=715).

This is the first large scale European cohort that assessed the association between skeletal muscle density and survival with cut-off points relevant for/defined in our cohort of early-stage CRC patients. Other strengths are the large sample size, the long follow-up time and the inclusion of exclusively early-stage CRC patients.

In conclusion, low skeletal muscle density was significantly associated with higher overall mortality and lower disease-free survival in early-stage CRC patients. Future observational studies are needed to study which factors determine low skeletal muscle density. Thereafter, intervention studies should be performed to study whether intervening on skeletal muscle density could improve prognosis of cancer patients e.g. with nutritional interventions and/or exercise training.

References

1. Goodpaster BH, Kelley DE, Thaete FL, He J, Ross R. Skeletal muscle attenuation determined by computed tomography is associated with skeletal muscle lipid content. Journal of Applied Physiology. 2000;89(1):104-10.

2. Goodpaster BH, Carlson CL, Visser M, Kelley DE, Scherzinger A, Harris TB, et al. Attenuation of skeletal muscle and strength in the elderly: The Health ABC Study. Journal of Applied Physiology. 2001;90(6):2157-65.

3. Lee S, Kuk JL, Davidson LE, Hudson R, Kilpatrick K, Graham TE, et al. Exercise without weight loss is an effective strategy for obesity reduction in obese individuals with and without type 2 diabetes. Journal of Applied Physiology. 2005;99(3):1220-5.

4. Anderson DE, D'Agostino JM, Bruno AG, Demissie S, Kiel DP, Bouxsein ML. Variations of CT-based trunk muscle attenuation by age, sex, and specific muscle. The Journals of Gerontology Series A: Biological Sciences and Medical Sciences. 2013;68(3):317-23.

5. Xiao J, Caan BJ, Weltzien E, Cespedes Feliciano EM, Kroenke CH, Meyerhardt JA, et al. Associations of pre-existing co-morbidities with skeletal muscle mass and radiodensity in patients with non-metastatic colorectal cancer. Journal of cachexia, sarcopenia and muscle. 2018.

6. Sjøblom B, Grønberg BH, Wentzel-Larsen T, Baracos VE, Hjermstad MJ, Aass N, et al. Skeletal muscle radiodensity is prognostic for survival in patients with advanced non-small cell lung cancer. Clinical Nutrition. 2016.

7. Fujiwara N, Nakagawa H, Kudo Y, Tateishi R, Taguri M, Watadani T, et al. Sarcopenia, intramuscular fat deposition, and visceral adiposity independently predict the outcomes of hepatocellular carcinoma. Journal of hepatology. 2015;63(1):131-40.

8. Rier HN, Jager A, Sleijfer S, van Rosmalen J, Kock MC, Levin M-D. Low muscle attenuation is a prognostic factor for survival in metastatic breast cancer patients treated with first line palliative chemotherapy. The Breast. 2017;31:9-15.

9. Dijk DP, Bakens MJ, Coolsen MM, Rensen SS, Dam RM, Bours MJ, et al. Low skeletal muscle radiation attenuation and visceral adiposity are associated with overall survival and surgical site infections in patients with pancreatic cancer. Journal of Cachexia, Sarcopenia and Muscle. 2016.

10. Blauwhoff-Buskermolen S, Versteeg KS, de van der Schueren MA, Den Braver NR, Berkhof J, Langius JA, et al. Loss of Muscle Mass During Chemotherapy Is Predictive for Poor Survival of Patients With Metastatic Colorectal Cancer. Journal of Clinical Oncology. 2016;34(12):1339-44.

11. Ebadi M, Martin L, Ghosh S, Field CJ, Lehner R, Baracos VE, et al. Subcutaneous adiposity is an independent predictor of mortality in cancer patients. British Journal of Cancer. 2017;117(1):148.

12. Antoun S, Lanoy E, Iacovelli R, Albiges-Sauvin L, Loriot Y, Merad-Taoufik M, et al. Skeletal muscle density predicts prognosis in patients with metastatic renal cell carcinoma treated with targeted therapies. Cancer. 2013;119(18):3377-84.

13. van Vugt JL, van den Braak RRC, Lalmahomed ZS, Vrijland WW, Dekker JW, Zimmerman DD, et al. Impact of Low Skeletal Muscle Mass and Density on Short and Long-Term Outcome after Resection of Stage I-III Colorectal Cancer. European Journal of Surgical Oncology. 2018.

14. McSorley ST, Black DH, Horgan PG, McMillan DC. The relationship between tumour stage, systemic inflammation, body composition and survival in patients with colorectal cancer. Clinical Nutrition. 2017.

15. Kroenke CH, Prado CM, Meyerhardt JA, Weltzien EK, Xiao J, Cespedes Feliciano EM, et al. Muscle radiodensity and mortality in patients with colorectal cancer. Cancer. 2018.

16. Siegel RL, Miller KD, Fedewa SA, Ahnen DJ, Meester RG, Barzi A, et al. Colorectal cancer statistics, 2017. CA: a cancer journal for clinicians. 2017;67(3):177-93.

17. Martin L, Birdsell L, MacDonald N, Reiman T, Clandinin MT, McCargar LJ, et al. Cancer cachexia in the age of obesity: skeletal muscle depletion is a powerful prognostic factor, independent of body mass index. Journal of Clinical Oncology. 2013:JCO. 2012.45. 722.

18. Winkels R, Heine-Broring R, Van Zutphen M, van Harten-Gerritsen S, Kok D, Van Duijnhoven F, et al. The COLON study: Colorectal cancer: Longitudinal, Observational study on Nutritional and lifestyle factors that may influence colorectal tumour recurrence, survival and quality of life. BMC Cancer. 2014;14(1):374.

19. van Roekel EH, Bours MJL, de Brouwer CPM, Ten Napel H, Sanduleanu S, Beets GL, et al. The Applicability of the International Classification of Functioning, Disability, and Health to Study Lifestyle and Quality of Life of Colorectal Cancer Survivors. Cancer Epidemiology Biomarkers & Prevention. 2014;23(7):1394-405.

20. Mitsiopoulos N, Baumgartner R, Heymsfield S, Lyons W, Gallagher D, Ross R. Cadaver validation of skeletal muscle measurement by magnetic resonance imaging and computerized tomography. Journal of applied physiology. 1998;85(1):115-22.

21. Williams BA. Finding optimal cutpoints for continuous covariates with binary and time-to-event outcomes. 2006.

22. Prado CM, Lieffers JR, McCargar LJ, Reiman T, Sawyer MB, Martin L, et al. Prevalence and clinical implications of sarcopenic obesity in patients with solid tumours of the respiratory and gastrointestinal tracts: a population-based study. Lancet Oncol. 2008;9(7):629-35.

23. Caan BJ, Meyerhardt JA, Kroenke CH, Alexeeff S, Xiao J, Weltzien E, et al. Explaining the Obesity Paradox: The Association between Body Composition and Colorectal Cancer Survival (C-SCANS Study). Cancer Epidemiology and Prevention Biomarkers. 2017;26(7):1008-15.

24. Daly LE, Ryan AM, Power DG. Response to "Loss of Muscle Mass During Chemotherapy Is Predictive for Poor Survival of Patients With Metastatic Colorectal Cancer". Journal of Clinical Oncology. 2016;34(31):3816-7.

25. Blauwhoff-Buskermolen S, Schueren MAEdvd, Langius JAE, Verheul HMW. Reply to L.E. Daly et al. Journal of Clinical Oncology. 2016;34(31):3817.

26. Taaffe DR, Henwood TR, Nalls MA, Walker DG, Lang TF, Harris TB. Alterations in muscle attenuation following detraining and retrainingin resistance trained older adults. Gerontology. 2009;55(2):217.

2

27. Poehlman ET, Dvorak RV, DeNino WF, Brochu M, Ades PA. Effects of Resistance Training and Endurance Training on Insulin Sensitivity in Nonobese, Young Women: A Controlled Randomized Trial 1. The Journal of Clinical Endocrinology & Metabolism. 2000;85(7):2463-8.

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Chapter 3

Associations of abdominal skeletal muscle mass, fat mass, and mortality among men and women with stage I-III colorectal cancer

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ABSTRACT

Background: The associations of abdominal skeletal muscle mass index (SMI), visceral and subcutaneous adipose tissue (VAT and SAT respectively) and mortality among stage I-III colorectal cancer (CRC) patients may differ for men and women, but only few studies stratified their data into men and women. We investigated associations of abdominal SMI, VAT and SAT with overall mortality among men and among women with stage I-III CRC.

Methods: SMI, VAT and SAT were assessed from abdominal computed tomography (CT) images for 1,998 stage I-III CRC patients diagnosed between 2006-2015. Restricted cubic splines (RCS) were used to investigate associations of SMI, VAT and SAT with overall mortality.

Results: Average age of the participants was 67.9 ± 10.6 years and 58% were men. During a median follow-up of 4.3 years, 546 (27%) patients died. Among men, the association of SMI and mortality was statistically significantly in a non-linear way in the RCS analyses, with lower SMI levels associated with higher mortality. SMI was not associated with mortality among women. SAT was associated with mortality in a non-linear way for men and for women, with lower SAT levels being associated with higher mortality. VAT was not significantly associated with mortality in men nor women.

Conclusion: Associations of abdominal skeletal muscle mass with mortality among CRC patients were not the same for men than for women.

Impact: This study stresses the importance for more attention for sex-related differences in body composition and cancer outcomes.

Introduction

Colorectal cancer (CRC) is one of the most common malignancies worldwide, with over 1.8 million new cases in 2018 (1). Various studies have investigated associations of abdominal skeletal muscle mass, amount of visceral adipose tissue (VAT) or subcutaneous adipose tissue (SAT) and mortality among CRC patients (2-18), but up till now the results are inconsistent. This inconsistency has several reasons. First, studies included a mix of stage I-III and stage IV CRC patients. Five year survival rate of patients with stage I-III CRC is generally above 70%, whereas in stage IV CRC patients this is generally less than 15% (19). Severe weight loss, and muscle wasting (cachexia) are highly prevalent among patients with stage IV cancer, due to metabolic alterations induced by the tumour (20). Among stage I-III cancer patients, cachexia is less common; thus, low skeletal muscle mass is more likely to be related with aging and/or a sedentary lifestyle. Analyses on body composition and mortality should therefore not include combined populations of stage I-III and stage IV patients.

A second possible explanation for the inconsistent results is the use of inappropriate cut-off values to identify patients with a low or high abdominal muscle or fat mass. When it comes to stage I-III colorectal cancer patients, five studies investigated the association of low abdominal skeletal muscle mass and mortality all using different cut-off values for low skeletal muscle mass: the used cut-offs ranged from <31.6 to <46.6 cm^2/m^2 for women and <43 to <54.3 cm^2/m^2 for men (9-12, 17). An Asian (n=220), a Canadian (n=968) and a large US study (n=3,262) (10, 12, 17) reported a significant association between low abdominal skeletal muscle mass and higher mortality, while in two European studies (n=816 & n=339) (9, 11), low abdominal skeletal muscle mass was not associated with higher mortality. Both European studies used cut-off levels for low skeletal muscle as defined by US or Canadian cohorts (12, 14). As noted by others (21, 22), the cut-off levels for low abdominal skeletal muscle as defined in US/Canadian populations, might not be appropriate for European populations.

VAT is known to be associated with chronic, low-grade inflammation, and a higher risk of worse cardiovascular outcomes (23, 24). So far there is no convincing evidence that VAT is also associated with mortality among stage I-III CRC patients (11, 12, 15-18). A relatively small study (n=62) (15) reported a statistically significant association of high VAT with increased overall mortality among patients receiving adjuvant chemotherapy. Four other studies observed no clear associations between low or high VAT and mortality (11, 12, 16, 17). One study determined a cut-off value for high VAT using optimal stratification (17), two other studies used tertiles (11, 12) and one other study applied a median split (16) to categorize VAT. All these categorizations are relatively crude, and these four studies may have failed to detect an association as a result of these crude categorizations. A sixth study

used cubic splines to investigate the association of VAT and mortality (18) and observed a non-linear association between VAT and mortality.

Compared to VAT, SAT contains a lower number of inflammatory and immune cells and is considered to be a less active fat tissue then VAT (25). Three studies (11, 12, 18) reported on the association of abdominal SAT and mortality among exclusively stage I-III CRC patients. Two studies did not observe significant associations while using tertiles to categorize SAT. Again Brown *et al* (18) observed a non-linear association between SAT and mortality using restricted cubic splines. This shows that dose-response analyses using restricted cubic spline (RCS) functions (26) could help identify possible linear or non-linear associations of VAT and SAT with mortality that might be missed when categorizing muscle or adipose tissue variables.

Another possible explanation for inconsistent results on associations of body composition and survival could be that not all studies stratified their data into men and women. Body composition differs between men and women, with men generally having more VAT and higher muscle mass, and women generally having more SAT and lower muscle mass (27). Age-related changes in skeletal muscle structure, function and metabolism differ between men and women, possibly mediated by sex hormones (28). Sex difference in body fat distribution might account for differences in inflammatory markers between men and women (29). Currently there is a gap in literature on whether these sex-based differences in body composition have an impact on the association between body composition and mortality in cancer patients. Of the previous studies among stage I-III CRC patients (9-12, 15-18) only two studies (both in the same dataset: men n=1,634, women n=1,628) (12, 18), stratified their data by sex. The stratified analyses suggest that associations of abdominal skeletal muscle mass, VAT and SAT with mortality differ between men and women.

The aim of the present study was to investigate the associations of abdominal skeletal muscle mass, VAT, and SAT with overall mortality among men and among women with stage I-III colorectal cancer.

Materials and Methods

Population
For this study, data of two ongoing prospective cohort studies among CRC patients, the COLON (30) and EnCoRe (31) study, were combined with registry-based data, as described previously (32). Briefly, for the COLON study, participants were recruited between August 2010 and January 2016; for the EnCoRe study participants were recruited between April 2012 and September 2015; and for the registry-based data the Netherlands Cancer Registry

was used to select all stage I–III CRC patients diagnosed between 2007-2010 in three hospitals. Patients had to be diagnosed with stage I-III CRC to be eligible for the current analysis. Additional exclusion criteria for the present analyses were missing computed tomography (CT) image, CT images of poor quality or missing data on stage of disease, see **Figure 1**. Only pre-treatment CT images assessed within 3 months of diagnosis were considered representative for body composition at diagnosis. Patients with a CT image assessed after treatment or outside this time period were excluded from analyses.

The study was performed in accordance with the Declaration of Helsinki. The COLON study (ClinicalTrials.gov Identifier: NCT03191110) was approved by the Committee on Research involving Human Subjects, region Arnhem-Nijmegen, the Netherlands. The EnCoRe study (Netherlands Trialregister number 7099) was approved by the Medical Ethics Committee of the University Hospital Maastricht and Maastricht University, the Netherlands. All participants of the COLON and EnCoRe study provided written informed consent. According to the Central Committee on Research involving Human Subjects (CCMO), studies using the data of the Netherlands Cancer Registry do not require approval from an ethics committee in the Netherlands. The study was approved by the Privacy Review Board of the Netherlands Cancer Registry. In addition, the ethical committees of the three participating hospitals gave permission to use additional data from CT images.

Body composition

Skeletal muscle, VAT and SAT cross-sectional areas were quantified on CT images at the level of the 3rd lumbar vertebrae with the use of Slice-O-Matic software version 5.0 (Tomovision, Montreal, Canada). Standard radiodensity thresholds, measured in Hounsfield units (HU), were used to quantify the cross-sectional areas of VAT, SAT, and skeletal muscle. For VAT the threshold values were between -150 and -50 HU, for SAT between -190 and -30 HU, and for skeletal muscle between -29 and +150 HU (33, 34). The skeletal muscle cross-sectional area (cm^2) was adjusted for height (cm^2/m^2) to calculate the skeletal muscle index (SMI). Skeletal muscle radiodensity (32) was assessed as the mean radiographic density of the total skeletal muscle cross-sectional area. Total adipose tissue (TAT) was calculated as the sum of VAT and SAT.

Medical history and mortality

For both the COLON and EnCoRe study, data on age, sex, type of cancer, stage of disease, comorbidities and date of surgery were collected from medical records. For the registry-based dataset, these data were retrieved from the Netherlands Cancer Registry. Mortality data (i.e. vital status and date of death) were retrieved from the Municipal Personal Records Database.

Statistical analyses

Demographic and clinical characteristics are presented as median and interquartile range (IQR) for continuous variables that were not normally distributed (SMI, VAT, SAT and follow-up time); mean and standard deviation (SD) are presented for continuous variables that followed a normal distribution; frequency and percentages are presented for categorical variables.

RCS analyses (26) were used to investigate associations of SMI, VAT and SAT with mortality, and to assess whether associations were non-linear. Knots were places at the 5th, 50th and 95th percentiles of SMI, VAT or SAT. Hazard Ratios (HR) for the RCS analyses were calculated using the median as the reference. All analyses were stratified into men and women. Based on literature, the RCS analyses for abdominal SMI, VAT and SAT were adjusted for age and stage of the disease. Other variables, i.e., tumor location (colon or rectum), number of comorbidities (0, 1 or ≥ 2), neo-adjuvant and/or adjuvant treatment (chemotherapy yes or no; radiotherapy yes or no), cohort (COLON or EnCoRe or Registry-based), height (continuous, in the VAT and SAT analyses), skeletal muscle radiodensity (continuous), SMI (continuous, in the VAT and SAT analyses), VAT (continuous, in the SMI and SAT analyses), SAT (continuous, in the SMI and VAT analyses) and TAT (continuous, in the SMI analyses) were included in the final models if they changed the HR for mortality in any of the models with at least 10% when the potential confounder was individually added to the model. Based on these analyses skeletal muscle radiodensity, SMI, chemotherapy and radiotherapy were identified as confounding variables. Additional to the RCS plots, HRs with 95% confidence intervals for specific SMI, VAT and SAT values were calculated using the RCS analyses.

A sensitivity analyses was performed excluding all patients who deceased within 30 days of surgery, as those patients most likely died due to post-operative complications.

Overall mortality was defined as number of days between date of the CT image and time of death or January 31, 2017. Patients still alive on this date were censored in the survival analyses.

Level of significance was set at 0.05. Analyses were performed using IBM SPSS Statistics software (version 23.0, SPSS Inc., Chicago, IL, USA) and SAS software (version 9.4, SAS Institute Inc, Cary, North Carolina, USA).

Results

In total, 2,618 eligible CRC patients were identified; 552 of them were excluded because no suitable CT image was available and 68 were excluded due to missing stage of disease data (**Figure 1**). The final dataset consisted of 1,998 patients; 703 of them were participants from the COLON study, 154 were participants from the EnCoRe study and 1,141 came from the registry-based dataset (**supplementary Table 2** describes baseline characteristics of the separate cohorts).
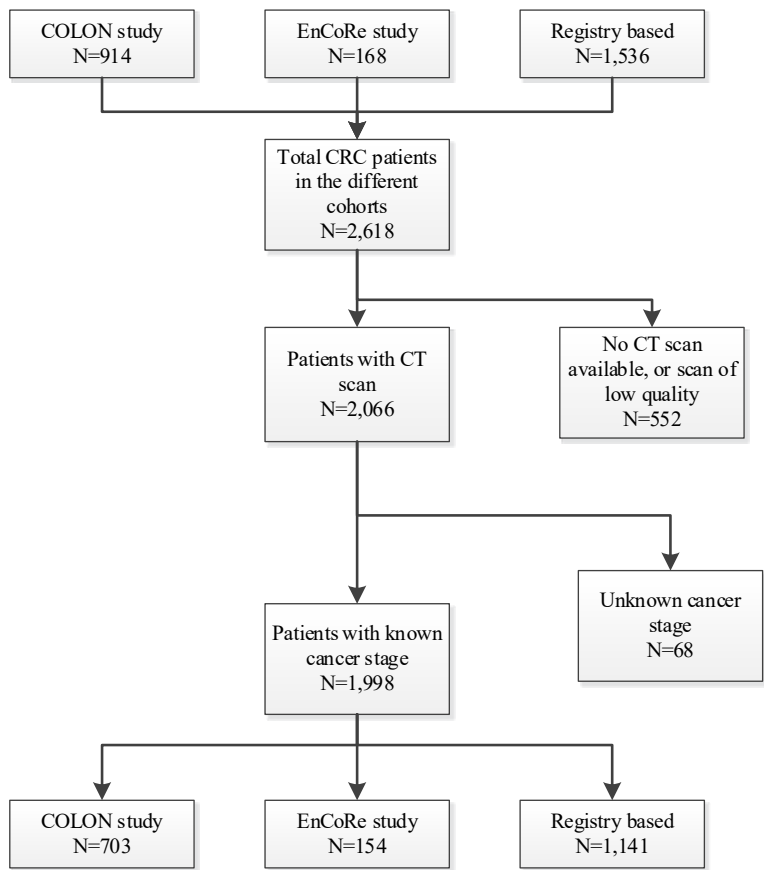
Figure 1: Flowchart for inclusion of stage I-III colorectal cancer patients in an observational study on body composition and mortality.

Table 1 shows the demographic and clinical characteristics for the total study population. The average age of the included patients was 67.9 ± 10.6 years and 58% were men, 33% of the patients was diagnosed with rectal cancer. The majority of the patients was diagnosed

with stage III of the disease (42%). Forty-two percent of the population was overweight (BMI 25-30 kg/m^2), and 17% was obese (BMI ≥ 30 kg/m^2). On average, men had more VAT and SMI and less SAT than women. The median follow-up time was 51 months and during this period 546 (27%) patients died.

Baseline characteristics were similar for included and excluded patients. Of the excluded patients, 59% were men, mean age was 70.6 years, 36% had rectal cancer and of the excluded patients with stage of disease data available (68%), 26% had stage I disease, 35% stage II and 40% stage III CRC.

Distribution of abdominal skeletal muscle, VAT and SAT by BMI categories among men and women are shown in **Figure 2.** These figures illustrate that among men, VAT tended to increase with increasing BMI, while for women, SAT tended to increase with increasing BMI. Moreover, the figure shows that with increasing BMI, absolute skeletal muscle index increased, but more so among men than among women.

In the RCS analyses, SMI was associated with mortality in a non-linear way among men (p=0.04), but no significant association between SMI and mortality was observed for women (p=0.21) (**Figure 3a&b). Table 2** shows estimated HR's for specific SMI values and illustrates that among men lower levels of SMI were significantly associated with higher mortality but that among women, no statistically significant associations are apparent.

For VAT, the RCS analyses showed that VAT was not significantly associated with mortality neither in men (p=0.21), nor in women (p=0.79) (**Figure 4a&b).**

The RCS analyses for SAT showed that among men and among women SAT was associated in a non-linear way with mortality (p<0.01 and p=0.02 respectively) (**Figure 5a&b). Table 2** shows that SAT levels below the median were associated with higher mortality in both men and women.

Sensitivity analyses excluding all patients who deceased within 30 days post-surgery (n=41) did not substantially alter the shape of any of the splines (**Supplementary Figure 1-3, Supplementary Table 1)**. However, the results for the analyses for SMI among men no longer reached statistical significance (p=0.10).

Table 1: Baseline characteristics of stage I-III CRC patients in an observational study on body composition and mortality

	Total population (n=1,998)	Women (n=832) (42%)	Men (n=1166) (58%)
Demographic characteristics			
Age [yrs, mean (SD)]	67.9 (10.6)	67.8 (11.6)	68.0 (9.8)
Follow-up time [months, median (IQR range)]	51 (28, 79)	54 (29, 82)	49 (27, 77)
Deceased patients [n (%)]	546 (27)	223 (27)	323 (28)
Study [n (%)]			
Prospective	857 (43)	323 (39)	534 (46)
Registry-based	1141 (57)	509 (61)	632 (54)
Clinical characteristics			
Cancer stage [n (%)]			
I	478 (24)	203 (24)	275 (23)
II	691 (35)	281 (34)	410 (35)
III	829 (42)	348 (42)	481 (41)
Tumor location [n (%)][+]			
Colon	1303 (67)	595 (74)	708 (62)
Rectal	648 (33)	214 (27)	434 (38)
Number of comorbidities [n (%)][++]			
0	622 (33)	288 (37)	334 (30)
1	474 (25)	209 (27)	265 (24)
≥ 2	796 (42)	2921(37)	505 (43)
Neo-adjuvant or adjuvant treatment			
Radiotherapy [No. (%)][+++]			
No	1395 (74)	616 (74)	779 (71)
Yes	488 (26)	173 (21)	315 (29)
Chemotherapy [No. (%)][#]			
No	1401 (73)	581 (72)	820 (74)
Yes	510 (27)	221 (28)	289 (26)
Body composition			
BMI [kg/m^2, n (%)][##]			
<20	67 (4)	44 (6)	23 (2)
20-24.9	629 (37)	278 (40)	351 (30)
25-29.9	728 (42)	254 (31)	474 (47)
≥30	295 (17)	124 (15)	171 (17)
Visceral adipose tissue [cm^2, median (IQR range)]	145 (76, 219)	93 (47, 150)	186 (117, 260)
Subcutaneous adipose tissue [cm^2, median (IQR range)]	156 (112, 215)	189 (137, 256)	136 (105, 183)

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Skeletal muscle index [cm^2/m^2, mean (SD)]	46.8 (8.8)	40.4 (5.9)	51.3 (7.7)
Skeletal muscle radiodensity [HU, mean (SD)]	34.2 (9.3)	32.0 (10.0)	35.7 (8.5)

[+] = Tumor location data of 47 patients missing; [++] = Comorbidity data of 106 patients missing; [+++]=Radiotherapy data of 115 patients missing; [#]=Chemotherapy data of 87 patients missing; [##]= BMI data of 279 patients missing.

Abbreviations: BMI, body mass index; SD, standard deviation; HU, Hounsfield unit
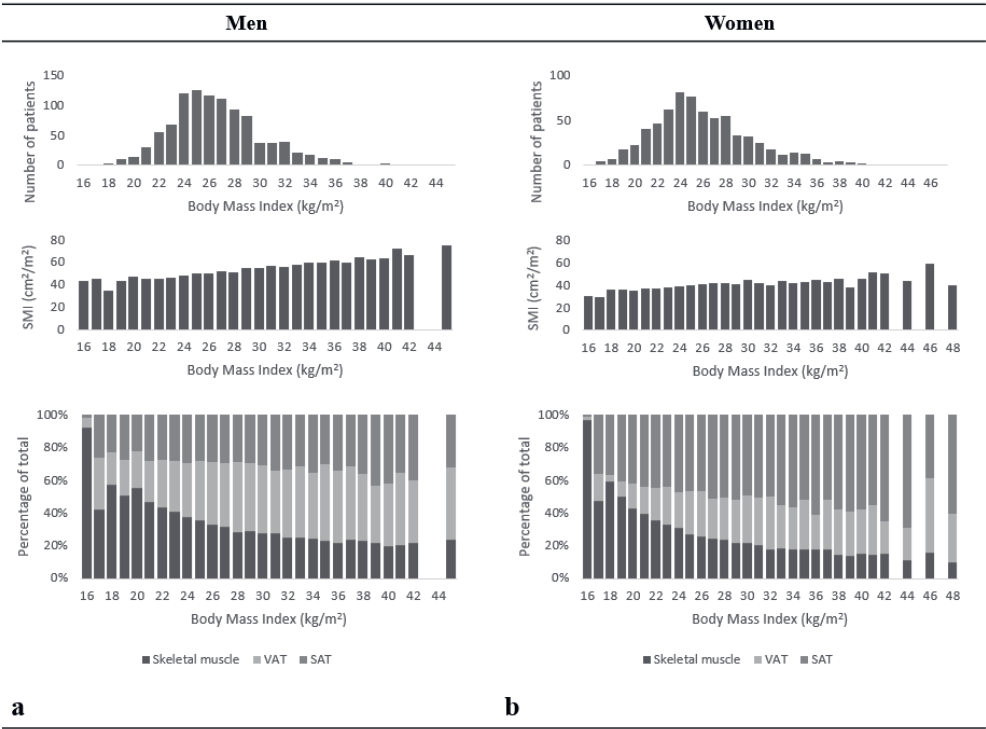
Figure 2: *Top:* Number of patients per BMI category. *Middle:* Mean absolute values of skeletal muscle index (SMI) per BMI category among (a) men and (b) women. *Bottom:* Mean relative distribution of skeletal muscle, visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT) by BMI categories among (a) men and (b) women.
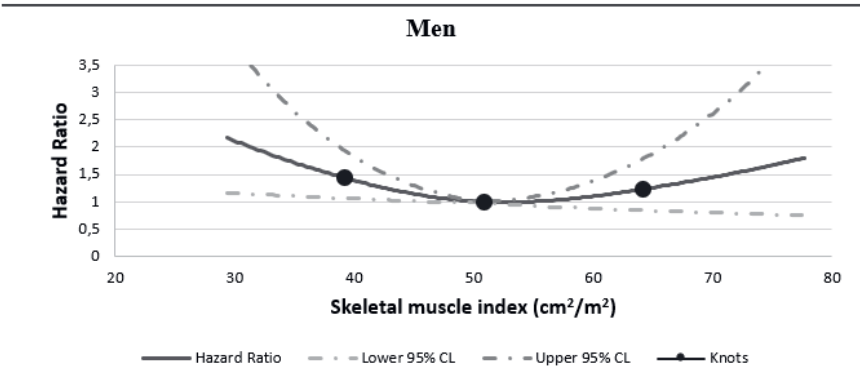
a. Test for overall association p=0.04. Test for non-linearity p=0.02.
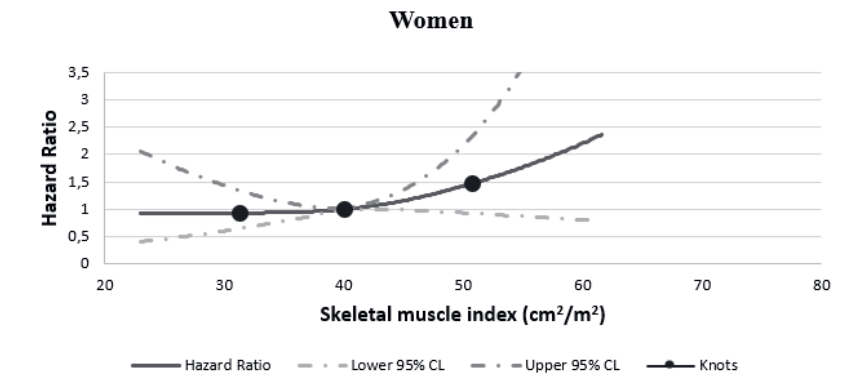
b. Test for overall association p=0.21. Test for non-linearity p=0.41.

Figure 3: The association of skeletal muscle index and mortality among colorectal cancer patients stratified into men (a) and women (b), adjusted for adjusted for age and stage of disease, radiotherapy, chemotherapy and skeletal muscle density with three knots located at the 5th, 50th (reference), and 95th percentiles of the distribution of skeletal muscle index.

Men

a. Test for overall association p=0.21. Test for non-linearity p=0.10.

Women

b. Test for overall association p=0.79. Test for non-linearity p=0.52.
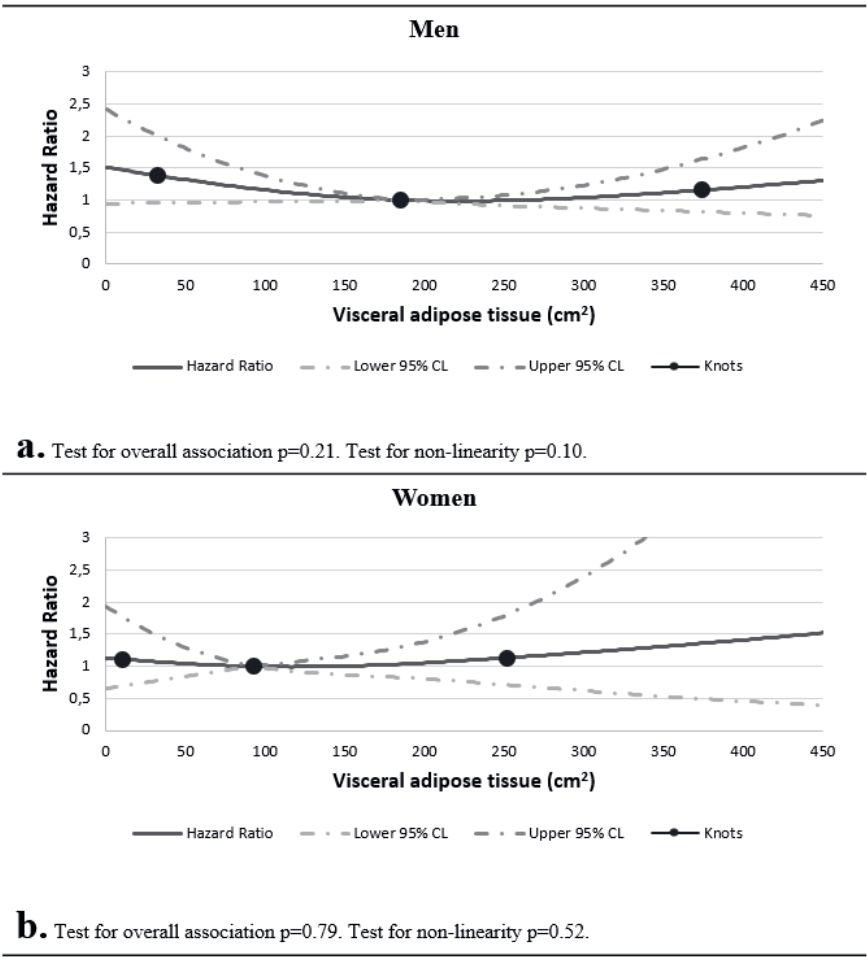
Figure 4: The association of visceral adipose tissue and mortality among colorectal cancer patients stratified into men (a) and women (b), adjusted for age and stage of disease, radiotherapy, chemotherapy, skeletal muscle density and skeletal muscle index with three knots located at the 5th, 50th (reference), and 95th percentiles of the distribution of visceral adipose tissue.

a. Test for overall association p<0.01. Test for non-linearity p<0.01

b. Test for overall association p=0.02. Test for non-linearity p<0.01
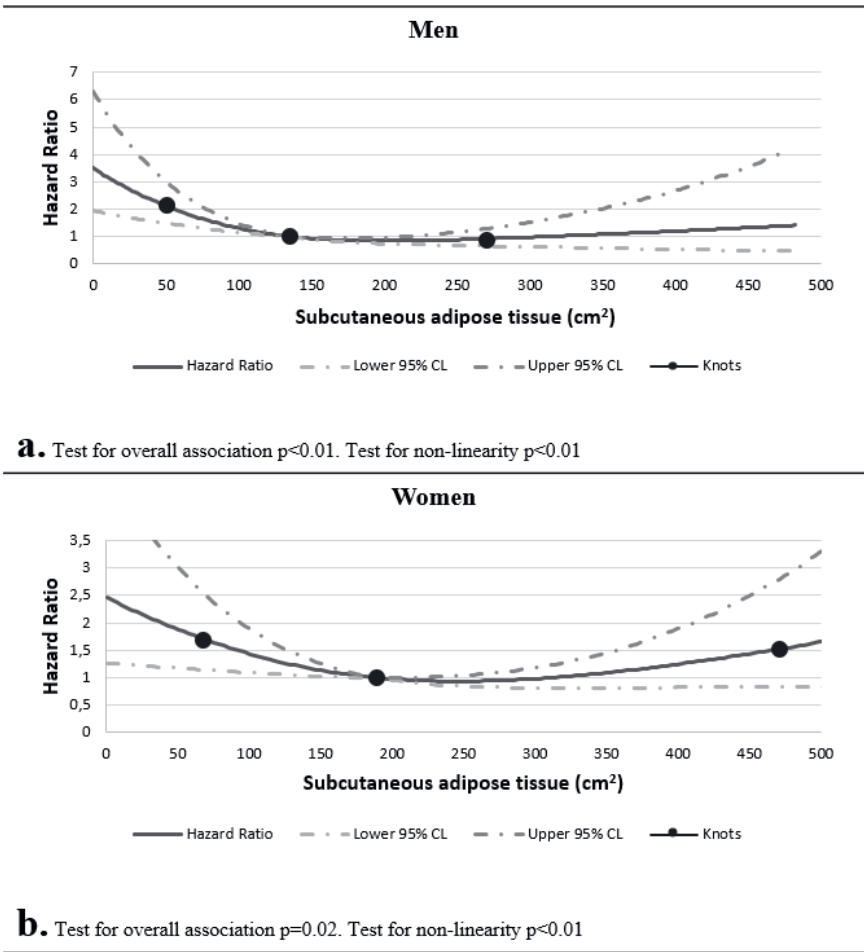
Figure 5: The association of subcutaneous adipose tissue and mortality among colorectal cancer patients stratified into men (a) and women (b), adjusted for age and stage of disease, radiotherapy, chemotherapy, skeletal muscle density and skeletal muscle index with three knots located at the 5th, 50th (reference), and 95th percentiles of the distribution of subcutaneous adipose tissue.

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Table 2: Estimated Hazard Ratios for specific skeletal muscle index, visceral adipose tissue and subcutaneous adipose tissue values and mortality, with median values as the reference category (i.e. for skeletal muscle index: men 50.8 cm^2/m^2, women 40.2 cm^2/m^2; for visceral adipose tissue: men 185.6 cm^2, women 93.3 cm^2; for subcutaneous adipose tissue: men 136.4 cm^2, women 188.3 cm^2).

	Men			Women	
	HR	95% CI		HR	95% CI
SMI (cm^2/m^2)*			SMI (cm^2/m^2)*		
40	1.39	1.06-1.83	30	0.93	0.60-1.44
45	1.15	1.01-1.30	35	0.94	0.78-1.13
50	1.01	1.00-1.02	40	1.00	0.99-1.00
55	1.00	0.92-1.09	45	1.15	0.98-1.36
60	1.10	0.88-1.38	50	1.42	0.93-2.16
VAT (cm^2)**			VAT (cm^2)**		
100	1.16	0.97-1.38	50	1.05	0.85-1.30
150	1.05	0.99-1.11	100	1.00	0.97-1.02
200	0.99	0.97-1.01	150	1.00	0.87-1.16
250	0.99	0.92-1.08	200	1.06	0.81-1.38
300	1.04	0.88-1.23	250	1.14	0.72-1.80
SAT (cm^2)**			SAT (cm^2)**		
50	2.12	1.49-3.01	100	1.44	1.09-1.90
100	1.31	1.16-1.48	150	1.14	1.03-1.26
150	0.94	0.91-0.97	200	0.98	0.95-1.00
200	0.85	0.74-0.97	250	0.94	0.84-1.04
250	0.89	0.63-1.17	300	0.98	0.81-1.18

*=adjusted for age and stage of disease, radiotherapy, chemotherapy and skeletal muscle density; **= adjusted for age and stage of disease, radiotherapy, chemotherapy, skeletal muscle density and SMI. HR, hazard ratio; 95% CI, 95% confidence interval; SMI, skeletal muscle index; VAT, visceral adipose tissue SAT, subcutaneous adipose tissue.

Discussion

In the present study, lower abdominal SMI levels were associated with higher overall mortality among men, but not among women. Lower abdominal SAT levels were associated with higher overall mortality among men and among women. VAT was not associated with overall mortality among men nor women.

Our findings that lower abdominal SMI levels were associated with higher mortality among men, but not among women, are in agreement with findings in a US cohort of stage I-III CRC patients (12). The splines presented in that paper also show no association between abdominal SMI and mortality among women (p for overall significance of the spline=0.72), and a significant association among men (p for overall significance of the spline =0.05). In both the current and the US study, the HR did not increase among women with lower SMI level. Interestingly, in the current study higher levels of SMI among women were associated with increased risk of mortality, although this was not statistically significant. Since higher SMI levels in general are correlated with higher levels of adipose tissue, one could hypothesize that the higher HR is actually a result of having more adipose tissue. However, adjustment for TAT (or VAT or SAT) did not change this HR and for that reason TAT, VAT, or SAT were not included as possible confounding factors in those analyses. Therefore, the potential higher risk of mortality among women with higher SMI was not explained by having more adipose tissue.

Other studies may have decided not to stratify by sex, because they were too small (9-11). Other cohorts of (healthy) elderly populations found differences between men and women in associations of body composition and mortality although data were not fully consistent (35). Sex-related differences in inflammation levels seen with muscle wasting (36) may be part of the mechanism of why there are sex-related differences in the association of skeletal muscle mass with mortality. Additionally, age-related changes in muscle mass have been hypothesized to differ between men and women, (28) which could shed further light on why associations with mortality could differ between men and women, but the exact mechanisms remain to be fully elucidated. Our findings stress the importance of the recent increase of attention for sex-related differences in biomedical research (37).

We showed that lower abdominal SAT levels were associated with higher mortality among men and among women with stage I-III CRC. This is in agreement with a recent study among stage I-III CRC patients where splines were used to investigate the association of abdominal SAT and mortality (18). Notably however, having higher levels of SAT was associated with lower risk of mortality in that study, which is something that we did not observe in our data. A possible explanation for this discrepancy can be that we chose to

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use the median as reference value in our splines, while the other study elected to use a SAT level of 50 cm^2 as reference value (18). Moreover, the variation in SAT levels in our study was lower in our cohort, than in the published study: in our study 95% of the men had a SAT level below 272.1 cm^2 and, 95% of the women a SAT level below 398.4 cm^2 while a substantial number of participants had SAT levels above 400 cm^2 in the other published study. This lower range of SAT values in our study, is the result of a lower average BMI in the population in the Netherlands, relative to the BMI distribution in the US (38). As a result, we had less statistical power to detect associations especially for SAT values in the upper/high range.

Our results for abdominal SAT are not in agreement with analyses in a cohort of CRC patients from the US (12). In that analysis, no associations were observed for abdominal SAT and mortality. We hypothesize that this inconsistency is the result of methodological differences in the statistical analysis between those results and the results presented in the current paper. The US study (12) only reported on risk associated with high SAT (highest tertile) versus lower SAT (reference group consisting of participants in the lowest two tertiles). This categorization may not have been optimal to detect a potential increased risk of mortality with lower levels of SAT. This underlines the importance of using splines to explore the potential associations of features of body composition and mortality (39). Studies among other cancer populations have also reported a statistically significant association between low abdominal SAT versus normal/high abdominal SAT and higher mortality (13, 40, 41). The potential mechanism of why low SAT may be associated with increased risk of mortality remains to be established, but low SAT areas may be a sign of minimal metabolic reserves, which may explain why these patients have a higher mortality (42).

VAT was not significantly associated with worse survival in our population, which is consistent with three other studies (11, 12, 16), but not with two other studies (15, 18). As mentioned before, one of those earlier studies used splines to investigate the association of VAT and mortality. That study observed that among women higher levels of VAT were associated with higher mortality relative to a reference value of 50 cm^2, and that among men having VAT levels of ~100-300 cm^2 was associated with lower mortality compared with the reference value of 50 cm^2. As also argued in the earlier paragraph on the SAT findings, an important factor to consider is what was chosen as the reference group. In our analyses, we chose the median value as reference value, while in the other study a (very low) VAT level of 50 cm^2 was chosen as reference value. Moreover, the range of values in VAT is smaller in our cohort than in the cohort from the US. From the splines reported in that paper it is apparent that the increased risk of mortality with increasing VAT levels reached statistical significance when levels of VAT were above ~200 cm^2. In our dataset only a limited number of women had VAT levels above that value, and thus our statistical

power to observe associations was limited. In addition, contrary to the other study, we adjusted our analyses for VAT for skeletal muscle radiodensity. This adjustment attenuated our HR with more than 10%, while the other study chose not to adjust for it. Thus, this discrepancy can partly explain the different findings. Although more research on this topic is warranted, a conclusion could be that the increased risk of higher levels of VAT is evident mostly among very high levels of VAT, and this is most relevant for populations with extremely high BMI.

The current study has some limitations. Almost one quarter of the patients in the original dataset had to be excluded, mostly due to unavailable or unusable CT images. Nevertheless, sex, age and stage distribution were largely similar for in- and excluded patients, so we are confident that excluding these patients did not affect our findings substantially. This study consisted of data from three independent studies. The size of the separate studies was too small to conduct separate analysis for the different studies. However, Supplementary Table 2 shows that characteristics are generally similar across cohorts, thus we are confident that results are similar across cohorts and across the total cohort. As in any observational study, we might miss data of unknown confounders which could influence the observed associations and have led to residual confounding.

Strengths of this study were the large study population and the use of CT images to assess body composition. Furthermore, this is the largest European study with information on body composition, clinical and personal factors thus far. Although earlier large-scale studies in US/Canadian populations have been performed, the differences in body composition (related to a generally lower BMI in Europe) (38) underline the necessity to study these associations in European populations. Further studies, including even more diverse populations, for example Asian or African cohorts, could shed further light on associations between body composition and colorectal cancer prognosis.

In conclusion, in this large-scale study among stage I-III CRC patients, lower abdominal SMI levels were associated with higher mortality among men, but not among women. Lower abdominal SAT levels were associated with higher overall mortality in both men and women. An association between VAT and mortality was not observed. Our results show the utility and importance of using splines to explore relationships of features of body composition and mortality. Furthermore, our findings underline the importance for more attention for sex-related differences in body composition and cancer outcomes.

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References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: A Cancer Journal for Clinicians. 2018;68(6):394-424.
2. Choi MH, Oh SN, Lee IK, Oh ST, Won DD. Sarcopenia is negatively associated with long-term outcomes in locally advanced rectal cancer. Journal of cachexia, sarcopenia and muscle. 2018;9(1):53-9.
3. Malietzis G, Currie AC, Athanasiou T, Johns N, Anyamene N, Glynne-Jones R, et al. Influence of body composition profile on outcomes following colorectal cancer surgery. British Journal of Surgery. 2016;103(5):572-80.
4. van der Kroft G, Bours M, Janssen-Heijnen M, van Berlo C, Konsten J. Value of Sarcopenia Assessed by Computed Tomography for the Prediction of Postoperative Morbidity Following Oncological Colorectal Resection: a Comparison with the Malnutrition Screening Tool. Clinical nutrition ESPEN. 2018;24:114-9.
5. Van Vledder MG, Levolger S, Ayez N, Verhoef C, Tran TCK, Ijzermans JNM. Body composition and outcome in patients undergoing resection of colorectal liver metastases. British Journal of Surgery. 2012;99(4):550-7.
6. Lodewick TM, Nijnatten TJ, Dam RM, Mierlo K, Dello SA, Neumann UP, et al. Are sarcopenia, obesity and sarcopenic obesity predictive of outcome in patients with colorectal liver metastases? HPB. 2015;17(5):438-46.
7. Prado CM, Lieffers JR, McCargar LJ, Reiman T, Sawyer MB, Martin L, et al. Prevalence and clinical implications of sarcopenic obesity in patients with solid tumours of the respiratory and gastrointestinal tracts: a population-based study. The lancet oncology. 2008;9(7):629-35.
8. Blauwhoff-Buskermolen S, Versteeg KS, de van der Schueren MA, Den Braver NR, Berkhof J, Langius JA, et al. Loss of Muscle Mass During Chemotherapy Is Predictive for Poor Survival of Patients With Metastatic Colorectal Cancer. Journal of Clinical Oncology. 2016;34(12):1339-44.
9. van Vugt J, van den Braak RC, Lalmahomed Z, Vrijland W, Dekker J, Zimmerman D, et al. Impact of Low Skeletal Muscle Mass and Density on Short and Long-Term Outcome after Resection of Stage I-III Colorectal Cancer. European Journal of Surgical Oncology. 2018;44(9):177.
10. Miyamoto Y, Baba Y, Sakamoto Y, Ohuchi M, Tokunaga R, Kurashige J, et al. Sarcopenia is a negative prognostic factor after curative resection of colorectal cancer. Annals of surgical oncology. 2015;22(8):2663-8.
11. Black D, Mackay C, Ramsay G, Hamoodi Z, Nanthakumaran S, Park KG, et al. Prognostic Value of Computed Tomography: Measured Parameters of Body Composition in Primary Operable Gastrointestinal Cancers. Annals of Surgical Oncology. 2017:1-11.
12. Caan BJ, Meyerhardt JA, Kroenke CH, Alexeeff S, Xiao J, Weltzien E, et al. Explaining the Obesity Paradox: The Association between Body Composition and Colorectal Cancer Survival (C-SCANS Study). Cancer Epidemiology and Prevention Biomarkers. 2017;26(7):1008-15.

3

13. Ebadi M, Martin L, Ghosh S, Field CJ, Lehner R, Baracos VE, et al. Subcutaneous adiposity is an independent predictor of mortality in cancer patients. British Journal of Cancer. 2017;117(1):148.

14. Martin L, Birdsell L, Macdonald N, Reiman T, Clandinin MT, McCargar LJ, et al. Cancer Cachexia in the Age of Obesity: Skeletal Muscle Depletion Is a Powerful Prognostic Factor, Independent of Body Mass Index. J Clin Oncol. 2013;31(12):1539-47.

15. Lee CS, Murphy DJ, McMahon C, Nolan B, Cullen G, Mulcahy H, et al. Visceral Adiposity is a Risk Factor for Poor Prognosis in Colorectal Cancer Patients Receiving Adjuvant Chemotherapy. Journal of Gastrointestinal Cancer. 2015;46(3):243-50.

16. Rickles AS, Iannuzzi JC, Mironov O, Deeb AP, Sharma A, Fleming FJ, et al. Visceral Obesity and Colorectal Cancer: Are We Missing the Boat with BMI? Journal of Gastrointestinal Surgery. 2013;17(1):133-43.

17. Hopkins JJ, Reif RL, Bigam DL, Baracos VE, Eurich DT, Sawyer MB. The Impact of Muscle and Adipose Tissue on Long-term Survival in Patients With Stage I to III Colorectal Cancer. Diseases of the Colon & Rectum. 2019;62(5):549-60.

18. Brown JC, Caan BJ, Prado CM, Cespedes Feliciano EM, Xiao J, Kroenke CH, et al. The Association of Abdominal Adiposity With Mortality in Patients With Stage I–III Colorectal Cancer. JNCI: Journal of the National Cancer Institute. 2019.

19. Siegel RL, Miller KD, Fedewa SA, Ahnen DJ, Meester RG, Barzi A, et al. Colorectal cancer statistics, 2017. CA: a cancer journal for clinicians. 2017;67(3):177-93.

20. Wie G-A, Cho Y-A, Kim S-Y, Kim S-M, Bae J-M, Joung H. Prevalence and risk factors of malnutrition among cancer patients according to tumor location and stage in the National Cancer Center in Korea. Nutrition. 2010;26(3):263-8.

21. Daly LE, Ryan AM, Power DG. Response to "Loss of Muscle Mass During Chemotherapy Is Predictive for Poor Survival of Patients With Metastatic Colorectal Cancer". Journal of Clinical Oncology. 2016;34(31):3816-7.

22. Blauwhoff-Buskermolen S, Schueren MAEdvd, Langius JAE, Verheul HMW. Reply to L.E. Daly et al. Journal of Clinical Oncology. 2016;34(31):3817.

23. Schorr M, Dichtel LE, Gerweck AV, Valera RD, Torriani M, Miller KK, et al. Sex differences in body composition and association with cardiometabolic risk. Biology of Sex Differences. 2018;9(1):28.

24. Liesenfeld DB, Grapov D, Fahrmann JF, Salou M, Scherer D, Toth R, et al. Metabolomics and transcriptomics identify pathway differences between visceral and subcutaneous adipose tissue in colorectal cancer patients: the ColoCare study. The American Journal of Clinical Nutrition. 2015;102(2):433-43.

25. Ibrahim MM. Subcutaneous and visceral adipose tissue: structural and functional differences. Obesity reviews. 2010;11(1):11-8.

26. Desquilbet L, Mariotti F. Dose-response analyses using restricted cubic spline functions in public health research. Statistics in medicine. 2010;29(9):1037-57.

27. Bredella MA. Sex differences in body composition. Sex and Gender Factors Affecting Metabolic Homeostasis, Diabetes and Obesity: Springer; 2017. p. 9-27.

28. Gheller BJF, Riddle ES, Lem MR, Thalacker-Mercer AE. Understanding Age-Related Changes in Skeletal Muscle Metabolism: Differences Between Females and Males. Annual Review of Nutrition. 2016;36(1):129-56.

29. Cartier A, Côté M, Lemieux I, Pérusse L, Tremblay A, Bouchard C, et al. Sex differences in inflammatory markers: what is the contribution of visceral adiposity? The American journal of clinical nutrition. 2009;89(5):1307-14.

30. Winkels R, Heine-Broring R, Van Zutphen M, van Harten-Gerritsen S, Kok D, Van Duijnhoven F, et al. The COLON study: Colorectal cancer: Longitudinal, Observational study on Nutritional and lifestyle factors that may influence colorectal tumour recurrence, survival and quality of life. BMC Cancer. 2014;14(1):374.

31. van Roekel EH, Bours MJL, de Brouwer CPM, Ten Napel H, Sanduleanu S, Beets GL, et al. The Applicability of the International Classification of Functioning, Disability, and Health to Study Lifestyle and Quality of Life of Colorectal Cancer Survivors. Cancer Epidemiology Biomarkers & Prevention. 2014;23(7):1394-405.

32. van Baar H, Beijer S, Bours M, Weijenberg M, van Zutphen M, van Duijnhoven F, et al. Low radiographic muscle density is associated with lower overall and disease-free survival in early-stage colorectal cancer patients. Journal of cancer research and clinical oncology. 2018:1-9.

33. Prado CMM, Birdsell LA, Baracos VE. The emerging role of computerized tomography in assessing cancer cachexia. Current opinion in supportive and palliative care. 2009;3(4):269-75.

34. Mitsiopoulos N, Baumgartner R, Heymsfield S, Lyons W, Gallagher D, Ross R. Cadaver validation of skeletal muscle measurement by magnetic resonance imaging and computerized tomography. Journal of applied physiology. 1998;85(1):115-22.

35. Lee DH, Giovannucci EL. Body composition and mortality in the general population: A review of epidemiologic studies. Experimental Biology and Medicine. 2018;243(17-18):1275-85.

36. Bano G, Trevisan C, Carraro S, Solmi M, Luchini C, Stubbs B, et al. Inflammation and sarcopenia: a systematic review and meta-analysis. Maturitas. 2017;96:10-5.

37. Clayton JA. Applying the new SABV (sex as a biological variable) policy to research and clinical care. Physiology & behavior. 2018;187:2-5.

38. Bassett DR, Pucher Jr J, Buehler R, Thompson DL, Crouter SE. Walking, cycling, and obesity rates in Europe, North America, and Australia. Journal of Physical Activity and Health. 2008;5(6):795-814.

39. Marrie RA, Dawson NV, Garland A. Quantile regression and restricted cubic splines are useful for exploring relationships between continuous variables. Journal of clinical epidemiology. 2009;62(5):511-7. e1.

40. Antoun S, Bayar A, Ileana E, Laplanche A, Fizazi K, di Palma M, et al. High subcutaneous adipose tissue predicts the prognosis in metastatic castration-resistant prostate cancer patients in post chemotherapy setting. European journal of cancer. 2015;51(17):2570-7.

41. Li X-T, Tang L, Chen Y, Li Y-L, Zhang X-P, Sun Y-S. Visceral and subcutaneous fat as new independent predictive factors of survival in locally advanced gastric carcinoma patients treated with neo-adjuvant chemotherapy. Journal of cancer research and clinical oncology. 2015;141(7):1237-47.

3

42. Lavie CJ, McAuley PA, Church TS, Milani RV, Blair SN. Obesity and cardiovascular diseases: implications regarding fitness, fatness, and severity in the obesity paradox. Journal of the American College of Cardiology. 2014;63(14):1345-54.

Supplemental tables and figures

Men
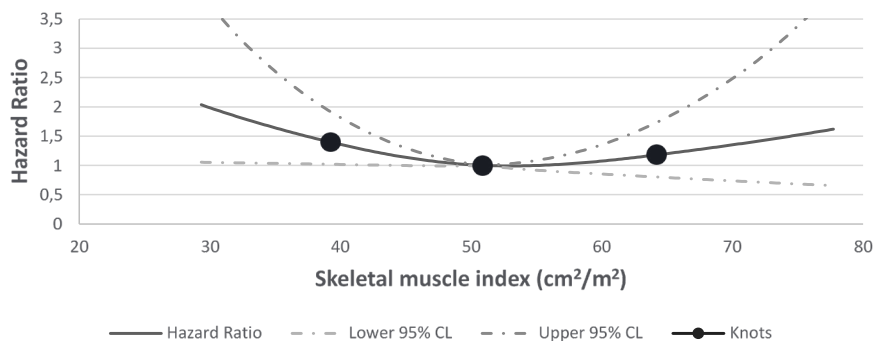
a. Test for overall association p=0.10. Test for non-linearity p=0.06.

Women
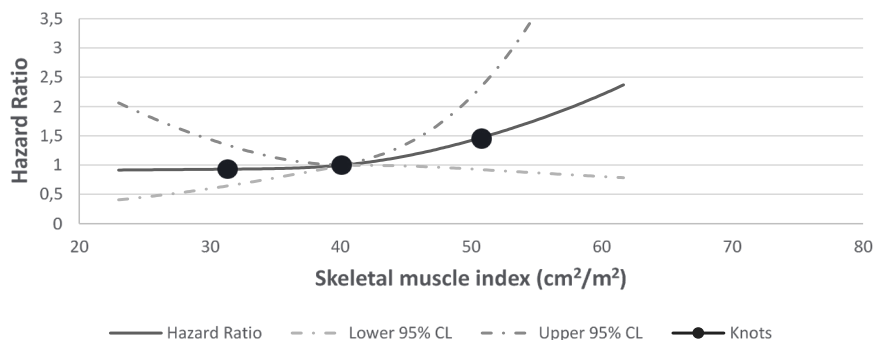
b. Test for overall association p=0.20. Test for non-linearity p=0.76.

Supplementary Figure 1: Results for the sensitivity analysis in which patients who deceased within 30 days of surgery were excluded (n=41 excluded). The association between skeletal muscle index and mortality among men (a) and women (b), adjusted for adjusted for age and stage of disease, radiotherapy, chemotherapy and skeletal muscle density with three knots located at the 5th, 50th (reference), and 95th percentiles of the distribution of skeletal muscle index.

Men
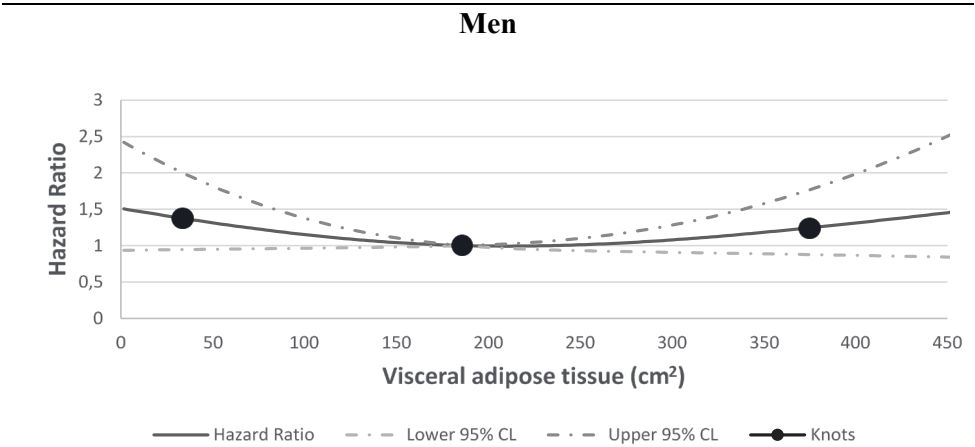
a. Test for overall association p=0.18. Test for non-linearity p=0.07.

Women
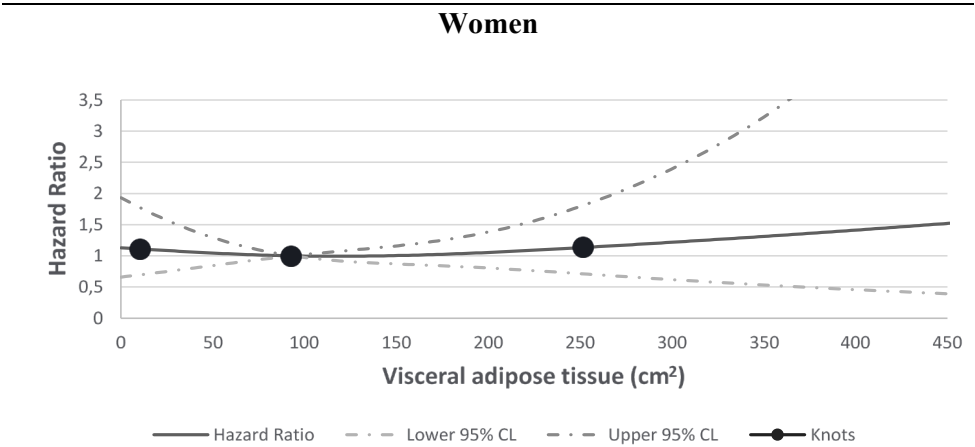
b. Test for overall association p=0.92. Test for non-linearity p=0.75.

Supplementary Figure 2: Results for the sensitivity analysis in which patients who deceased within 30 days of surgery were excluded (n=41 excluded). The association between visceral adipose tissue and mortality in men (a) and women (b), adjusted for age and stage of disease, radiotherapy, chemotherapy, skeletal muscle density and skeletal muscle index with three knots located at the 5th, 50th (reference), and 95th percentiles of the distribution of visceral adipose tissue.

Men
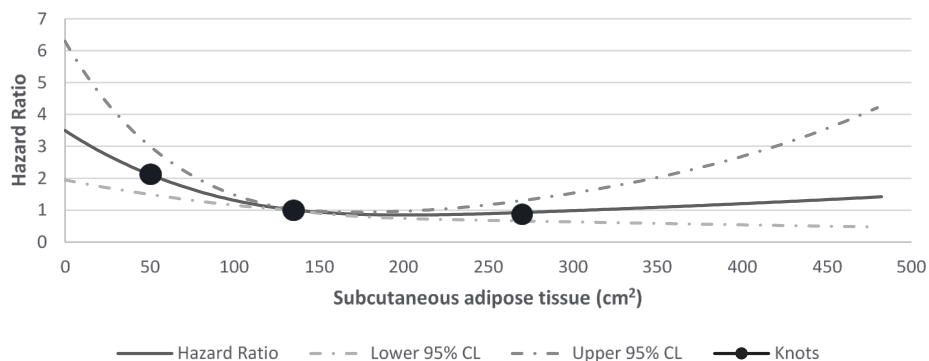
a. Test for overall association p<0.01. Test for non-linearity p<0.01

Women
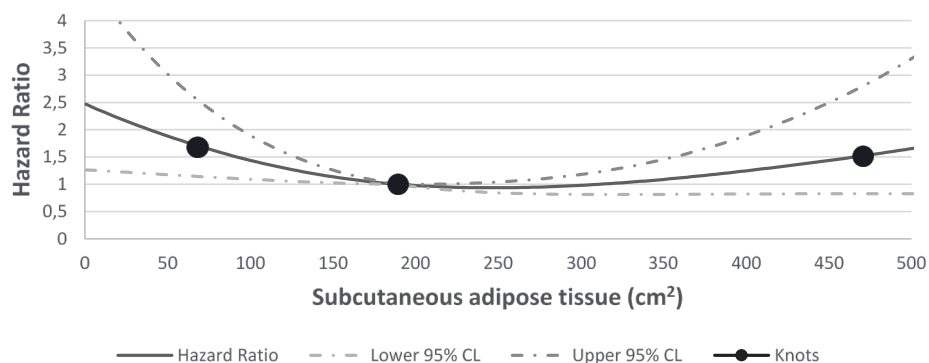
b. Test for overall association p=0.04. Test for non-linearity p=0.02.

Supplementary Figure 3: Results for the sensitivity analysis in which patients who deceased within 30 days of surgery were excluded (n=41 excluded). The association between subcutaneous adipose tissue and mortality in men (a) and women (b), adjusted for age and stage of disease, radiotherapy, chemotherapy, skeletal muscle density and skeletal muscle index with three knots located at the 5th, 50th (reference), and 95th percentiles of the distribution of subcutaneous adipose tissue.

Supplementary Table 1: Results for the sensitivity analysis in which patients who deceased within 30 days of surgery were excluded (n=41 excluded). Estimated Hazard Ratios for specific skeletal muscle index, visceral adipose tissue and subcutaneous adipose tissue values and mortality with median values as the reference category (i.e. for skeletal muscle index: men 51.4 cm^2/m^2, women 40.5 cm^2/m^2; for visceral adipose tissue: men 186.5 cm^2, women 92.9 cm^2; for subcutaneous adipose tissue: men 136.2 cm^2, women 189.8 cm^2).

| Men			Women		
	HR	95% CI		HR	95% CI
SMI (cm^2/m^2)[*]			SMI (cm^2/m^2)[*]		
40	1.36	1.02-1.81	30	0.93	0.60-1.44
45	1.14	1.00-1.30	35	0.94	0.78-1.13
50	1.01	0.99-1.03	40	1.00	0.99-1.00
55	1.00	0.92-1.08	45	1.15	0.98-1.36
60	1.07	0.85-1.35	50	1.42	0.93-2.16
VAT (cm^2)[**]			VAT (cm^2)[**]		
100	1.15	0.96-1.38	50	1.02	0.82-1.27
150	1.04	0.98-1.11	100	1.00	0.97-1.03
200	0.99	0.98-1.01	150	1.01	0.87-1.18
250	1.01	0.93-1.10	200	1.05	0.78-1.40
300	1.08	0.91-1.28	250	1.15	0.672-1.80
SAT (cm^2)[**]			SAT (cm^2)[**]		
50	2.04	1.43-2.93	100	1.44	1.08-1.92
100	1.29	1.14-1.46	150	1.14	1.02-1.27
150	0.94	0.91-0.97	200	0.98	0.96-1.00
200	0.86	0.76-0.99	250	0.92	0.83-1.03
250	0.92	0.70-1.21	300	0.95	0.78-1.15

[*]=adjusted for age and stage of disease, radiotherapy, chemotherapy and skeletal muscle density; [**]= adjusted for age and stage of disease, radiotherapy, chemotherapy, skeletal muscle density and SMI. HR, hazard ratio; 95% CI, 95% confidence interval; SMI, skeletal muscle index; VAT, visceral adipose tissue SAT, subcutaneous adipose tissue.

Supplementary Table 2: Baseline characteristics of stage I-III CRC patients stratified into the three contributing studies.

	COLON Study (n=703)	EnCoRe Study (n=154)	Registry-Based (n=1141)
Demographic characteristics			
Age [yrs, mean (SD)]	65.9 (9.0)	65.4 (10.1)	69.5 (11.2)
Men [n (%)]	431 (61)	103 (67)	632 (55)
Follow-up time [months, median (IQR range)]	37 (26, 54)	28 (21, 39)	75 (41, 91)
Clinical characteristics			
Cancer stage [n (%)]			
I	209 (30)	38 (25)	231 (20)
II	204 (29)	44 (29)	443 (39)
III	290 (41)	72 (47)	467 (41)
Tumor location [n (%)]			
Colon	483 (69)	92 (60)	154 (66)
Rectal	218 (31)	62 (40)	368 (34)
Body composition			
BMI [kg/m^2, n (%)]			
<20	24 (3)	3 (2)	40 (5)
20-24.9	253 (36)	38 (25)	338 (39)
25-29.9	308 (44)	66 (43)	354 (41)
≥30	118 (17)	46 (30)	131 (15)

Abbreviations: BMI, body mass index; SD, standard deviation.

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Chapter 4

Body composition and its association with fatigue in the first 2 years after colorectal cancer diagnosis

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Purpose: Persistent fatigue among colorectal cancer (CRC) patients might be associated with unfavorable body composition, but data are sparse and inconsistent. We studied how skeletal muscle index (SMI), skeletal muscle radiodensity (SMR), visceral adipose tissue (VAT), and subcutaneous adipose tissue (SAT) at diagnosis are associated with fatigue up to 24 months post-diagnosis in stage I-III CRC patients.

Methods: SMI, SMR, VAT and SAT were assessed among 646 CRC patients using pre-treatment computed tomography images. Fatigue at diagnosis, at 6 and 24 months post-diagnosis was assessed using the European Organization for Research and Treatment of Cancer Quality of Life Questionnaire. The association of SMI, SMR, VAT, and SAT with fatigue (yes/no) was assessed using confounder-adjusted restricted cubic spline analyses.

Results: Prevalence of fatigue at diagnosis was 18%, at 6 months 25% and at 24 months 12%. At diagnosis, a significant (p=0.01) non-linear association of higher levels of SAT with higher prevalence of fatigue was observed. Lower levels of SMR were linearly associated with higher prevalence of fatigue at 6 months post-diagnosis (overall association p=0.02). None of the body composition parameters were significantly associated with fatigue at 24 months.

Conclusion: Having more SAT was associated with more fatigue at diagnosis, while low levels of SMR were associated with more fatigue at 6 months post-diagnosis.

Implications for Cancer Survivors: Assessing SMR at diagnosis might help identify patients with higher risk of fatigue at a later stage. However, more knowledge is needed to understand the mechanisms behind the association of SMR with fatigue.

ABSTRACT

Introduction

Fatigue is highly prevalent among stage I-III colorectal cancer (CRC) patients. Fatigue is often already experienced before diagnosis (1) and can persist for years after treatment. The highest prevalence (up to 85%) of fatigue is seen during and shortly after treatment (2, 3), and although the prevalence decreases in the years after treatment, up to 40% of CRC patients experience fatigue in the first 5 years after diagnosis (4). Fatigue is one of the most debilitating side-effects of cancer and has a substantial negative impact on mood, work, social relationships and overall quality of life (5).

Fatigue among cancer survivors is thought to have a multi-factorial etiology. Fatigue has been associated with: treatment (radio- and chemotherapy), stage of disease, presence of (multiple) comorbidities, specific medications with sedating side effects, psychological factors (e.g. depression), decreased physical activity, and malnutrition (5-8). Associations between body composition and fatigue in cancer survivors have been studied in only a few studies, with mixed results. Two studies conducted among 734 stage IIIb/IV non-small cell lung cancer patients (9) and 151 advanced colorectal, breast, or prostate cancer patients (10), respectively, both observed that less skeletal mass at diagnosis was associated with higher levels of pre-treatment fatigue among men. Both studies did not observe significant associations among women. Another study conducted among 96 stage I-III CRC survivors who were on average 5.2 years post-diagnosis, did not find an association between skeletal muscle mass at diagnosis and levels of fatigue at that point post-diagnosis (11). The latter study also investigated visceral adipose tissue (VAT), and skeletal muscle radiodensity (SMR, an indicator of fat infiltration within the muscle) and did not find an association between those body composition parameters and fatigue (11). The association between SMR at diagnosis and pretreatment fatigue was also investigated among the earlier quoted study among non-small cell lung cancer patients, and no significant association was observed there either (9). To our knowledge, no study has investigated the association between subcutaneous adipose tissue (SAT) and fatigue among cancer patients.

The aim of the current study was to investigate the association of body composition parameters (Skeletal Muscle Index (SMI), SMR, VAT and SAT) at diagnosis with fatigue at diagnosis, and at 6 and 24 months post-diagnosis in stage I-III CRC patients.

Methods

Study population

For this study, we used data of the ongoing COLON study: Colorectal cancer: Longitudinal, Observational study on Nutritional and lifestyle factors that may influence colorectal tumor recurrence, survival and quality of life (12). In eleven participating hospitals, hospital staff invited eligible patients to participate in the COLON study shortly after diagnosis and before scheduled surgery. Patients were not eligible if they had a history of CRC, a previous (partial) bowel resection, known hereditary CRC, inflammatory bowel disease, dementia or another mental condition limiting their ability to fill out surveys, or were non-Dutch speaking. For the present study, participants diagnosed between 2010 and 2015, from seven of the eleven participating hospitals were included. For the participants from the other hospitals and participants included after 2015, no CT images had been retrieved from the medical records at time of the current analyses. Exclusion criteria for the present analyses were: missing data on fatigue, height, stage of disease or comorbidities; stage IV CRC; and missing or unusable CT images (i.e., CT images of poor quality or scans where muscle tissue was partly cutoff). In addition, we only used data of patients who had a pretreatment CT image performed no longer than 3 months before diagnosis, as we considered that to be representative for body composition at diagnosis. The study population consisted of 960 patients, of which we had to exclude: 192 patients because no suitable CT image was available; 68 patients because of stage IV disease; 11 patients because of missing information on stage of disease; and 63 patients because no fatigue data at diagnosis were available (**Figure 1**). This resulted in a final dataset of 646 patients with fatigue data at diagnosis, 581 patients with also fatigue data at 6 months, and 496 patients with fatigue data at 24 months.

The COLON study was approved by the Committee on Research involving Human Subjects, region Arnhem–Nijmegen, the Netherlands, and all study participants provided written informed consent.

Fatigue

Fatigue was assessed using the European Organization for Research and Treatment of Cancer Quality of Life Questionnaire C30 (EORTC QLQ-C30) version 3.0 (13) at three points in time: at diagnosis, and 6 and 24 months after diagnosis. In the EORTC QLQ-C30, the fatigue subscale is comprised of three items (During the past week: Did you need to rest?, Have you felt weak?, Were you tired?), with four response options used to score the items: "Not at all", "A little", "Quite a bit", and "Very much". The raw score for fatigue was linearly transformed into a score of 0-100 points as described earlier (13), with a higher score indicating higher levels of fatigue. A score of >39 was defined as having clinically relevant fatigue as recommended elsewhere (14, 15).

Body composition

Cross-sectional areas (cm^2) of skeletal muscle, VAT, and SAT were assessed using standard radiodensity thresholds measured in Hounsfield Units (HU) in preoperative CT images at the level of the 3rd lumbar vertebrae using Slice-O-Matic 5.0 (Tomovision, Montreal, Canada). For skeletal muscle the threshold values were between -29 and +150 HU, for VAT between -150

and -50 HU, and for SAT between -190 and -30 HU (16, 17). The skeletal muscle cross-sectional area was adjusted for height squared (cm^2/m^2) to calculate Skeletal Muscle Index (SMI). SMR was assessed as the mean radiodensity of the total skeletal muscle cross-sectional area at the level of the 3rd lumbar vertebrae.

Demographic, lifestyle and clinical data

Demographic information including age at diagnosis, sex, and weight and height were collected using self-administered questionnaires. Physical activity was assessed using the validated Short QUestionnaire to ASsess Health enhancing physical activity (SQUASH) (18). Data on stage of disease, tumor site, treatment, comorbidities, complications after surgery, and stoma placement after surgery were retrieved from the Dutch ColoRectal Audit (19).

Data analyses

Demographic, clinical and lifestyle characteristics are presented as mean and standard deviation (SD) for continuous variables with normal distribution, median and interquartile range (IQR) for continuous variables without normal distribution, or frequency and percentage for categorical variables.

Restricted cubic spline (RCS) analyses (20) were used to investigate associations of SMI, SMR, VAT and SAT with fatigue at all time-points, and to assess whether associations were non-linear. Knots were placed at the 5th, 50th and 95th percentiles of SMI, SMR, VAT or SAT. Prevalence ratios (PR) and 95% confidence intervals (CI) for the associations of SMI, SMR, VAT and SAT with fatigue were estimated using RCS functions in Cox proportional hazard regression models with a fixed time-point. PRs were chosen instead of odd ratios since the latter tend to overestimate the size of the association when the outcome is common (21). Median value for each body composition parameter was set as the reference in each model. As complement to the graphic presentation of the RCS graphs, PRs with 95% confidence intervals for specific SMI, SMR, VAT and SAT values were calculated using the RCS analyses.

All analyses were tested for effect modification by gender, by calculating the p value for interaction. In none of the analyses gender was identified as effect modifier. Potential
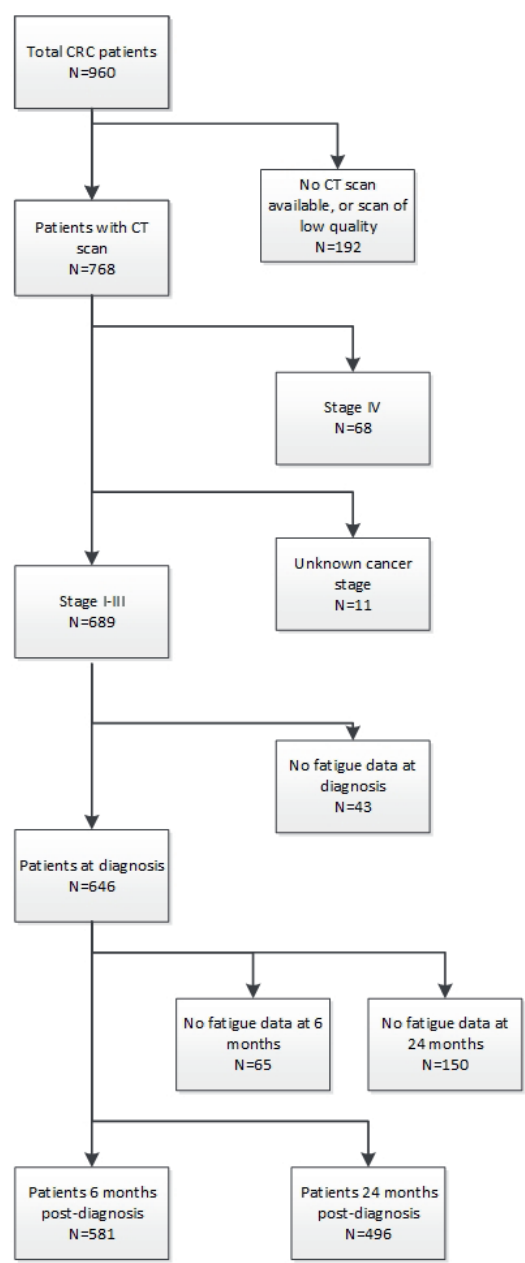
Figure 1 Flowchart of the inclusion process of patients in this study

confounders were included in the final model if they changed the PR for fatigue with 10% or more when the variable was individually added to a crude model including SMI/SMR/VAT/SAT, age, stage of disease and gender. For all analyses, potential confounders were: SMI (continuous, in the model for SMR, VAT and SAT), SMR (continuous, in the model for SMI, VAT and SAT), total adipose tissue (calculated as sum of VAT and SAT, continuous), comorbidities (0, 1 and ≥ 2), tumor site (colon/rectum), and physical activity (minutes per week of moderate-to-vigorous physical activity). For the analyses at 6 and 24 months, additional potential confounders were: chemotherapy (yes/no), radiotherapy (yes/no), stoma placement after surgery (yes/no), complications post-surgery (yes/no).

The statistical significance level for the analyses was set at $p < 0.05$. Statistical analyses were carried out using IBM SPSS v.23 (SPSS, Chicago, IL).

Results

Study population
Baseline characteristics of the total study population and of patients with and without fatigue at the various time-points are presented in **Table 1.** The average age of the total study population at diagnosis was 66.1 ± 8.8 years, 63% of the patients were men, and in 66% of the patients the tumor was located in the colon. The majority of the patients had stage III disease (46%), while the percentages of patients with stage I or II disease were 27% and 28%, respectively. At diagnosis, and at 6 and 24 months post-diagnosis, 18%, 25% and 12% of the patients, respectively, experienced fatigue.

Difference between patients with and without fatigue
At diagnosis, age was similar for patients with and without fatigue, women more often reported fatigue, and patients with colon cancer more often experienced fatigue (**Table 1**).

Six months post-diagnosis, the patients who experienced fatigue were slightly younger, more often women, and received more often chemotherapy and/or radiotherapy. At six month post-diagnosis the percentage of patients with colon cancer was almost similar between the group with and without fatigue.
At 24 months post-diagnosis, age was similar for patients with or without fatigue and the percentage of men was higher among patients with fatigue. The percentage of patients who received chemotherapy was almost similar between the group with and without fatigue, while slightly more patients with fatigue 24 months post-diagnosis received radiotherapy.

At all three time-points, the number of hours of moderate-to-vigorous physical activity was lower among the patients with fatigue, and the percentage of patients with two or more comorbidities at diagnosis was higher among patients with fatigue.

Association between body composition and fatigue

No association was observed between SMI and fatigue at any of the three time-points (test for overall association at diagnosis and at 6 and 24 months post-diagnosis: p=0.36, p=0.80, and p=0.54, respectively), **Figure 2a**. PRs with 95% confidence intervals for specific SMI values can be found in **Table 2**.

A significant linear association between SMR and fatigue was observed at 6 months post-diagnosis (test for overall association p=0.02, test for non-linearity p=0.14), where lower levels of SMR were associated with higher prevalence of fatigue, see **Figure 2b**. **Table 2** shows that relative to the median, SMR levels below the median were significantly associated with higher fatigue prevalence. No significant associations were observed between SMR and fatigue at diagnosis (test for overall association p=0.74), and at 24 months post-diagnosis (test for overall association at diagnosis p=0.17).

For VAT, no significant association with fatigue was observed at any of the time-points (test for overall association at diagnosis and at 6 and 24 months post-diagnosis: p=0.63, p=0.56, and p=0.22, respectively), see **Figure 3a**.

Levels of SAT were non-linearly associated with fatigue at diagnosis, where higher levels of SAT were significantly associated with higher prevalence of fatigue (test for overall association p<0.01, test for non-linearity p=0.01), see **Figure 3b**. **Table 2** also illustrates that higher SAT levels relative to the median were associated with higher fatigue prevalence at baseline. At both 6 and 24 months, SAT was not associated with fatigue (test for overall association at 6 and 24 months post-diagnosis: p=0.63 and p=0.22, respectively).

Table 1: Characteristics of stage I-III colorectal cancer patients with or without fatigue at diagnosis, 6 months post-diagnosis and 24 months post-diagnosis

|	Total population at diagnosis	Fatigue					
		Diagnosis		6 months post-diagnosis		24 months post-diagnosis	
	n=646	Yes n=114 (18%)	No n=532 (82%)	Yes n=148 (25%)	No n=433 (75%)	Yes n=61 (12%)	No n=435 (88%)
Age at diagnosis [yrs, mean (SD)]	66.1 (8.8)	66.3 (10.1)	66.0 (8.6)	63.4 (10.3)	66.6 (8.3)	66.0 (11.6)	65.5 (8.4)
Men [n (%)]	406 (63)	61 (54)	345 (65)	86 (58)	283 (65)	42 (69)	270 (62)
Cancer stage [n (%)]							
I	174 (27)	26 (23)	148 (28)	17 (12)	144 (33)	15 (25)	133 (31)
II	178 (28)	40 (35)	138 (26)	22 (15)	139 (32)	13 (21)	124 (29)
III	294 (46)	48 (42)	246 (46)	109 (74)	150 (35)	33 (54)	178 (41)
Tumor location [n (%)]							
Colon	426 (66)	94 (83)	332 (62)	92 (62)	288 (67)	37 (61)	282 (65)
Rectal	220 (34)	20 (18)	200 (38)	56 (38)	145 (34)	24 (39)	153 (35)
Number of comorbidities at diagnosis [n (%)]							
0	230 (36)	34 (30)	196 (37)	50 (34)	163 (38)	17 (28)	168 (39)
1	167 (26)	26 (23)	141 (27)	30 (20)	116 (27)	9 (15)	113 (26)
≥ 2	246 (38)	53 (47)	193 (36)	67 (45)	153 (35)	34 (56)	153 (35)
Radiotherapy [n (%)][a]							
Yes				50 (34)	104 (24)	21 (35)	114 (26)
No				97 (66)	325 (75)	39 (65)	319 (74)
Chemotherapy [n (%)][b]							
Yes				74 (50)	87 (20)	18 (30)	113 (26)
No				73 (50)	344 (79)	43 (70)	320 (74)
Physical activity [moderate-vigorous min/wk, median (IQR)][c]							
Diagnosis	600 (818)	505 (754)	630 (840)				
6 months				245 (450)	510 (630)		
24 months						420 (698)	570 (690)
Skeletal muscle index [cm²/m², mean (SD)]	47.7 (8.7)	46.4 (7.8)	48.1 (8.8)	47.4 (8.9)	48.0 (8.6)	48.2 (7.6)	47.9 (8.7)
Skeletal muscle radiodensity [HU, mean (SD)]	37.0 (8.2)	36.2 (8.4)	37.3 (8.2)	35.8 (9.3)	37.7 (7.8)	35.5 (8.2)	37.8 (7.9)
Visceral adipose tissue [cm², median (IQR)]	142 (144)	131 (124)	146 (144)	140 (143)	145 (139)	173 (140)	135 (143)
Subcutaneous adipose tissue [cm², median (IQR)]	163 (97)	162 (135)	163 (94)	183 (108)	157 (88)	175 (104)	163 (92)

a = at diagnosis data of 5 patients missing; b = at diagnosis data of 4 patients missing; c = at diagnosis data of 7 patients missing

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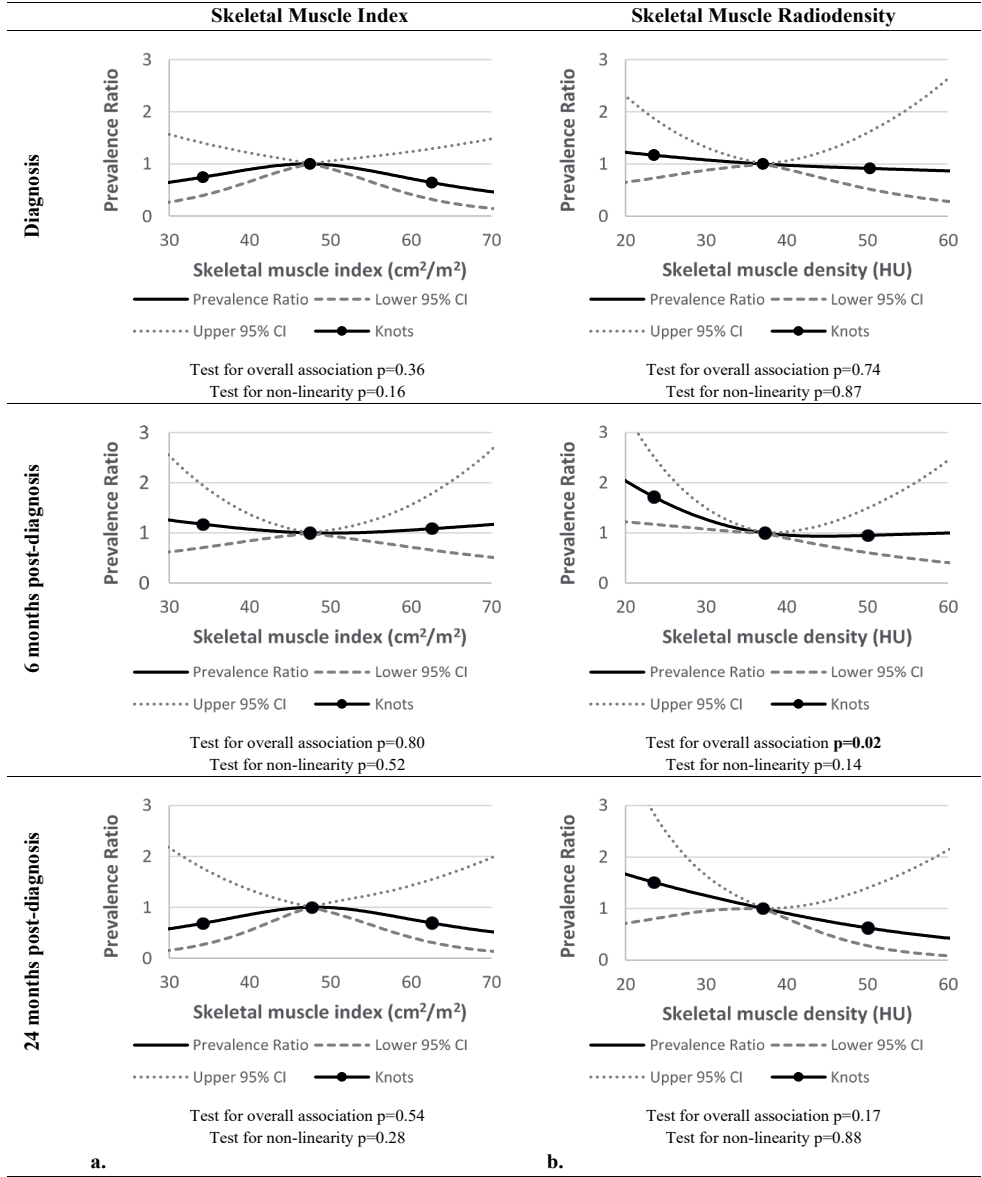
Figure 2 The association between skeletal muscle index (SMI)(a), skeletal muscle radiodensity (SMR)(b) and fatigue at diagnosis, at 6 months and 24 months post-diagnosis, adjusted for age, gender and stage of disease (SMI at all time-points; SMR at diagnosis) and age, gender, stage of disease and physical activity (minutes per week of moderate-to-vigorous physical activity, for SMR at 6 and 24 months post-diagnosis) with three knots located at the 5th, 50th (reference), and 95th percentiles of the distribution of SMI or SMR.
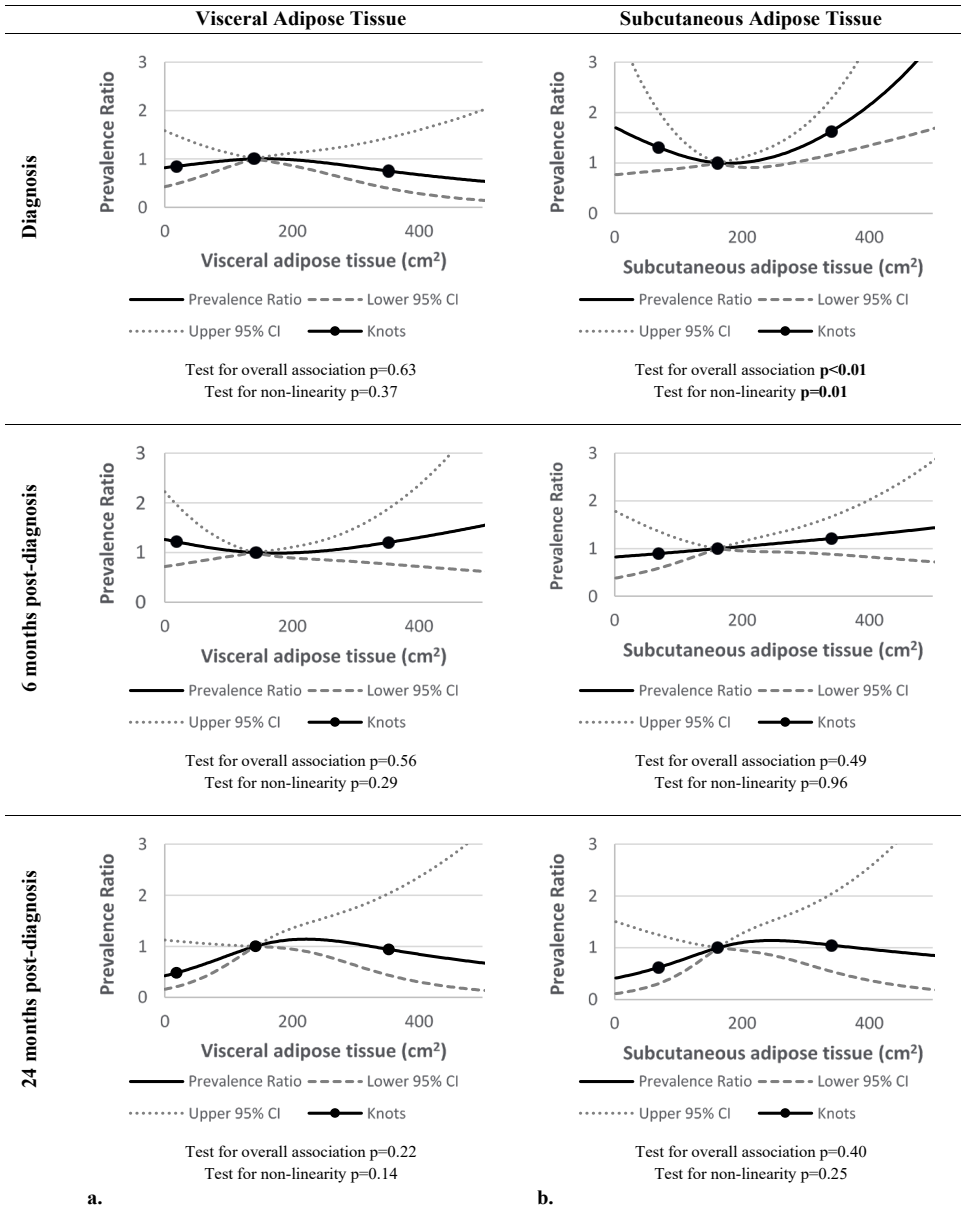
Figure 3: The association between visceral adipose tissue (VAT)(a), subcutaneous adipose tissue (SAT)(b) and fatigue at diagnosis, at 6 months and 24 months post-diagnosis, adjusted for age, gender and stage of disease and skeletal muscle radiodensity at all time-points, with three knots located at the 5th, 50th (reference), and 95th percentiles of the distribution of VAT or SAT.

Table 2: Estimated prevalence ratios for specific skeletal muscle index, skeletal muscle radiodensity, visceral adipose tissue and subcutaneous adipose tissue values and fatigue, with median values as the reference category (i.e. for skeletal muscle index: 47.5 cm^2/m^2; for skeletal muscle radiodensity: 37.2 HU; for visceral adipose tissue: 142.6 cm^2; for subcutaneous adipose tissue: 162.9 cm^2).

	Diagnosis		6 months		24 months	
	PR	**95% CI**	**PR**	**95% CI**	**PR**	**95% CI**
SMI (cm^2/m^2)*						
30	0.65	0.27-1.56	1.25	0.62-2.54	0.58	0.15-2.17
40	0.90	0.66-1.21	1.07	0.84-1.37	0.86	0.54-1.34
50	0.98	0.91-1.06	1.00	0.94-1.06	0.99	0.90-1.09
60	0.72	0.42-1.24	1.06	0.72-1.56	0.76	0.41-1.42
SMR (HU) **						
20	1.22	0.65-2.30	**2.04**	**1.22-3.40**	1.67	0.71-3.90
30	1.08	0.88-1.32	**1.27**	**1.08-1.49**	1.25	0.96-1.64
40	0.98	0.90-1.06	0.96	0.89-1.02	0.91	0.81-1.02
50	0.92	0.53-1.60	0.95	0.61-1.49	0.63	0.28-1.40
VAT (cm^2) ***						
50	0.90	0.61-1.32	1.14	0.82-1.60	0.60	0.34-1.07
150	1.00	0.98-1.02	1.00	0.98-1.01	1.03	1.00-1.06
250	0.92	0.71-1.19	1.04	0.86-1.25	1.13	0.82-1.55
350	0.75	0.40-1.43	1.20	0.77-187	0.95	0.44-2.02
SAT (cm^2) ***						
50	1.41	0.82-2.41	0.87	0.52-1.47	0.55	0.23-1.31
150	1.02	0.97-1.06	0.99	0.94-1.03	0.95	0.89-1.02
250	1.12	0.94-1.34	1.10	0.93-1.31	1.14	0.85-1.53
350	**1.70**	**1.20-2.42**	1.22	0.87-1.72	1.04	0.51-2.11

*= adjusted for age, gender and stage of disease at all timepoints. **= at diagnosis adjusted for age, gender and stage of disease; at 6 and 24 months additionally adjusted for physical activity (minutes per week of moderate-to-vigorous physical activity). ***= adjusted for age, gender and stage of disease and skeletal muscle radiodensity at all time-points. PR, prevalence ratio; 95% CI, 95% confidence interval; SMI, skeletal muscle index; SMR, skeletal muscle radiodensity; VAT, visceral adipose tissue SAT, subcutaneous adipose tissue.

Discussion

The present study is the first study to investigate associations of body composition at time of diagnosis with fatigue at diagnosis, and 6 and 24 months post-diagnosis in stage I-III CRC patients. Having more SAT at diagnosis was associated with higher prevalence of fatigue at diagnosis, while lower SMR levels at diagnosis were associated with fatigue at 6 months post-diagnosis.

We did not observe an association between low SMI and fatigue at diagnosis, while such an association of low SMI and more fatigue at cancer diagnosis was observed among men in two earlier studies among different cancer populations (9, 10). Those two studies included mainly late-stage cancer patients, while the present study included stage I-III CRC patients. In late-stage cancer patients, low SMI is often the result of tumour-induced muscle degradation, while low SMI among stage I-III is most likely age and lifestyle related since cachexia is not common within patient group among these earlier stages. Since fatigue is more prevalent among late-stage cancer patients (6), the association between low SMI and fatigue among late-stage patients might be driven by the progressive tumor instead of low SMI itself. This might explain why no association was observed at diagnosis in the present study. In the present study, we also did not observe an association between SMI at diagnosis and fatigue two years after diagnosis. This is similar to what was observed in a study of Van Roekel *et al* (11). In that study among stage I-III CRC patients, SMI was not associated with fatigue 2-10 years post-diagnosis.

In the present study, we observed that lower SMR levels were associated with higher prevalence of fatigue 6 months post-diagnosis, but not at diagnosis or 24 months post-diagnosis. Nevertheless, at 24 months, the shape of the RCS appeared similar to the shape of the association at 6 months, but did not reach statistical significance at 24 months. This may be the result of lower statistical power at the 24 month timepoint: at 24 months, the total number of participants in the dataset was lower than at the earlier timepoints, while the prevalence of fatigue at this timepoint was also lower than at the earlier time-points.

Low SMR is associated with an increased pro-inflammatory cytokine production (22) and this may potentially explain the association of low SMR with fatigue (5). Another explanation might be that lower levels of SMR are usually seen in patients with an inferior overall condition (i.e. multiple comorbidities, higher ASA score)(23, 24). Because of this, patients with low SMR may experience more (long-term) side-effects from treatment. Further studies are needed to understand the mechanisms behind the association of low SMR with fatigue.

To the best of our knowledge, the present study was the first study to investigate the association of SAT with fatigue among cancer patients. The results from our study suggest that at time of diagnosis high levels of SAT are associated with higher prevalence of fatigue. Interestingly, the association was found only for SAT and not for VAT. This was somewhat unexpected, since VAT is known to be more metabolically active than SAT (25)

and a higher inflammatory response seen with higher levels of VAT could have explained a potential association with fatigue. Several authors have questioned the use of a single slice CT image to assess VAT because the natural movement of abdominal soft tissue might influence the amount of VAT shown at the level of L3 (26, 27). This might have influenced our findings for VAT.

A limitation of the study was that body composition data were only available at diagnosis, as in the Netherlands CT images are only standard of care for diagnosis/staging, but not in the period of follow-up (28). Therefore, we could not assess how SMI, SMR, VAT and/or SAT change post-diagnosis and whether any changes impact the association with fatigue. A second limitation was that statistical power was lower at 24 months post-diagnosis than at the earlier time-points. Strengths of this study were the use of CT images to assess body composition and the availability of fatigue data at multiple time-points.

In conclusion, having more SAT was associated with more fatigue at diagnosis, while low levels of SMR were associated with more fatigue at 6 months post-diagnosis. Assessing SMR at diagnosis might help identify patients with higher risk of fatigue at a later stage. However, more knowledge is needed to understand the mechanisms behind the association of SMR and fatigue.

References

1. Hickok JT, Roscoe JA, Morrow GR, Mustian K, Okunieff P, Bole CW. Frequency, severity, clinical course, and correlates of fatigue in 372 patients during 5 weeks of radiotherapy for cancer. Cancer. 2005;104(8):1772-8.
2. Bower JE. Cancer-related fatigue—mechanisms, risk factors, and treatments. Nature reviews Clinical oncology. 2014;11(10):597.
3. Hofman M, Ryan JL, Figueroa-Moseley CD, Jean-Pierre P, Morrow GR. Cancer-related fatigue: the scale of the problem. The oncologist. 2007;12(Supplement 1):4-10.
4. Thong MS, Mols F, Wang XS, Lemmens VE, Smilde TJ, van de Poll-Franse LV. Quantifying fatigue in (long-term) colorectal cancer survivors: a study from the population-based patient reported outcomes following initial treatment and long term evaluation of survivorship registry. European journal of cancer. 2013;49(8):1957-66.
5. Bower JE. Cancer-related fatigue—mechanisms, risk factors, and treatments. Nature Reviews Clinical Oncology. 2014;11:597.
6. Mitchell SA. Cancer-related fatigue: state of the science. PM&R. 2010;2(5):364-83.
7. Aapro M, Scotte F, Bouillet T, Currow D, Vigano A. A Practical Approach to Fatigue Management in Colorectal Cancer. Clinical colorectal cancer. 2017;16(4):275-85.
8. Vissers PAJ, Thong MSY, Pouwer F, Zanders MMJ, Coebergh JWW, van de Poll-Franse LV. The impact of comorbidity on Health-Related Quality of Life among cancer survivors: analyses of data from the PROFILES registry. Journal of Cancer Survivorship. 2013;7(4):602-13.
9. Bye A, Sjøblom B, Wentzel-Larsen T, Grønberg BH, Baracos VE, Hjermstad MJ, et al. Muscle mass and association to quality of life in non-small cell lung cancer patients. Journal of Cachexia, Sarcopenia and Muscle. 2017.
10. Neefjes ECW, Hurk RM, Blauwhoff-Buskermolen S, Vorst MJDL, Becker-Commissaris A, Schueren MAE, et al. Muscle mass as a target to reduce fatigue in patients with advanced cancer. Journal of Cachexia, Sarcopenia and Muscle. 2017;8(4):623-9.
11. van Roekel EH, Bours MJL, te Molder MEM, Breedveld-Peters JJL, Olde Damink SWM, Schouten LJ, et al. Associations of adipose and muscle tissue parameters at colorectal cancer diagnosis with long-term health-related quality of life. Quality of Life Research. 2017;26(7):1745-59.
12. Winkels R, Heine-Broring R, Van Zutphen M, van Harten-Gerritsen S, Kok D, Van Duijnhoven F, et al. The COLON study: Colorectal cancer: Longitudinal, Observational study on Nutritional and lifestyle factors that may influence colorectal tumour recurrence, survival and quality of life. BMC Cancer. 2014;14(1):374.
13. Aaronson NK, Ahmedzai S, Bergman B, Bullinger M, Cull A, Duez NJ, et al. The European Organization for Research and Treatment of Cancer QLQ-C30: a quality-of-life instrument for use in international clinical trials in oncology. JNCI: Journal of the National Cancer Institute. 1993;85(5):365-76.

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14. Giesinger JM, Kuijpers W, Young T, Tomaszewski KA, Friend E, Zabernigg A, et al. Thresholds for clinical importance for four key domains of the EORTC QLQ-C30: physical functioning, emotional functioning, fatigue and pain. Health and Quality of Life Outcomes. 2016;14:87.

15. Giesinger JM, Loth FL, Aaronson NK, Arraras JI, Caocci G, Efficace F, et al. Thresholds for clinical importance were established to improve interpretation of the EORTC QLQ-C30 in clinical practice and research. Journal of clinical epidemiology. 2019.

16. Prado CMM, Birdsell LA, Baracos VE. The emerging role of computerized tomography in assessing cancer cachexia. Current opinion in supportive and palliative care. 2009;3(4):269-75.

17. Mitsiopoulos N, Baumgartner R, Heymsfield S, Lyons W, Gallagher D, Ross R. Cadaver validation of skeletal muscle measurement by magnetic resonance imaging and computerized tomography. Journal of applied physiology. 1998;85(1):115-22.

18. Wendel-Vos GW, Schuit AJ, Saris WH, Kromhout D. Reproducibility and relative validity of the short questionnaire to assess health-enhancing physical activity. Journal of clinical epidemiology. 2003;56(12):1163-9.

19. Van Leersum N, Snijders H, Henneman D, Kolfschoten N, Gooiker G, Ten Berge M, et al. The Dutch surgical colorectal audit. European Journal of Surgical Oncology (EJSO). 2013;39(10):1063-70.

20. Desquilbet L, Mariotti F. Dose-response analyses using restricted cubic spline functions in public health research. Statistics in medicine. 2010;29(9):1037-57.

21. Tamhane AR, Westfall AO, Burkholder GA, Cutter GR. Prevalence odds ratio versus prevalence ratio: choice comes with consequences. Statistics in medicine. 2016;35(30):5730-5.

22. Van Hollebeke RB, Cushman M, Schlueter EF, Allison MA. Abdominal Muscle Density is Inversely Related to Adiposity Inflammatory Mediators. Medicine & Science in Sports & Exercise. 2018;50(7):1495-501.

23. Margadant C, Bruns E, Sloothaak D, van Duijvendijk P, van Raamt A, van der Zaag H, et al. Lower muscle density is associated with major postoperative complications in older patients after surgery for colorectal cancer. European Journal of Surgical Oncology (EJSO). 2016;42(11):1654-9.

24. Xiao J, Caan BJ, Weltzien E, Cespedes Feliciano EM, Kroenke CH, Meyerhardt JA, et al. Associations of pre-existing co-morbidities with skeletal muscle mass and radiodensity in patients with non-metastatic colorectal cancer. Journal of cachexia, sarcopenia and muscle. 2018.

25. Ibrahim MM. Subcutaneous and visceral adipose tissue: structural and functional differences. Obesity reviews. 2010;11(1):11-8.

26. Thomas EL, Bell JD. Influence of undersampling on magnetic resonance imaging measurements of intra-abdominal adipose tissue. International journal of obesity. 2003;27(2):211-8.

27. Shuster A, Patlas M, Pinthus JH, Mourtzakis M. The clinical importance of visceral adiposity: a critical review of methods for visceral adipose tissue analysis. The British journal of radiology. 2012;85(1009):1-10.

28. Netherlands CCCt. Oncoline, Cancer Clinical Practice Guidelines. 2017.

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Chapter 5

Inflammation is a mediating factor in the association between lifestyle and fatigue in colorectal cancer patients

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Fatigue is very common among colorectal cancer (CRC) patients. We examined the association between adherence to the WCRF/AICR lifestyle recommendations and fatigue among stage I-III CRC patients, and whether inflammation mediated this association. Data of two prospective cohort studies were used. Adherence to the WCRF/AICR recommendations was expressed as a score ranging from 0-7 and assessed shortly after diagnosis. Six months post-diagnosis, fatigue was assessed with the EORTC QLQ-C30 questionnaire and in a subpopulation plasma levels of inflammation markers (IL6, IL8, TNFα and hsCRP), were assessed. Multiple linear regression analyses were performed to investigate the association between adherence to the WCRF/AICR recommendations and fatigue. To test mediation by inflammation, the PROCESS analytic tool developed by Hayes was used. A higher WCRF/AICR adherence score was associated with less fatigue six months after diagnosis (n=1417, β -2.22, 95%CI -3.65; -0.78). In the population of analysis for the mediation analyses (n=551), the total association between lifestyle and fatigue was (β -2.17, 95% CI -4.60; 0.25). A statistically significant indirect association via inflammation was observed (β -0.97, 95% CI -1.92; -0.21), explaining 45% of the total association between lifestyle and fatigue (-0.97/-2.17*100). Thus, inflammation is probably one of the underlying mechanisms linking lifestyle to fatigue.

ABSTRACT

Introduction

Fatigue is one of the most common adverse effects colorectal cancer (CRC) survivors experience and can be present for years after treatment (1). During and shortly after treatment, prevalence of fatigue is reported to range from 25% to 99%, depending on cancer stage and/or type of treatment, patient population and method of assessment (1, 2).

Several factors have been identified which may contribute to fatigue among cancer patients, including a more advanced stage of disease, type of treatment (radio- and chemotherapy compared to only surgery), recurrences, more comorbidities and decreased physical activity (1, 3-6). A healthier lifestyle has been associated with less fatigue among breast and colorectal cancer survivors (7-9), where better lifestyle was operationalized as better adherence to the World Cancer Research Fund/American Institute for Cancer Research (WCRF/AICR) recommendations for cancer prevention, which include guidelines on body weight, physical activity and a healthy diet (10).

Better adherence to the WCRF/AICR lifestyle recommendations for cancer prevention on physical activity, body weight and healthy diet may potentially impact fatigue by attenuating systemic inflammatory processes (11-13). Firstly, physical activity, obesity and diet have been associated with fatigue. Being more physically active, is known to lower fatigue in cancer patients (14). Underweight as well as excessive body weight might be related to more fatigue (15-17) although this was not observed in all studies (9, 18, 19). Limited data support a potential role for nutrition in managing fatigue after cancer diagnosis (20). Secondly, physical activity, obesity and diet have been associated with inflammatory markers such as interleukins (IL). A meta-analysis on physical activity and fatigue among breast cancer survivors (12) showed that exercise decreased levels of IL6, IL8 and tumour necrosis factor alfa (TNFα). Furthermore, exercise might lead to a decrease in C-Reactive Protein (CRP) levels (21). A higher BMI has been associated with higher circulating levels of IL6, IL8, TNF-α and CRP (22, 23). Furthermore, several studies suggest that dietary intake is associated with inflammation (24, 25). Diets high in red and processed meat and fast foods are associated with higher levels of systemic inflammation, expressed by amongst others IL6, TNFα and CRP, while diets high in fruit, vegetables and unsaturated fatty acids were associated with lower levels of inflammation (24, 25). Thirdly, inflammatory markers such as IL6, IL8, CRP and TNFα are associated with fatigue in cancer survivors (1, 21). Finally, an anti-inflammatory diet characterized by a high intake of fruit, vegetables, whole grains, and omega-3 fatty acid-rich foods compared to non-dietary related advice sessions reduced fatigue in an experimental study among 30 breast cancer patients (26). Taken together, this could imply a possible role for inflammation as a

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mediating mechanism in the association between lifestyle and fatigue, although this has, to the best of our knowledge, not been investigated to date.

The aim of the current study was, to examine whether adherence to the WCRF/AICR recommendations for cancer prevention at diagnosis was associated with fatigue experienced six months post-diagnosis among patients with stage I-III CRC. Furthermore, the study aimed to determine whether inflammation (plasma levels of IL6, IL8, TNFα and hsCRP) at six months post-diagnosis (partly) mediated this association.

Materials and Methods

Study population
For this study, we used data of two prospective cohort studies: the COLON study (27) and the EnCoRe study (28). For the COLON study, hospital staff of eleven participating hospitals invited eligible patients with stage I-IV of disease shortly after diagnosis and before scheduled surgery to participate in the study. Patients were not eligible if they had a history of CRC, a previous (partial) bowel resection, known hereditary CRC, inflammatory bowel disease, dementia or another mental condition limiting their ability to fill out surveys, or were non-Dutch speaking. For the EnCoRe study, patients with stage I to III CRC from three hospitals were enrolled at diagnosis. Exclusion criteria for the EnCoRe study were diagnosis of stage IV disease; current home address not in the Netherlands; inability to understand the Dutch language in speech as well as in writing; and presence of comorbidities that may obstruct successful participation, including cognitive disorders such as Alzheimer disease, and severe visibility or hearing disorders such as complete blindness and/or deafness (28). In total 1417 CRC patients were included, 1156 from the COLON study and 261 from the EnCoRe study, see **Figure 1**. Additional exclusion criteria for the present analyses were: missing data on lifestyle (to build the WCRF/AICR score) (n=272), fatigue six months after diagnosis (n=162), stage of disease (n=138), stage IV CRC (n=107).

The study was performed in accordance with the Declaration of Helsinki. The COLON study (ClinicalTrials.gov Identifier: NCT03191110) was approved by the Committee on Research involving Human Subjects, region Arnhem-Nijmegen, the Netherlands. The EnCoRe study (Netherlands Trialregister number NL6904) was approved by the Medical Ethics Committee of the University Hospital Maastricht and Maastricht University, the Netherlands. All participants of the COLON and EnCoRe study provided written informed consent.

WCRF/AICR score

We followed the approach as published previously (10) to score adherence to the 2018 WCRF/AICR lifestyle recommendations for cancer prevention (29). The score includes seven lifestyle recommendations: 'maintain a healthy weight'; 'be physically active'; 'eat a diet rich in wholegrains, vegetables, fruits and beans'; 'limit consumption of "fast foods" and other processed foods high in fat, starches or sugars'; 'limit consumption of red and processed meat'; 'limit consumption of sugar-sweetened drinks'; and 'limit alcohol consumption'. Two recommendations were excluded: the recommendation 'for mothers: to breastfeed their baby, if they can' was excluded as it is only specific to mothers who are able to breastfeed; the recommendation about dietary supplement use was not included, since it was not operationalized in the standard WCRF/AICR score (10). For complete adherence to a recommendation 1 point was assigned, for moderate adherence 0.5 point and 0 points otherwise. Total scores could range from 0 to 7. In supplementary Table S1 the exact breakdown of the scoring system is depicted.

Data on body weight, height, and waist circumference were self-reported in the COLON study. In the EnCoRe study, body weight, height and waist circumference were measured by a research dietician during a home visit. An extended validated semi-quantitative food frequency questionnaire (FFQ) of 204 items for the COLON study (30, 31) and 253 items for the EnCoRe study (32) was used to assess habitual dietary intake and consumption of alcoholic drinks in the preceding month (COLON) or year (EnCoRe). Intake of foods and beverages in grams per day was calculated for all food groups (fruit and vegetables, red and processed meat, fast food, alcohol, sugar-sweetened beverages). Energy and nutrient intakes were calculated using the Dutch Food Composition Table 2011 (NEVO table) (33). Physical activity was assessed using the self-reported Short QUestionnaire to ASsess Health-enhancing physical activity (SQUASH) in both cohorts (34). Moderate-to-vigorous physical activity in minutes per week was assessed from the data of that questionnaire in order to score adherence to the physical activity guideline (see table 1).

Fatigue

Fatigue was assessed using the fatigue domain of the validated European Organization for Research and Treatment of Cancer Quality of Life Questionnaire C30 (EORTC QLQ-C30 version 3.0). Fatigue scores were based on three items in this questionnaire, namely: 'Did you need to rest?'; 'Have you felt weak?'; and 'Were you tired?'. Participants could answer on a 4-point Likert scale ranging from 'not at all' to 'very much'; 1 to 4 points were assigned accordingly. First, a 'RawScore' was calculated as the average of the three items. Next, this score was linearly converted to the total fatigue score with a 0-100 scale, as recommended for this questionnaire, where a higher score indicates more fatigue (35). Within the COLON study fatigue was assessed six months after diagnosis, within the EnCoRe study fatigue was assessed six weeks and six months after finalizing treatment. For EnCoRe, therefore, fatigue data closest to six months after diagnosis was selected for the analyses (median

6.8 months, IQR 6.4-8.3). To identify the number of patients with fatigue six months after diagnosis, a fatigue score of >39 was defined as having fatigue (36).

Blood collection and Inflammatory markers

Non-fasting blood samples were drawn into EDTA tubes during a regular clinical visit in the hospital (COLON study) or during a home visit (EnCoRe study) around six months after diagnosis. All blood samples were centrifuged and plasma was aliquoted and stored in a freezer at -80°C until further analysis.

Levels of IL6, IL8 and TNFα were quantified in plasma using a custom-made multiplex assay using electrochemiluminescence detection (Meso Scale Diagnostics, Rockville, Maryland, USA) in our lab at Wageningen University & Research as described previously (37). Samples were analysed in duplicate according to instructions by the manufacturer on a QuickPlex SQ 120 plate reader (Meso Scale Diagnostics). Each sample plate contained a calibration curve and quality control samples provided by the manufacturer with different levels of each inflammation marker. Target values for the quality control samples were provided. Inter batch and intra-batch coefficients of variation were <8% and observed values deviated less than 15% from target values. Analyses of inflammation markers were done in a sub-population (**Figure 1**) as cytokines were previously shown to remain stable in plasma for a period up to 2 years of storage at -80 °C (38). Therefore, samples stored > 2 years were not analysed within the COLON study.

High sensitive C-Reactive Protein (hsCRP) was measured using an immuno-MALDI mass spectrometry method (39) (BEVITAL, Bergen, Norway). The inter-assay coefficient of variation ranged between 3-6%.

Covariates

Demographic information (including age and sex) and smoking history were collected with self-reported questionnaires. Clinical data, such as stage of disease, tumour location (colon/rectum), type of treatment (surgery, neo-adjuvant/adjuvant chemotherapy or radiotherapy) and presence of comorbidities (diabetes, endocrine disorders, cardiovascular, infectious, gastro-intestinal, muscular and joint, neurologic, pulmonary and urogenital diseases) were derived from the Dutch ColoRectal Audit (DCRA) (40).

Data analysis

Population characteristics are described as numbers with percentages and medians with interquartile range (IQR) for the total population and stratified by tertiles of adherence to the WCRF/AICR recommendations. Levels of inflammatory markers were log-transformed to obtain normally distributed data.

Descriptive statistics were used to describe demographic, lifestyle and clinical characteristics of the total population and patients of whom data about inflammation levels were available and those of whom not.

Multiple linear regression analyses were used to assess the association of adherence to the WCRF/AICR recommendations at diagnosis (continuous, 1 point increment) and fatigue (continuous, 1 point increment) six months after diagnosis. Betas (β) and 95% confidence intervals (CIs) were reported to describe the associations. All analyses were tested for effect modification by sex, stage of disease and cohort by calculating the p value for interaction. None of these variables were identified as effect modifiers. Potential confounders were: age (years), stage of disease (stage I/II/III), sex (male/female), comorbidities (yes/no), smoking (current/former/never), stoma at time of fatigue assessment (yes/no), chemotherapy (yes/no), radiotherapy (yes/no), recurrence within one year after diagnosis and cohort (COLON/EnCoRe). Variables were included in the final model if they changed the β for fatigue or inflammation with 10% or more when the variable was individually added to a crude model. Age, stage of disease, sex, chemotherapy (yes/no) and cohort (COLON/EnCoRe) were identified as confounders and included in all models.

Mediation analyses
Mediation analyses were conducted to analyse whether inflammation six months after diagnosis is a mediator in the association between adherence to the WCRF/AICR recommendations at diagnosis and fatigue six months after diagnosis.

Before performing the mediation analyses, the associations between adherence to the WCRF/AICR recommendations and inflammation markers (exposure-mediator) and the association between inflammation markers and fatigue (mediator-outcome) were assessed using multiple linear regression analyses. The same confounders as identified above for the total associations were included in all models created for the mediation analyses.

Two paths are important in mediation analyses. First, the path from lifestyle to fatigue without passing through inflammation, which is referred to as the direct association between lifestyle and fatigue. Second, the path between lifestyle and fatigue passing through inflammation, which is referred to as the indirect association. The summed effect of the direct and indirect paths is referred to as the total path. We used the PROCESS analytic tool developed by Hayes to assess whether inflammation is a mediator in the association between lifestyle and fatigue. This analyses is based on multiple linear regression path analysis (41). Model 4 of the PROCESS macro version 3.5 for SAS was used to assess indirect, direct and total effects of the association between lifestyle and

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fatigue. A 95% percentile bootstrap confidence interval for the indirect effect using 10.000 bootstrap samples was generated.

For the mediation analyses the following sensitivity analyses were done, excluding data of plasma samples that had been stored in the -80 freezer for more than 2 years before analysis and excluding extreme values (> 3SD above the mean). Cytokines were previously shown to remain stable in plasma for a period up to 2 years of storage at -80 °C (38). Therefore, samples stored > 2 years were excluded in a sensitivity analyses.

All data analyses were performed using the statistical software program SAS (version 9.4: SAS Institute Inc.).

Results

For the analyses regarding the association between lifestyle and fatigue in total, 1417 CRC patients were included, 1156 from the COLON study and 261 from the EnCoRe study, (Figure 1). Of these patients 514 (36%) were female (Table 1) and median age was 66.1 (IQR 61.2-71.5) years. Two thirds of all patients had colon cancer, 44% were diagnosed with stage III disease, and 30% received chemotherapy of which 75% received adjuvant chemotherapy. The median WCRF/AICR score at diagnosis was 3.5 (IQR 2.8-4.0) and 26% experienced fatigue at six months post-diagnosis. Patients with the highest adherence to the WCRF/AIRC recommendations compared to the lowest adherence were more often female, were more often highly educated, experienced less often fatigue and had slightly lower levels of inflammation markers. Baseline characteristics of patients with inflammatory data available (n=607 for cytokines and n=1116 for hsCRP) were similar as characteristics of patients with no inflammatory data available (Table S2).

In the total population (n=1417), higher adherence to the WCRF/AICR recommendations was statistically significantly associated with less fatigue (β=-2.22, 95% CI: -3.65; -0.78).

For the mediation analyses, we first investigated if a potential mediator was associated with both WCRF/AICR score and fatigue. A healthier lifestyle (higher adherence to the WCRF/AICR recommendations) at diagnosis was associated with lower levels of IL6, TNFα and hsCRP six months post-diagnosis (β -0.10, 95%CI -0.17; -0.03, β -0.07, 95%CI -0.10; -0.04, β -0.20, 95%CI -0.28; -0.12, respectively). For IL8 the association was not statistically significant (Table 2). IL6 and hsCRP at six months post-diagnosis were statistically significantly associated with higher fatigue levels six months after diagnosis (β 5.05, 95% CI 2.54; 7.57 and β 3.57, 95% CI 2.37; 4.78, respectively), while IL8 and TNFα were not (Table 3).

In the population of analysis for the mediation analyses (n=551), the total association between lifestyle and fatigue was β -2.17, 95% CI -4.60; 0.25. A statistically significant indirect association (via inflammation) was observed (β -0.97, 95% CI -1.92; -0.21), indicating that a statistically significant part of the association between lifestyle and fatigue goes through inflammation. Forty-five percent of the total association between lifestyle and fatigue was mediated by inflammation (-0.97/-2.17*100). This mediation was mainly driven by IL6 and hsCRP. The indirect association via IL6 was observed to be β -0.48, 95% CI -1.05; -0.18, indicating a contribution of 22% of IL6 to the total association. The indirect association via hsCRP was observed to be β -0.68 95% CI -1.10; -0.34, 32% of the total association was mediated by hsCRP (Table 4).

Sensitivity analyses excluding old samples (> 2 years stored before analyses) n=47 and outliers (>3SD above the mean) n=10 showed similar results compared to the total population for analyses (data not shown).

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Table 1: Baseline characteristics of colorectal cancer patients stratified for a low, medium and high WCRF/AICR-score

	Total population (n=1417)	Low WCRF/AICR-score ≤3 (tertile 1) (n=548)	Medium WCRF/AICR- score >3 – 3.75 (tertile 2) (n=485)	High WCRF/AICR-score ≥ 3.75 (tertile 3) (n=384)
Age (years)	66.1 (61.2-71.5)	65.8 (60.4-71.2)	66.5 (61.8-71.5)	66.8 (61.4-72.0)
Sex (female)	514 (36)	175 (32)	170 (35)	169 (44)
Education level[*]				
low	536 (38)	215 (39)	180 (37)	141 (37)
medium	412 (29)	168 (31)	147 (30)	97 (25)
high	467 (33)	164 (30)	157 (32)	146 (38)
Smoking[*]				
current	155 (11)	60 (11)	58 (12)	37 (10)
former	811 (58)	322 (60)	280 (58)	209 (55)
never	436 (31)	155 (29)	144 (30)	137 (36)
BMI (kg/m^2)[*]	26.4 (24.2-29.4)	28.4 (26.2-31.5)	26.0 (24.1-28.4)	24.3 (22.5-26.4)
Waist circumference (cm)	97 (90-105)	103 (96-110)	95 (89-103)	91 (83-97)
Total moderate-vigorous physical activity (hours/week)	11.0 (5.5-19.0)	8.5 (3.5-17.5)	12 (7.0-20.3)	13 (6.5-21.0)
Dietary intake				
Fruit and vegetable (g/day)	260 (160-366)	187 (121-298)	260 (179-348)	352 (244-447)
Total fibre (g/day)	21 (17-26)	19 (16-24)	21 (17-25)	23 (18-28)
Percentage of total kcal from ultra-processed foods	28 (21-35)	33 (26-39)	27 (21-33)	22 (18-28)
Processed meat (g/day)	29 (13-46)	37 (22-52)	28 (15-46)	14 (4-34)
Red meat (g/day)	38 (23-56)	44 (29-61)	39 (23-56)	31 (17-49)
Sugary drinks (g/day)	72 (14-167)	108 (32-245)	67 (13-152)	42 (0-131)
Alcohol (g/day)	8 (1-21)	12 (2-24)	8 (1-19)	5 (0-17)
Inflammation markers				
IL6 (pg/ml)	1.0 (0.7-1.6)	1.1 (0.7-1.7)	0.9 (0.7-1.6)	0.9 (0.6-1.4)
IL8 (pg/ml)	5.6 (4.2-8.0)	5.6 (4.1-7.9)	5.8 (4.3-8.1)	5.6 (4.3-8.0)
TNFα (pg/ml)	2.0 (1.6-2.6)	2.1 (1.7-2.6)	2.0 (1.5-2.6)	1.9 (1.5-2.4)
hsCRP (µg/ml)	2.7 (1.2-6.5)	3.1 (1.5-7.4)	2.6 (1.2-5.9)	2.2 (1.0-6.3)
Type of Cancer				
colon	929 (66)	357 (65)	322 (67)	250 (65)
rectal	488 (34)	191 (35)	163 (33)	134 (35)
Type of treatment[*]				
Surgery only	756 (54)	292 (55)	248 (52)	216 (57)
chemotherapy	301 (22)	117 (22)	109 (23)	75 (20)
radiotherapy	224 (16)	89 (17)	72 (15)	63 (16)
chemoradiation	107 (8)	34 (6)	47 (10)	26 (7)
Tumour stage				
I	385 (27)	141 (26)	131 (27)	113 (29)
II	403 (28)	149 (27)	143 (29)	111 (29)
III	629 (44)	258 (47)	211 (44)	160 (42)
Comorbidities (yes)[*]	968 (69)	389 (71)	337 (70)	242 (63)
Fatigue (yes)	365 (26)	157 (29)	115 (24)	93 (24)
Recurrence within one year				
(yes)	81 (6)	31 (6)	25 (5)	25 (7)
Unknown	67	26	19	22

Daily use of NSAIDs (yes)	110 (8)	45 (8)	37 (8)	28 (7)
Cohort				
COLON	1156 (82)	421 (77)	399 (82)	336 (88)
EnCoRe	261 (18)	127 (23)	86 (18)	48 (13)

Values presented are median (quartile 1 – quartile 3) or number (percentages). Abbreviations: IL, interleukin; TNF, tumour necrosis factor; hsCRP, high sensitive c-reactive protein; NSAIDs, non-steroid anti-inflammatory drugs. *=For 2 patients data about education levels was missing, for 15 patients data about smoking status was missing and for 1 patients BMI was missing, for 29 patients treatment data was missing and for 6 patients data about comorbidities was missing.
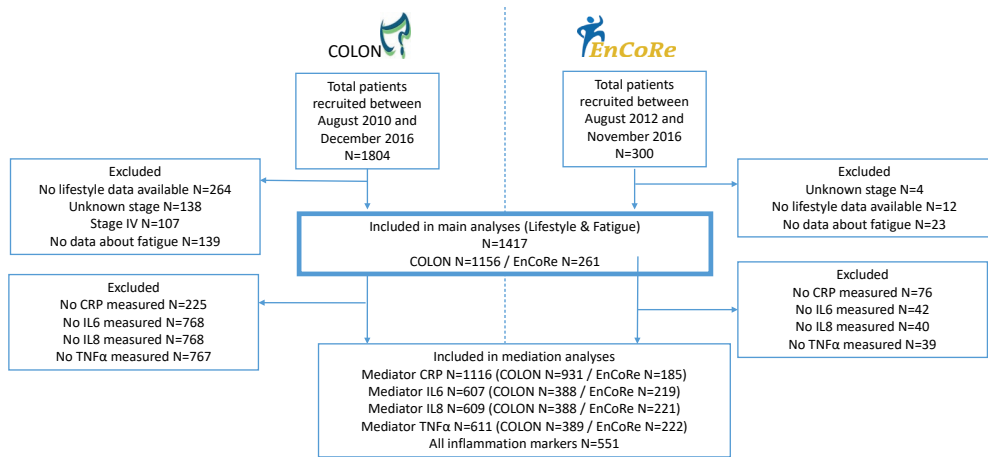
Figure 1: Flow diagram of patients included in this study.

Table 2: The association between lifestyle at diagnosis and inflammatory marker levels six months after diagnosis in colorectal cancer patients

Inflammation 6 months after diagnosis	N	Crude Beta 95%CI	Adjusted* Beta 95%CI
IL6			
WCRF/AICR-score	607	-0.11 (-0.19; -0.04)	-0.10 (-0.17; -0.03)
IL8			
WCRF/AICR-score	609	-0.05 (-0.10; -0.00)	-0.05 (-0.09; 0.00)
TNFα			
WCRF/AICR-score	611	-0.08 (-0.11; -0.05)	-0.07 (-0.10; -0.04)
hsCRP			
WCRF/AICR-score	1116	-0.20 (-0.28; -0.13)	-0.20 (-0.28; -0.12)

* adjusted for age, sex, stage of disease, chemotherapy (yes/no) and cohort. To interpret the beta coefficient of the regression line, the exponential of the beta should be taken (EXP^β), since a natural log transformation was done on the outcome variable.

Table 3: The association between inflammation marker levels and fatigue six months after diagnosis

Fatigue after 6 months	N	Crude Beta 95% CI	Adjusted* Beta 95% CI
IL6	607	5.49 (3.03; 7.95)	5.05 (2.54; 7.57)
IL8	609	1.41 (-2.34; 5.15)	1.62 (-2.07; 5.31)
TNFα	611	1.01 (-4.50; 6.51)	3.03 (-2.84; 8.90)
hsCRP	1116	3.57 (2.30; 4.83)	3.57 (2.37; 4.78)

* adjusted for age, sex, stage of disease, chemotherapy (yes/no) and cohort.

Table 4: The association between lifestyle and fatigue (total association) divided in an direct path independent of inflammation (direct association) and an indirect path via inflammation (indirect association).

Fatigue 6 months after diagnosis		*N*	*B (95%CI)*
IL6	Total association	607	-2.17 (-4.44; 0.10)
	Direct association	607	-1.69 (-3.95; 0.56)
	Indirect association	607	-0.48 (-1.05; -0.10)
	Proportion mediated*		22%
IL8	Total association	609	-2.19 (-4.45; 0.08)
	Direct association	609	-2.12 (-4.39; 0.15)
	Indirect association	609	-0.06 (-0.31; 0.12)
	Proportion mediated*		3%
TNFα	Total association	611	-2.19 (-4.45; 0.07)
	Direct association	611	-2.05 (-4.35; 0.24)
	Indirect association	611	-0.14 (-0.69; 0.21)
	Proportion mediated*		6%
hsCRP	Total association	1116	-2.11 (-3.74; -0.48)
	Direct association	1116	-1.42 (-3.05; 0.21)
	Indirect association	1116	-0.68 (-1.10; -0.34)
	Proportion mediated*		32%
Total inflammation (IL6, IL8, TNFα and hsCRP)	Total association	551	-2.17 (-4.60; 0.25)
	Direct association	551	-1.21 (-3.64; 1.25)
	Indirect association	551	-0.97 (-1.92; -0.21)
	Proportion mediated*		45%

Assessed using the PROCESS method version 3.5 developed by Andrew F. Hayes [41].

Models were adjusted for age, sex, stage of disease, chemotherapy (yes/no) and cohort. In the model for total inflammation all four inflammation markers were entered as potential mediators.

* Proportion mediation is calculated by dividing the indirect effect by the total effect e.g. beta indirect association / beta total association * 100.

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Discussion

In the present study we observed a statistically significant association between better adherence to the WCRF/AICR lifestyle recommendations at diagnosis and lower fatigue levels six months post-diagnosis among CRC patients. Furthermore, mediation analyses showed that inflammation markers, most importantly, IL6, hsCRP were mediators in the association between lifestyle and fatigue.

Our results for the association between adherence to the WCRF/AICR recommendations at diagnosis and lower fatigue levels six months post-diagnosis are in line with the results of two previous observational studies that investigated the association between adherence to the WCRF/AICR recommendations and long-term fatigue in patients with stage I-III CRC (8, 9). Consistently, our results and the results of those studies showed that a favourable lifestyle at and after diagnosis is associated with less fatigue on the short and long term in CRC patients.

Our study was the first to assess the mediating effect of inflammation on the association between lifestyle and fatigue. Lifestyle was associated with levels of IL6 and hsCRP as well as with fatigue. Previous studies showed that nutrition as well as physical activity (both part of the WCRF/AICR score) are associated with inflammation (14, 24) and/or fatigue (1, 13, 21). Results of our mediation analysis, combining those three factors for the first time (i.e. lifestyle, inflammation and fatigue), showed that 45% of the association observed between lifestyle and fatigue could be attributed to inflammation (mainly driven by IL6 and hsCRP). In other words, a healthier lifestyle may lead to less fatigue in part by lowering inflammation. Of note, we did measure the exposure variable (lifestyle at diagnoses) before the outcome (fatigue six months after diagnosis), while, the mediator and outcome were measured at the same time-point. Ideally, to investigate causality, inflammation should be measured several times between exposure and outcome (42). Despite this limitation, our results clearly indicate a role for inflammation in the association between lifestyle and fatigue.

The exact biological pathways by which inflammation mediates the association between lifestyle and fatigue in cancer patients are not completely understood. As described before, a healthier lifestyle (e.g. healthy weight, exercise and a healthy diet) are associated with lower circulating levels of inflammation markers. Inflammation, in turn, is hypothesized to induce fatigue via two main pathways. The first suggested pathway is via neuronal signalling (1). Pro-inflammatory cytokines, such as IL6, IL8, hsCRP or TNFα, can signal the central nervous system to generate symptoms of fatigue via alterations in neural processes (43-46). Another pathway could be an increased activity of nuclear factor kappa beta (NFkB), which plays a key role in controlling expression of pro-inflammatory genes

(47). On a mechanistic base, we cannot explain why IL6 and hsCRP were observed to be mediators, while IL8 and TNFα were not, since all four are associated with a lifestyle and all four could potentially activate both pathways described above. From a statistic point of view, these non-findings can be explained by the fact that in our study population IL8 and TNFα were not statistically significantly associated with fatigue, and could therefore not be mediators. Despite the inconsistency between the specific markers of inflammation, results of our study showed that systemic low grade inflammation is a mediator in the association between lifestyle and fatigue.

Given the supposed role of inflammation in fatigue, preventing or reversing low grade inflammation is of importance. However, before interventions aimed to reduce inflammation can be conducted the role of inflammation in fatigue should be further investigated. It should be investigated in an intervention study whether adopting a healthier lifestyle during and after cancer treatment results in lower levels of inflammation markers (IL6, TNFα) and ultimately less fatigue. If so, person-made lifestyle advices can be given to patients with higher levels of pro-inflammatory cytokines.

This study has several limitations and strengths. First, inflammation data were only available for a subset of our original population which lowered our statistical power in the mediation analyses. Nevertheless, both IL6 and hsCRP were identified to have a mediating role in the association between adherence to the WCRF/AICR recommendations and fatigue. Furthermore, we used the existing WCRF/AICR recommendation score, in which all recommendations weigh equally (10). Although the used WCRF/AICR scoring is simple and relatively easy to apply, one could debate whether all components of the score contribute equally to fatigue and inflammation. Future studies should assess whether assigning different weighing factors for each recommendation is more appropriate. Strengths of the study are that we had extensive information about lifestyle, clinical variables, circulating levels of inflammation markers, and patient-reported outcomes (such as fatigue) which allowed us to study for the first time the mediating effect of inflammation in the association between lifestyle and fatigue. Our data complement existing evidence regarding lifestyle and fatigue and the role of inflammation as a mediating factor.

Conclusions

The present study showed that a higher adherence to the WCRF/AICR recommendations at diagnosis was associated with less fatigue among CRC survivors six months after diagnosis, and that inflammation partly mediated this association. Future intervention studies should investigate if improving lifestyle directly after diagnosis, for example by improving adherence to WCRF/AICR recommendations, can reduce fatigue during and after treatment.

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References

1. Bower JE. Cancer-related fatigue—mechanisms, risk factors, and treatments. Nature reviews Clinical oncology. 2014;11(10):597.
2. Lawrence DP, Kupelnick B, Miller K, Devine D, Lau J. Evidence report on the occurrence, assessment, and treatment of fatigue in cancer patients. Jnci Monographs. 2004;2004(32):40-50.
3. Mitchell SA. Cancer-related fatigue: state of the science. PM&R. 2010;2(5):364-83.
4. Aapro M, Scotte F, Bouillet T, Currow D, Vigano A. A Practical Approach to Fatigue Management in Colorectal Cancer. Clinical colorectal cancer. 2017;16(4):275-85.
5. Vissers PAJ, Thong MSY, Pouwer F, Zanders MMJ, Coebergh JWW, van de Poll-Franse LV. The impact of comorbidity on Health-Related Quality of Life among cancer survivors: analyses of data from the PROFILES registry. Journal of Cancer Survivorship. 2013;7(4):602-13.
6. Mustian KM, Alfano CM, Heckler C, Kleckner AS, Kleckner IR, Leach CR, et al. Comparison of pharmaceutical, psychological, and exercise treatments for cancer-related fatigue: a meta-analysis. JAMA oncology. 2017;3(7):961-8.
7. Lei Y-Y, Ho SC, Cheng A, Kwok C, Lee C-KI, Cheung KL, et al. Adherence to the World Cancer Research Fund/American Institute for Cancer Research Guideline is associated with better health-related quality of life among Chinese patients with breast cancer. Journal of the National Comprehensive Cancer Network. 2018;16(3):275-85.
8. Breedveld-Peters JJ, Koole JL, Müller-Schulte E, van der Linden BW, Windhausen C, Bours MJ, et al. Colorectal cancers survivors' adherence to lifestyle recommendations and cross-sectional associations with health-related quality of life. British Journal of Nutrition. 2018;120(2):188-97.
9. van Veen MR, Mols F, Bours MJ, Weijenberg MP, Kampman E, Beijer S. Adherence to the World Cancer Research Fund/American Institute for Cancer Research recommendations for cancer prevention is associated with better health–related quality of life among long-term colorectal cancer survivors: results of the PROFILES registry. Supportive Care in Cancer. 2019;27(12):4565-74.
10. Shams-White MM, Brockton NT, Mitrou P, Romaguera D, Brown S, Bender A, et al. Operationalizing the 2018 World Cancer Research Fund/American Institute for Cancer Research (WCRF/AICR) Cancer Prevention Recommendations: A Standardized Scoring System. Nutrients. 2019;11(7):1572.
11. Baguley JB, Bolam AK, Wright RLO, Skinner LT. The Effect of Nutrition Therapy and Exercise on Cancer-Related Fatigue and Quality of Life in Men with Prostate Cancer: A Systematic Review. Nutrients. 2017;9(9).
12. Meneses-Echávez JF, Correa-Bautista, J.E., González-Jiménez, E., Schmidt Río-Valle, J., Elkins, M.R., Lobelo, F., Ramírez-Vélez, R. The Effect of Exercise Training on Mediators of Inflammation in Breast Cancer Survivors: A Systematic Review with Meta-analysis. Cancer Epidemiology Biomarkers & Prevention. 2016;25(7):1009.

5

13. Himbert C, Ose J, Lin T, Warby CA, Gigic B, Steindorf K, et al. Inflammation-and angiogenesis-related biomarkers are correlated with cancer-related fatigue in colorectal cancer patients: Results from the ColoCare Study. European journal of cancer care. 2019;28(4):e13055.

14. Kessels E, Husson O, Van der Feltz-Cornelis CM. The effect of exercise on cancer-related fatigue in cancer survivors: a systematic review and meta-analysis. Neuropsychiatric disease and treatment. 2018;14:479.

15. Schmidt ME, Chang-Claude J, Seibold P, Vrieling A, Heinz J, Flesch-Janys D, et al. Determinants of long-term fatigue in breast cancer survivors: results of a prospective patient cohort study. Psycho-Oncology. 2015;24(1):40-6.

16. Courneya KS, Karvinen KH, Campbell KL, Pearcey RG, Dundas G, Capstick V, et al. Associations among exercise, body weight, and quality of life in a population-based sample of endometrial cancer survivors. Gynecologic oncology. 2005;97(2):422-30.

17. Oldenburg CS, Boll D, Nicolaije KA, Vos MC, Pijnenborg JM, Coebergh J-W, et al. The relationship of body mass index with quality of life among endometrial cancer survivors: a study from the population-based PROFILES registry. Gynecologic oncology. 2013;129(1):216-21.

18. Smits A, Lopes A, Das N, Bekkers R, Galaal K. The impact of BMI on quality of life in obese endometrial cancer survivors: does size matter? Gynecologic oncology. 2014;132(1):137-41.

19. Basen-Engquist K, Scruggs S, Jhingran A, Bodurka DC, Lu K, Ramondetta L, et al. Physical activity and obesity in endometrial cancer survivors: associations with pain, fatigue, and physical functioning. American journal of obstetrics and gynecology. 2009;200(3):288. e1-. e8.

20. Baguley BJ, Skinner TL, Wright OR. Nutrition therapy for the management of cancer-related fatigue and quality of life: a systematic review and meta-analysis. British Journal of Nutrition. 2019;122(5):527-41.

21. LaVoy EC, Fagundes CP, Dantzer R. Exercise, inflammation, and fatigue in cancer survivors. Exercise immunology review. 2016;22:82.

22. Ibrahim MM. Subcutaneous and visceral adipose tissue: structural and functional differences. Obesity reviews. 2010;11(1):11-8.

23. Bruun JM, Lihn AS, Madan AK, Pedersen SB, Schiøtt KM, Fain JN, et al. Higher production of IL-8 in visceral vs. subcutaneous adipose tissue. Implication of nonadipose cells in adipose tissue. American Journal of Physiology-Endocrinology and Metabolism. 2004;286(1):E8-E13.

24. Galland L. Diet and inflammation. Nutrition in Clinical Practice. 2010;25(6):634-40.

25. Barbaresko J, Koch M, Schulze MB, Nöthlings U. Dietary pattern analysis and biomarkers of low-grade inflammation: a systematic literature review. Nutrition reviews. 2013;71(8):511-27.

26. Zick SM, Colacino J, Cornellier M, Khabir T, Surnow K, Djuric Z. Fatigue reduction diet in breast cancer survivors: a pilot randomized clinical trial. Breast cancer research and treatment. 2017;161(2):299-310.

27. Winkels R, Heine-Broring R, Van Zutphen M, van Harten-Gerritsen S, Kok D, Van Duijnhoven F, et al. The COLON study: Colorectal cancer: Longitudinal, Observational study on Nutritional and lifestyle factors that may influence colorectal tumour recurrence, survival and quality of life. BMC Cancer. 2014;14(1):374.

28. van Roekel EH, Bours MJL, de Brouwer CPM, Ten Napel H, Sanduleanu S, Beets GL, et al. The Applicability of the International Classification of Functioning, Disability, and Health to Study Lifestyle and Quality of Life of Colorectal Cancer Survivors. Cancer Epidemiology Biomarkers & Prevention. 2014;23(7):1394-405.

29. World Cancer Research Fund / American Institute for Cancer Research. Continuous Update Project Report 2018. Recommendations and public health and policy implications. 2018.

30. Feunekes I, Van Staveren W, Graveland F, Vos JD, Burema J. Reproducibility of a semiquantitative food frequency questionnaire to assess the intake of fats and cholesterol in The Netherlands. International journal of food sciences and nutrition. 1995;46(2):117-23.

31. Verkleij-Hagoort AC, de Vries JH, Stegers MP, Lindemans J, Ursem NT, Steegers-Theunissen RP. Validation of the assessment of folate and vitamin B 12 intake in women of reproductive age: the method of triads. European journal of clinical nutrition. 2007;61(5):610.

32. Koole JL, Bours MJ, Breedveld-Peters JJ, van Roekel EH, van Dongen MC, Eussen SJ, et al. Evaluating the validity of a food frequency questionnaire in comparison with a 7-day dietary record for measuring dietary intake in a population of survivors of colorectal cancer. Journal of the Academy of Nutrition and Dietetics. 2020;120(2):245-57.

33. Center NN. NEVO Nederlandse Voedingsmiddelen Tabel 2011 (In Englisch: Dutch Food Composition Table).

34. Wendel-Vos GCW, Schuit AJ, Saris WHM, Kromhout D. Reproducibility and relative validity of the short questionnaire to assess health-enhancing physical activity. Journal of Clinical Epidemiology. 2003;56(12):1163-9.

35. Fayers PM, Aaronson, N.K., Bjordal, K., Groenvold, M., Curran D., Bottomley, A.,. The EORTC QLQ-C30 Scoring Manual (3rd Edition). Brussels: European Organisation for Research and Treatment of Cancer; 2001.

36. Giesinger JM, Kuijpers W, Young T, Tomaszewski KA, Friend E, Zabernigg A, et al. Thresholds for clinical importance for four key domains of the EORTC QLQ-C30: physical functioning, emotional functioning, fatigue and pain. Health and Quality of Life Outcomes. 2016;14:87.

37. Wesselink E, Balvers M, Bours MJL, de Wilt JHW, Witkamp RF, van Baar H, et al. The association between circulating levels of vitamin D and inflammatory markers in the first 2 years after colorectal cancer diagnosis. Therapeutic Advances in Gastroenterology. 2020;13:1756284820923922.

38. de Jager W, Bourcier K, Rijkers GT, Prakken BJ, Seyfert-Margolis V. Prerequisites for cytokine measurements in clinical trials with multiplex immunoassays. BMC immunology. 2009;10(1):1-11.

39. Meyer K, Ueland PM. Targeted quantification of C-reactive protein and cystatin c and its variants by immuno-MALDI-MS. Analytical chemistry. 2014;86(12):5807-14.

40. Van Leersum N, Snijders H, Henneman D, Kolfschoten N, Gooiker G, Ten Berge M, et al. The Dutch surgical colorectal audit. European Journal of Surgical Oncology (EJSO). 2013;39(10):1063-70.

5

41. Hayes AF. Introduction to mediation, moderation, and conditional process analysis second edition: A regression-based approach. New York, NY: Ebook The Guilford Press Google Scholar. 2018.

42. Turnes PB, Ernst R. The Use of longitudinal mediation models for testing causal effects and measuring direct and indirect effects. China USA Bus Rev. 2016;15(1):1-13.

43. Dantzer R, O'Connor JC, Freund GG, Johnson RW, Kelley KW. From inflammation to sickness and depression: when the immune system subjugates the brain. Nature reviews neuroscience. 2008;9(1):46-56.

44. Haroon E, Raison CL, Miller AH. Psychoneuroimmunology meets neuropsychopharmacology: translational implications of the impact of inflammation on behavior. Neuropsychopharmacology. 2012;37(1):137-62.

45. Miller AH, Ancoli-Israel S, Bower JE, Capuron L, Irwin MR. Neuroendocrine-immune mechanisms of behavioral comorbidities in patients with cancer. Journal of clinical oncology: official journal of the American Society of Clinical Oncology. 2008;26(6):971.

46. Cleeland CS, Bennett GJ, Dantzer R, Dougherty PM, Dunn AJ, Meyers CA, et al. Are the symptoms of cancer and cancer treatment due to a shared biologic mechanism? A cytokine-immunologic model of cancer symptoms. Cancer: Interdisciplinary International Journal of the American Cancer Society. 2003;97(11):2919-25.

47. Bower JE, Ganz PA, Irwin MR, Arevalo JM, Cole SW. Fatigue and gene expression in human leukocytes: increased NF-κB and decreased glucocorticoid signaling in breast cancer survivors with persistent fatigue. Brain, behavior, and immunity. 2011;25(1):147-50.

Supplemental tables

Table S1: Breakdown of the official 2018 WCRF/AICR Score (10) and the percentage of our cohort of colorectal cancer patients that adhered to the specific recommendation.

2018 WCRF/AICR Recommendations	Operationalisation of Recommendations	Points	Adherence in total population (%)
	BMI (kg/m²)		
1. Be a healthy weight (Lifestyle questionnaire)	18.5-24.9	0.5	34
	25-29.9	0.25	44
	<18.5 or ≥30	0	22
	Waist circumference (cm)		
	Men: <94; Women: <80	0.5	22
	Men: 94-<102; Women: 80-<88	0.25	28
	Men: ≥102; Women: ≥88	0	50
2. Be physically active (SQUASH)	Total moderate-vigorous physical activity (min/week)		
	≥150	1	90
	75-<150	0.5	4
	<75	0	6
3. Eat a diet rich in wholegrains, vegetables, fruit and beans (FFQ)	Fruits and vegetables (g/day)		
	≥400	0.5	12
	200-<400	0.25	72
	<200	0	16
	Total fibre (g/day)		
	≥30	0.5	19
	15-<30	0.25	46
	<15	0	35
4. Limit consumption of "fast foods" and other processed foods high in fat, starches or sugars (FFQ)	Percent of total kcal from ultra-processed foods (aUPFs)		
	Tertile 1	1	32
	Tertile 2	0.5	33
	Tertile 3	0	36

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5.	Limit consumption of red and processed meat (FFQ)	Total red meat (g/week) and processed meat (g/week)		
		Red meat <500 and processed meat <21	1	8
		Red meat <500 and processed meat 21-<100	0.5	17
		Red meat >500 or processed meat ≥100	0	75
6.	Limit consumption of sugar-sweetened drinks (FFQ)	Total sugar-sweetened drinks (g/day)		
		0	1	14
		>0-≤250	0.5	70
		>250	0	16
7.	Limit alcohol consumption (FFQ)	Total ethanol (g/day)		
		0	1	14
		>0-≤28 (2 drinks) males and ≤14 (1 drink) females	0.5	53
		>28 (2 drinks) males and >14 (1 drink) females	0	32
	Total score		0-7	

Table S2: Baseline characteristics of the total population, patients of whom hsCRP levels were available and patients of whom IL6 levels were available and for patient without data about inflammation markers available.

	Total population (n=1417)	hsCRP levels available (n=1116)	IL6 levels available (n=607)	No hsCRP or IL6 levels available (n=866)
Age (years)	66.1 (61.2-71.5)	66.3 (61.4-71.4)	66.8 (61.8-72.5)	65.7 (60.9-71.0)
Sex (female)	514 (36)	401 (36)	195 (32)	337 (39)
Education level[*]				
- low	536 (38)	434 (39)	216 (36)	333 (39)
- medium	412 (29)	318 (29)	192 (32)	245 (28)
- high	467 (33)	363 (33)	198 (33)	287 (33)
Smoking[*]				
- current	155 (11)	123 (11)	66 (11)	92 (11)
- former	811 (58)	642 (58)	248 (59)	494 (57)
- never	436 (31)	336 (31)	179 (30)	279 (32)
BMI (kg/m²)[*]	26.4 (24.2-29.4)	26.3 (24.1-29.3)	26.9 (24.5-30.1)	26.1 (24.0-28.7)
Total moderate-vigorous physical activity (hours/week)	11.0 (5.5-19.0)	11.0 (5.5-19.0)	12.0 (5.3-21)	11.0 (5.5-18.5)
Type of Cancer				
- colon	929 (66)	743 (67)	394 (65)	564 (65)
- rectal	488 (34)	373 (33)	213 (35)	302 (35)
Type of treatment[*]				
- surgery	756 (54)	589 (53)	333 (56)	454 (53)
- chemotherapy	301 (22)	250 (23)	121 (20)	185 (22)
- radiotherapy	224 (16)	178 (16)	78 (13)	153 (18)
- chemoradiation	107 (8)	80 (7)	54 (9)	61 (7)
Tumour stage				
I	385 (27)	296 (27)	177 (29)	225 (26)
II	403 (28)	334 (30)	179 (29)	241 (28)
III	629 (44)	486 (44)	251 (41)	400 (46)
Comorbidities (yes)[*]	968 (69)	767 (69)	455 (75)	559 (65)
Daily use of NSAIDs (yes)	110 (8)	90 (8)	42 (7)	70 (8)
Cohort				
- COLON	1156 (82)	931 (84)	388 (64)	784 (91)
- EnCoRe	261 (18)	184 (17)	219 (36)	82 (9)

Values presented are median (quartile 1 – quartile 3) or number (percentages). Abbreviations: IL, interleukin; TNF, tumour necrosis factor; hsCRP, high sensitive c-reactive protein; NSAIDs, non-steroid anti-inflammatory drugs. For 2 patients data about education levels was missing, for 15 patients data about smoking status was missing and for 1 patients BMI was missing, for 29 patients treatment data was missing and for 6 patients data about comorbidities was missing.

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Chapter 6

General discussion

The overall objectives of this thesis were 1) to improve knowledge about the association of body composition in stage I-III CRC patients with both mortality and fatigue by using computed tomography (CT) images to quantify skeletal muscle mass, skeletal muscle radiodensity and adipose tissue, and 2) to improve knowledge about the association of adherence to the WCRF/AICR lifestyle recommendations with fatigue among stage I-III CRC and to elucidate the underlying mechanism. Specific aims were:

1. To investigate the association of skeletal muscle mass, skeletal muscle radiodensity and adipose tissue with mortality among a large population of stage I-III CRC patients.

2. To investigate the association of skeletal muscle mass, skeletal muscle radiodensity and adipose tissue with fatigue among stage I-III CRC patients.

3. To investigate the association between adherence to the WCRF/AICR lifestyle recommendations and fatigue experienced during or shortly after treatment among stage I-III CRC patients and to determine if inflammation is (partly) mediating this association.

This general discussion will first give an overview of the main findings of this thesis. After this, these findings will be discussed in light of existing literature. This will be done for each body composition component separately, followed by the findings for aim 3. Next, the methodological considerations will be discussed, followed by the implications for clinical practice and suggestions for future research. Finally, this general discussion ends with an overall conclusion.

Main Findings

Table 1 provides an overview of the main findings of this thesis per chapter.

First, the association between body composition components assessed at diagnosis and mortality was investigated (**Chapter 2 and 3**). Low skeletal muscle radiodensity (SMR) was significantly associated with higher mortality among stage I-III CRC patients. Furthermore, low SMR was significantly associated with poorer disease-free survival, but not with CRC-specific mortality (**Chapter 2**). Skeletal muscle index (SMI) was associated with mortality in a non-linear way among men, with lower SMI levels associated with higher mortality. SMI was not associated with mortality among women. Subcutaneous adipose tissue (SAT) was associated with mortality in a non-linear way for men and for women, with less SAT being associated with higher mortality. Visceral adipose tissue (VAT) was not associated with mortality in men and women (**Chapter 3**).

Table 1: Overview of the main findings of this thesis

Exposure measurement	Mortality (Chapter 2, 3)	Fatigue (Chapter 4, 5)
Body composition		
SMR	• Low SMR was significantly associated with higher overall mortality and worse disease-free survival. • Low SMR was not significantly associated with CRC-specific mortality.	• Lower SMR was associated with more fatigue at 6 months post-diagnosis.
SMI	• Among men, lower SMI was associated with higher mortality. • Among women, lower SMI was not associated with mortality.	• SMI was not associated with fatigue.
VAT	• VAT was not significantly associated with mortality in men and women.	• VAT was not associated with fatigue.
SAT	• Less SAT was associated with higher mortality in men and women.	• Having more SAT was associated with more fatigue at diagnosis.
Lifestyle		
WCRF/AICR	*Not investigated in this thesis.*	• A higher WCRF/AICR adherence score was associated with less fatigue six months after diagnosis. • A statistically significant indirect association via inflammation was observed, explaining 45% of the total association between lifestyle and fatigue.

Abbreviations: SMR, skeletal muscle radiodensity; SMI, skeletal muscle index; VAT, visceral adipose tissue; SAT, subcutaneous adipose tissue; CRC, colorectal cancer; WCRF/AICR, World Cancer Research Fund / American Institute for Cancer Research

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Second, the association of SMI, VAT, SAT and SMR at diagnosis with fatigue at time of diagnosis, and at 6 and 24 months post-diagnosis was investigated (**Chapter 4**). More SAT was associated with higher fatigue prevalence at time of diagnosis. Lower SMR was associated with higher fatigue prevalence 6 months post-diagnosis. SMI nor VAT were associated with fatigue at time of diagnosis, and at 6 and 24 months post-diagnosis.

Lastly, it was investigated 1) whether a better adherence to the WCRF/AICR lifestyle recommendations was associated with less fatigue among CRC patients, and 2) whether inflammation was mediating this association (**Chapter 5**). A higher WCRF/AICR adherence score was associated with less fatigue six months after diagnosis. An association via inflammation was observed explaining 45% of the total association between lifestyle

and fatigue, indicating that inflammation is probably one of the underlying mechanisms linking lifestyle to fatigue.

Comparison with other studies

In this section our findings will be compared with the findings from similar studies in the literature.

Skeletal muscle radiodensity

In **Chapter 2,** we observed an association of low SMR with increased mortality risk, and in **Chapter 4** we observed an association between lower SMR and more fatigue six months post-diagnosis.

At present, seven other studies have investigated the association between SMR and overall mortality among stage I-III CRC patients (1-7). Four of these studies observed a statistically significant association between low SMR and increased mortality risk, while in three studies no statistically significant association was observed. The average follow-up time of these studies varied between 3.6–6.9 years, and almost all studies used different cut-off values to determine low SMR. Kroenke *et al* (n=3262, low vs normal SMR: HR 1.61; 95% CI 1.36-1.90) (3), Hopkins *et al* (n=968, low vs normal SMR: HR 1.53; 95% CI, 1.19–1.97) (4), Xiao *et al* (n=1630, low vs normal SMR: HR 1.44; 95% CI, 1.12-1.85) (5), and Sueda *et al* (n=211, low vs normal SMR: HR 2.94; 95% CI, 1.32-7.17) (6) observed an association between low SMR and increased mortality risk. No statistically significant associations between low SMR and mortality were observed in the studies of Van Vugt *et al* (n=815, low vs normal SMR: HR 0.91, 95% CI 0.65-1.29) (1), McSorley *et al* (n=322, low vs normal SMR: adjusted HR not presented in paper, unadjusted HR 2.47; 95% CI, 1.49-4.10) (7), and Dolan *et al* (n=650, low vs normal SMR: HR 1.42, 95% CI 0.98–2.05) (2). Although the association between low SMR and increased mortality did not reach statistical significance in the study of Dolan *et al*, the point estimate is similar to the point estimates from the studies of Hopkins *et al* and Xiao *et al*. It is unclear why the results of Van Vugt *et al* and McSorley *et al* deviate from the other studies since the study set up was comparable to the rest of the studies and the percentage of patients that died was comparable among studies. One possible explanation could be the cut-off values that were used in these studies. Both Van Vugt *et al* and McSorley *et al* used the cut-off values from Martin *et al* (8) that were determined in a mixed cancer population, while the majority of the other studies determined their own cut-off values using their own study population. It is possible that the cut-off values of Martin *et al* were not optimal for the study populations of both Van Vugt *et al* and McSorley. The possibilities and challenges of categorizing a study population based on body composition will be discussed in the "Methodological considerations" section.

The results of the studies discussed above and our own results indicate that low SMR at diagnosis is associated with higher overall mortality risk among stage I-III CRC patients. The association between SMR and fatigue among stage I-III CRC patients has only been studied in one study (n=96) (9). In this study by Van Roekel *et al* no association between low SMR and fatigue was observed (per SD increase, β = -0.06; 95% CI −7.6 to 6.4). In our study fatigue was assessed at 6 and 24 months post-diagnosis, while in the study of Van Roekel *et al* fatigue was assessed on average 5.2 years post-diagnosis. Because of the retrospective collection of CT scans from 2-10 year CRC survivors in the study of Van Roekel *et al*, survival bias might have occurred in the study. People that passed away before they could participate in the study might have suffered from more fatigue than those who did participate. Because of this, the observed association may have been underestimated in the study of Van Roekel. Lack of studies makes it difficult to draw firm conclusions about the association between low SMR and fatigue post-diagnosis. However, the findings from our study suggest that low SMR at diagnosis is associated with fatigue experienced during or shortly after cancer treatment.

Skeletal muscle index

In **Chapter 3** the association of SMI and mortality was statistically significant in a non-linear way among men, with lower SMI levels associated with higher mortality. SMI was not associated with mortality among women. In **Chapter 4** no association of SMI with fatigue at time of diagnosis, and at 6 and 24 months post-diagnosis was observed.

At present eight other relatively large studies investigated the association between low SMI and overall mortality among stage I-III CRC patients (1, 2, 4-6, 10-12). In six of these studies an association between low SMR and increased mortality risk was observed, while in one study no association was observed. The average follow-up time of these studies varied between 3.5–6.4 years, and almost all studies used different cut-off values to determine low SMI. An association between low SMI and increased overall mortality risk was observed in the studies of Caan *et al* (n = 3,262, low vs normal + high SMI: HR 1.27, 95% CI 1.09-1.48) (10); Dolan *et al* (n=650, low vs normal + high SMI: HR 1.50, 95% CI 1.04–2.18) (2); Hopkins *et al* (n=968, low vs normal + high SMI: HR 1.45; 95% CI, 1.16-1.84) (4); Xiao *et al* (n=1630, low vs normal + high SMI: HR 1.40; 95% CI 1.13-1.74) (5); Sueda *et al* (n=211, low vs normal SMI: HR 2.29; 95% CI, 1.04-5.41) (13) and Miyamoto *et al* (n=220, low vs normal SMI: HR 2.18; 95% CI, 1.20-3.94) (11). Van Vugt *et al* (n=816, low vs normal + high SMI: HR 1.06, 95% CI 0.80-1.42) (1) and Black *et* al (n=339, low vs normal + high SMI: HR 1.21, 95% CI 0.81-1.79) (12) did not find a significant association between low SMI and mortality. It is unclear why the results in the study of Van Vugt *et al* and Black *et al* deviate since the study set up was comparable to the rest of the studies and the percentage of patients who died was comparable among studies. As with SMR, one possible explanation could be the cut-off values that were used in these studies. As described above, both van Vugt *et al* and Black *et al* used the cut-off values from Martin *et al* (8) that were determined in a mixed

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cancer population, while the majority of the other studies determined their own cut-off values using their own study population. See the "Methodological considerations" section for a discussion about the possibilities and challenges of categorizing a study population based on body composition. The results from the studies discussed above in combination with our study suggest that low SMI at diagnosis is associated with higher overall mortality risk. Our results suggest that this association might be strongest or only present in men. The difference in findings between men and women was also observed in restricted cubic spline plots that were presented in the publication of Caan *et al* (10). Unfortunately, none of the other studies investigated gender differences in the association between SMI and overall mortality.

The association of low SMI and fatigue among stage I-III CRC has only been studied in one other study (9). In this study conducted among 96 stage I–III CRC survivors, who were on average 5.2 years post-diagnosis, no association was observed between low SMI at diagnosis and levels of fatigue 2-10 years post-diagnosis (per SD increase, β = -4.4; 95% CI −12.1 to 3.4). Another study in a mixed cancer population did investigate the association between SMI and fatigue at time of diagnosis or during treatment (14). In this study lower SMI was not associated with higher fatigue levels (per 1 cm^2/m^2 increase, β -0.01; 95% CI -0.22 to 0.20). Only in the study of Kilgour *et al* (15) a significant association between low SMI and increased fatigue levels was observed among 84 stage III–IV gastrointestinal or non-small cell lung cancer patients (per 1 kg/m^2 increase, β −4.8; 95% CI −8.4 to −1.3). However, in this study SMI was assessed using dual-energy X-ray absorptiometry (DEXA) instead of CT.

Although the evidence for a possible association between low SMI and fatigue is currently not convincing, it has been suggested in the literature that there is a strong relationship between skeletal muscle mass and fatigue experienced by cancer patients (16, 17). In one review it is suggested that fatigue in cancer patients is a result from progressive reduction of physical activity, reducing skeletal muscle mass and skeletal muscle strength which gradually affects the ability to carry out simple activities of daily living (17). However, there is not enough evidence to support this hypothesis. In another review, fatigue is suggested to be a symptom of cachexia-related muscle loss, possible due to mitochondrial dysfunction in the skeletal muscle cells (16). These suggestions and the results from the study of Kilgour *et al* (15) indicate that it would be useful to perform more studies on the association between low SMI and fatigue in stage I-III CRC patients. Whether an association between low SMI and fatigue is causal, as is suggested in the two reviews (16, 17), should be tested with randomized controlled trials. In these studies it should be investigated whether improving SMI leads to lower fatigue levels among cancer survivors.

Visceral adipose tissue

In this thesis it was observed that VAT was not significantly associated with mortality (**Chapter 4**) nor with fatigue at time of diagnosis, and at 6 and 24 months post-diagnosis.

At present only four other studies have investigated the association of VAT with mortality in stage I-III CRC patients (4, 10, 12, 18). One study observed a statistically significant association between VAT and mortality risk, with higher VAT levels being associated with increased mortality risk (18); in one study the association did not reach statistical significance (10); and in two studies no association was observed (4, 12). The average follow-up time of these studies varied between 5.2-6.9 years and three of those studies used cut-off values to determine high VAT (4, 10, 12), while one study used restricted cubic splines to investigate the association (18). Two of these studies were performed using the same study population (10, 18).

No clear association of high VAT with increased mortality was observed in the studies of Hopkins *et al* (n=968, high vs medium + low VAT: unadjusted HR 0.98; 95% CI 0.80–1.21) (4); Black *et* al (n=339, high vs medium + low VAT: HR 1.00, 95% CI 0.80–1.26) (12); and Caan *et al* (n=3262, high vs normal + low VAT: HR 1.22; 95 CI 0.99 - 1.49) (10). Restricted cubic spline analyses of the latter study, showed that a non-linear association was observed between VAT and mortality, where high to extremely high levels of VAT were associated with increased mortality risk relative to having lower levels of VAT (18). It is possible that the association of VAT and mortality was not observed in our study because people with extreme VAT levels were not present in our study, most likely because the number of people with an extremely high BMI is still low in The Netherlands. The study of Brown *et al* (18) was performed in the US. In the Netherlands 15% of the adults is obese (19), while in the US more than 40% is obese and 9% of the adults is severely obese (20).

Our results and the results from the studies mentioned above, suggest that only extremely high levels of VAT may be associated with increased mortality risk among stage I-III CRC patients.

Currently, only one study (n=92) (9) has investigated the association between VAT and fatigue among stage I-III CRC patients. In this study high VAT at diagnosis was not associated with higher levels of fatigue 2-10 years after diagnosis (per SD increase, β = -0.5; 95% CI −7.2 to 8.2). Lack of studies makes it hard to draw a conclusion. However, our results also suggest that high VAT is not associated with fatigue at time of diagnosis and beyond.

Subcutaneous adipose tissue

In **Chapter 3** SAT was associated with mortality in a non-linear way for men and for women, with lower SAT levels being associated with higher mortality. In **Chapter 4** higher levels of SAT at time of diagnosis were observed to be associated with higher fatigue prevalence at time of diagnosis.

Three other papers reported on the association of SAT with mortality (10, 12, 18) and only one study observed an association between SAT and mortality, with lower levels of SAT being associated with increased mortality risk. Two studies were conducted with the same study population. No association between high SAT and mortality was observed in the

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study of Caan *et al* (n=3262, high vs normal + low SAT: HR 0.94; 95 CI 0.77 - 1.15) (10). However, when Brown *et al* investigated the association within the same study population using restricted cubic splines instead of cut-off points, lower levels of SAT were observed to be significantly associated with higher mortality risk. No association between high SAT and mortality was also observed in the study of Black *et al* (n=339, high vs medium + low SAT: HR 0.85, 95% CI 0.66–1.08) (12). The low number of studies investigating the association between SAT and mortality makes it hard to draw conclusions. Yet, both our study (**Chapter 3**) and the study of Brown *et al* (18) showed that low levels of SAT were associated with a higher mortality risk compared to medium SAT levels. An explanation why patients with low SAT, but not low VAT, have a higher mortality risk is that low SAT may be a sign of minimal metabolic reserves (13).

No other study investigated the association between SAT and fatigue among stage I-III CRC patients. Lack of studies makes it hard to draw a conclusion. However, our results suggest that high SAT is associated with fatigue at time of diagnosis, but possibly not with fatigue during treatment and beyond.

The findings for SAT discussed above suggest that there might be an optimal range for SAT as too low SAT is associated with increased mortality and too high SAT is associated with more fatigue. More research is needed to determine the optimal range for SAT for men and women.

Adherence to the WCRF/AICR lifestyle recommendations

In **Chapter 5** a higher WCRF/AICR adherence score was associated with less fatigue six months after diagnosis among 1,417 stage I-III CRC patients. An association via inflammation was observed, explaining 45% of the total association between lifestyle and fatigue. This indicates that inflammation is probably one of the underlying mechanisms linking lifestyle to fatigue.

Two other studies (n=1096 and 155) have investigated the association between adherence to the WCRF/AICR recommendations and fatigue among CRC patients (21, 22). In both studies an association between better adherence to the WCRF/AICR recommendations and less fatigue was observed. The results from these studies and our study suggest that better adherence to the WCRF/AICR recommendations is indeed associated with lower fatigue.

Currently, it is unknown if increasing adherence to the WCRF/AICR recommendations leads to less fatigue. However, the WCRF/AICR recommendations have been developed to lower cancer risk and not specifically to lower fatigue risk. Currently, it is unclear if the WCRF/AICR recommendations are effective lifestyle recommendations to prevent or alleviate fatigue complaints. Of all of the seven WCRF/AICR recommendations studied in our and the previously mentioned studies, only being more physically active is known to lower fatigue in cancer patients (23). Therefore, one could argue whether this recommendation should contribute equally to the total WCRF/AICR score when investigating the association

with fatigue. Another recommendation is "be a healthy weight". In the study of **Chapter 4** only SMR was associated with fatigue post-diagnosis. Therefore, the "be a healthy weight" recommendation might not be specific enough, since low SMR is seen in overweight, obese and people with a healthy weight. Five of the recommendations are about nutrition. Currently, only limited data support a potential role for nutrition in managing fatigue after cancer diagnosis (24). Therefore, it is unclear to what extent a healthy diet can decrease fatigue, and if certain components of a healthy diet are more effective for decreasing fatigue than other.

With the current knowledge it would be better to use adjusted WCRF/AICR lifestyle recommendations when investigating if improving lifestyle can decrease fatigue. In these recommendations, physical activity should contribute equally to diet and instead of focussing on BMI, the focus should be on improving SMR and SMI. Lastly, since it is unknown which components of a diet can have an influence on fatigue, a total diet score should be determined based on the five WCRF/AICR recommendations on diet.

The role of inflammation in a possible association between improved lifestyle and lower fatigue among CRC patients is currently unknown. It has been observed that exercise improved inflammation markers in breast cancer survivors (25), but it is unknown if these improvements led to less fatigue. No changes in inflammation markers were seen among breast cancer survivors after a 12-month commercially available web-based lifestyle program (26). However, healthy lifestyle changes were associated with a decrease in systemic inflammation in 1,794 patients with stable cardiovascular disease (27). Although this study was performed in a non-cancer population, it suggests that improving lifestyle can improve inflammation markers. Therefore, it may be possible that improving lifestyle has a similar effect on inflammation markers among CRC patients, which may have an effect on fatigue.

Methodological considerations

The strengths and limitations of the various studies described in this thesis have already been discussed in each chapter. In this section additional methodological considerations will be discussed in more detail, i.e.:

1. The possibilities and challenges of categorizing study populations based on body composition
2. Excluding stage IV CRC patients from the analyses
3. Overall limitations of our studies
4. Generalizability of our findings

The possibilities and challenges of categorizing study populations based on body composition

Currently, there is no consensus on cut-off points to determine unfavorable body composition related to mortality, fatigue or other cancer-related health outcomes using CT. Therefore, different studies used different categorizations for body composition measures: 1) median splits or tertiles to compare groups; 2) cut-off points determined within their own study population using the optimal stratification technique (28); or 3) existing cut-off points from literature. These different cut-off points makes it difficult to compare results between studies. In the study described in **Chapter 2**, we decided to determine cut-off points for low SMR. The main reason for defining our own cut-off points was that the published cut-off points were determined in study populations that were not comparable to our population, because they were determined either in a mixed group of cancer patients of various diagnosis, mostly with very poor prognosis (8), or in a US cancer population. Possible differences in body composition and prevalence of (extreme) obesity between European and US cancer patients might make the US cut-off points not representative for a European cohort (29, 30).

Table 2 shows the various cut-off points for SMR to categorize study populations that were published, showing large differences across studies. The use of the published cut-off values would most likely have influenced our findings, because a different number of people would have been identified with low SMR. To check this, I repeated the adjusted mortality analyses within the study population described in **Chapter 2** (n=1,681) using not only our own cut-off values, but also those published by Martin *et al* (8) and Kroenke *et al* (3). The analyses were adjusted for the same confounders that were used in the study of **Chapter 2.** By using our cut-off values only 38% of patients were classified as having low SMR while for Martin this was 57% and for Kroenke 49%. The use of the other cut-off points resulted in substantially different HRs, as can be seen in **Table 3**. The highest HR is observed when using our own cut-off values and the HR observed when using cut-off values from the literature are much lower. Even though the association between low SMR and mortality remains statistically significant when the cut-off values of Martin *et al* or Kroenke *et al* are used within our study population, this might not be the case when they are used in other (smaller) study populations. This does not mean, however, that every study should determine their own cut-off values. What might be a better alternative, is to use restricted cubic splines instead of cut-points. With restricted cubic splines it is possible to investigate the shape of the curve describing the associations between body composition (i.e. SMI, VAT and SAT) and for instance mortality or fatigue. This visually shows how for instance mortality risk changes with decreasing SMI levels, and shows if an association is linear or non-linear. We started using restricted cubic splines instead of cut-off values in the studies of **Chapter 3-4.** Simultaneously to our study in **Chapter 3**, other studies started using restricted cubic splines as well to explore the association between body composition and

mortality (3, 10, 18). These studies found similar relations, i.e. an association of low SMR, low SMI and low SAT with increased mortality risk.

What can be observed from our results and from the other studies using restricted cubic splines (3, 10, 18), and what is missed in studies using cut-off values, is that for neither SMI, nor SMR nor VAT nor SAT there is a point where mortality risk suddenly rises. This suggests that it is not possible to define a specific point where mortality risk starts to increase, which means that is not possible to categorize a study population into low or high risk groups based on their body composition.

Table 2. Cut-off points for low skeletal muscle radiodensity measured in Hounsfield Units (HU) determined in various populations of cancer patients and in stage I-III colorectal cancer patients

	Van Baar Chapter 2		Martin (8)		Kroenke (3)	
BMI category, kg/m²	Men (HU)	Women (HU)	Men (HU)	Women (HU)	Men (HU)	Women (HU)
<25	36.4	31.1	41	41	35.5	32.5
≥25	31.6	29.3	33	33	35.5	32.5

Abbreviations: HU, Hounsfield Units; BMI, body mass index.

Table 3. Association between skeletal muscle density and overall mortality using various published cut-off points within the study population of **Chapter 2**

	No. of patients (%)	No. of deaths *n* (%)	HR (95% CI)*
SMR (cut-off value van Baar, **Chapter 2**)			
Normal	1033 (62)	153 (15)	REF
Low	648 (38)	261 (40)	1.91 (1.53-2.38)
SMR (cut-off value Martin *et al*) (8)			
Normal	727 (43)	106 (15)	REF
Low	954 (57)	308 (32)	1.38 (1.08-1.75)
SMR (cut-off value Kroenke *et al*) (3)			
Normal	852 (51)	132 (16)	REF
Low	829 (49)	282 (34)	1.40 (1.12-1.75)

HR, hazard ratio; 95% CI, 95% confidence interval; SMR, skeletal muscle radiodensity; REF, reference value, *Adjusted for age, stage of disease, gender

The exclusion of stage IV CRC

For the studies described in **Chapter 2-4** only patients diagnosed with stage I-III CRC were included, excluding all patients diagnosed with stage IV. This was done because in the majority of stage I-III CRC patients, body composition is mostly determined by lifestyle and age, while in many patients with stage IV CRC, low SMI and low SMR will often be caused by metabolic alterations induced by advanced cancer. The association between low SMI and/or SMR might therefore be more the consequence of tumor progression than of the muscle mass/quality loss itself. Furthermore, there is a large difference in survival

rate between stage I-III and stage IV. We argued that analyses on body composition and mortality should therefore not include combined populations of stage I–III and stage IV patients. Various systematic reviews have been published on the association between low SMI (31) and mortality or low SMR and mortality (32, 33) among CRC patients. Studies including stage I-III, stage I-IV and exclusively stage IV CRC patients were included in these systematic reviews. The majority of these studies did find an association between low SMI and increased mortality risk and/or low SMR and increased mortality risk. When looking at the HRs between studies among stage I-III, stage I-IV or exclusively stage IV CRC patients, no clear difference in HRs can be observed between these studies (31-33). Because we already excluded all stage IV CRC patients before collecting the CT images, it was not possible to perform sensitivity analyses, to investigate if our findings are generalizable to a stage I-IV or exclusively stage IV CRC population.

Overall limitations of our studies

First, although we were able to test various variables for possible confounding (e.g. tumor location, treatment, body composition), there is still the possibility of residual confounding by unmeasured variables. A potential unmeasured variable is muscle strength. Muscle strength is related to both SMI (34) and SMR (35) and it might also be associated with fatigue (15) and mortality (36) in cancer patients. If muscle strength is indeed a confounder, the observed associations of low SMR and mortality and fatigue might be overestimated. Currently, it is not common practice to measure muscle strength at time of diagnosis, therefore it was not available in the medical records

Second, in all our studies data from observational studies were used. One limitation of observational studies is that these studies identify associations between independent variables and the outcome interest, but causality cannot be established even when adjusting for potential confounders. Therefore it is unknown if an unfavorable body composition causes higher mortality risk or more fatigue. Randomized controlled studies among cancer patients are needed to investigate the causal relationship between low SMR and fatigue. Ideally, these studies should include participants who suffer from fatigue and have low SMR. The study population should be divided in two groups: one intervention group and one control group. Since it has been observed that SMR can be improved with strength and endurance training (37-39), the intervention group should follow an exercise program aimed at improving SMR and the control group should not follow an exercise program. Both SMR and fatigue should be assessed at baseline and post-intervention to determine changes in SMR and changes in levels of fatigue. If there is a causal relationship, it is expected that fatigue improves in the participants with increased SMR levels. The same applies for the association of higher adherence to the WCRF/AICR recommendations with less fatigue six months post-diagnosis. Instead of one group following an exercise program, the intervention group follows a healthy lifestyle program aimed at improving both physical activity and overall diet.

Third, about one fifth of the patients in our studies had to be excluded because no CT-image was available or the CT-image was of low quality. As was mentioned in **Chapter 2**, the mean age, percentage of women, stage of disease and percentage of colon tumors were comparable between the included and excluded patient group. Therefore, we do not expect that excluding these patients affected our results.

Fourth, body composition was only measured at time of diagnosis. It has been observed that body composition, including SMR, can change post-diagnosis (40, 41). Possible causes of this are cancer progression, cancer therapy, physical inactivity, nutritional deficiency and deteriorating health.

Fifth, because the CT images used in our studies were retrieved from the medical records and not assessed for the study, the CT scans were not performed using a standardized protocol. Because of this, different CT scanners were used and the use of contrast fluid and radiation dose differed between scans. Both contrast fluid (42-44) and radiation dose (45) have been observed to influence the measurement of SMR and SMI. For the majority of the our patients it was unknown whether contrast fluid was used, and what the exact radiation dose was. However, based on the data we do have, it appears that there was no specific group that received no contrast fluid and/or a different radiation dose. Therefore, it is expected that the differences in contrast fluid and differences in radiation dose were equally distributed in our study population, reducing the chance of differential misclassification.

Generalizability of the findings

All studies described in this thesis consisted of one or more study populations from the Netherlands. The possibilities and challenges of combining the various studies have already been described in each individual chapter. Based on the stage of disease -, tumor site -, and gender distribution, the study populations used in this thesis are comparable with the general stage I-III CRC patients diagnosed in the Netherlands (46).

One topic that still needs to be discussed in more detail though, is the difference in body composition between countries/continents and how this might affect the generalizability of our findings. For instance, in the Netherlands, 15% of the adults is currently obese (19), while in the US more than 40% is obese and 9% of the adults has severe obesity (20). Based on the results from our studies and the results from similar studies, it appears that the results for SMR, SMI and SAT are similar to what was seen in studies from Europe, US and Canada, indicating that our results for SMR, SMI and SAT are generalizable to populations from Europe, US and Canada. Only our results for VAT deviated with the results from the US, where extreme high VAT levels were observed to be associated with higher mortality risk (18). Our results for VAT might therefore not be generalizable to CRC patients with extreme obesity.

It should be noted that our study was performed in a predominately white study population. There are known differences in body composition between ethnicities (47),

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which could indicate that the association of body composition with mortality and fatigue could differ between patients with a different ethnical background. Meta-analyses are needed to investigate possible ethnic differences in the association of body composition with mortality.

Implications for clinical practice

The studies in this thesis show that unfavorable body composition is associated with both increased mortality and increased prevalence of fatigue in stage I-III CRC patients. In other studies low SMR has been observed to be associated with higher odds of having major complications after surgery (5) and prolonged hospital stay (5) among stage I-III CRC patients. Associations of low SMI with longer hospital stay, higher incidence of total postoperative morbidity and infection (31) have also been observed among stage I-III CRC patients. To identify patients who have an increased mortality risk, risk of developing fatigue and higher risk of having post-surgery complications, it is valuable to quantify and monitor body composition in clinical practice. These patients might need to receive prehabilitation prior to surgery or receive more personalized cancer treatment. Unfortunately, for clinical practice the currently used method of finding the CT image at the level of the third lumbar vertebrae and quantifying body composition by hand is very time-consuming. Automated CT segmentation might solve this problem. With automated segmentation the CT images at the level of the third lumbar vertebrae is identified by the computer software and quantifying SMI, SMR, VAT and SAT is also done automatically by the software (48). The accuracy of automated CT segmentation is improving and hopefully quantifying body composition by hand, as was done for this study, will no longer be needed in the near future.

The study from **Chapter 5** showed that an unhealthy lifestyle is associated with more fatigue during and/or shortly after treatment. Being more physically active is already known to lower fatigue in cancer patients (23) and exercise programs, if possible, should be used as therapy against fatigue. Not much is known about the effect of increasing adherence to the WCRF/AICR lifestyle recommendations or other lifestyle recommendations on fatigue among cancer patients. However, it has been shown that healthy lifestyle changes can have a positive effect on QOL in CRC patients (49). Furthermore, cardiovascular disease is a major cause of death after CRC diagnosis (50). Healthy lifestyle changes can play an important role in lowering the risk of death from cardiovascular disease. Therefore, it is valuable to already implement programs to improve lifestyle in clinical practice.

Future research

An important question that needs to be resolved is: what can we do with the knowledge that low SMR and low SMI are associated with increased mortality risk and that especially low SMR is associated with more fatigue? Future studies should investigate what the effect is of improving SMR and SMI in stage I-III CRC patients. These studies should focus on the effect of improving SMR and SMI pre-treatment (e.g. with prehabilitation) and the effect of improving SMR and SMI post-treatment (e.g. with healthy lifestyle changes).

Currently, there is an increasing interest in the effect of prehabilitation on surgical outcomes (51). With prehabilitation, patients follow a short exercise program combined with nutritional and psychological support (52) to improve physical fitness in patients prior to surgery. Changes in SMR and SMI should be measured in future studies. Because follow-up CT scans are needed to assess SMR, it might be difficult to implement this in these studies. An alternative that could be used is echo intensity measured by ultrasonography, a promising, affordable and non-invasive alternative for SMR (53, 54). Compared to SMR assessed by CT, it is more difficult to obtain consistent echo intensity measurements using ultrasonography because consistency relies on probe placement, pressure, and angle of incidence (55). However, with trained personnel it is possible to take reliable measurements.

Besides investigating the effect of pre-treatment changes in SMR and SMI, the effect of improving SMR and SMI post-treatment on fatigue and other QOL components should be investigated. Studies have shown that SMR and SMI can be improved by exercising (37-39, 56). Unfortunately, the exercise programs used in these intervention studies are often hard to replicate in a non-research setting as people do not have the right equipment to perform the exercises. Furthermore, these programs are often aimed at getting high improvements in a short time period of only a few weeks. In a non-research setting (i.e. real life) you should not only aim at improving SMR and SMI, but also aim at maintaining higher SMR and SMI levels over time. Therefore, the focus should be on improving SMI and SMR by adopting a healthy lifestyle that has a positive influence on SMR and SMI.

In the proposed studies above, the differences in effect between men and women should be taken into account as we observed a different association of low SMI with mortality between men and women. The effect of low SMI appears to be less among women and the impact of improving SMI might therefore also be different.

In this thesis an association between low adherence to the WCRF/AICR lifestyle recommendations and more fatigue was observed. To be able to give lifestyle advice to combat fatigue, more knowledge is needed on the association between diet and fatigue. Furthermore, the role of inflammation in the association between lifestyle and fatigue should be further investigated. Changes in inflammation markers should be measured in intervention studies investigating if fatigue can be improved with healthy lifestyle changes. Since body composition is highly related to lifestyle and also related to

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inflammation markers (57), changes in the SMR, SMI, VAT and SAT should be taken into account in these studies

Overall conclusion

In conclusion, results presented in this thesis show that low SMR, low SAT and among men, low SMI are associated with higher mortality risk among stage I-III CRC patients. For both men and women with low VAT and among women with low SMI, no statistically significant association with mortality risk was observed. High levels of SAT were associated with more fatigue at diagnosis and low levels of SMR were associated with more fatigue at six months post-diagnosis. Furthermore, a higher WCRF/AICR adherence score was associated with less fatigue six months after diagnosis among stage I-III CRC patients. This association was observed to be partly mediated by inflammation. The next step will be investigating the effect of improving pre-treatment body composition in a controlled setting (i.e. prehabilitation) on fatigue, QOL and surgical outcomes; and investigating the effect of improving body composition post-treatment by healthy lifestyle changes on fatigue and other QOL components.

References

1. van Vugt J, van den Braak RC, Lalmahomed Z, Vrijland W, Dekker J, Zimmerman D, et al. Impact of Low Skeletal Muscle Mass and Density on Short and Long-Term Outcome after Resection of Stage I-III Colorectal Cancer. European Journal of Surgical Oncology. 2018;44(9):177.

2. Dolan RD, Almasaudi AS, Dieu LB, Horgan PG, McSorley ST, McMillan DC. The relationship between computed tomography-derived body composition, systemic inflammatory response, and survival in patients undergoing surgery for colorectal cancer. Journal of Cachexia, Sarcopenia and Muscle. 2019;10(1):111-22.

3. Kroenke CH, Prado CM, Meyerhardt JA, Weltzien EK, Xiao J, Cespedes Feliciano EM, et al. Muscle radiodensity and mortality in patients with colorectal cancer. Cancer. 2018.

4. Hopkins JJ, Reif RL, Bigam DL, Baracos VE, Eurich DT, Sawyer MB. The Impact of Muscle and Adipose Tissue on Long-term Survival in Patients With Stage I to III Colorectal Cancer. Diseases of the Colon & Rectum. 2019;62(5):549-60.

5. Xiao J, Caan BJ, Feliciano EMC, Meyerhardt JA, Peng PD, Baracos VE, et al. Association of Low Muscle Mass and Low Muscle Radiodensity With Morbidity and Mortality for Colon Cancer Surgery. JAMA surgery. 2020.

6. Sueda T, Takahasi H, Nishimura J, Hata T, Matsuda C, Mizushima T, et al. Impact of Low Muscularity and Myosteatosis on Long-term Outcome After Curative Colorectal Cancer Surgery: A Propensity Score–Matched Analysis. Diseases of the Colon & Rectum. 2018;61(3):364-74.

7. McSorley ST, Black DH, Horgan PG, McMillan DC. The relationship between tumour stage, systemic inflammation, body composition and survival in patients with colorectal cancer. Clinical nutrition. 2018;37(4):1279-85.

8. Martin L, Birdsell L, MacDonald N, Reiman T, Clandinin MT, McCargar LJ, et al. Cancer cachexia in the age of obesity: skeletal muscle depletion is a powerful prognostic factor, independent of body mass index. Journal of Clinical Oncology. 2013:JCO. 2012.45. 722.

9. van Roekel EH, Bours MJL, te Molder MEM, Breedveld-Peters JJL, Olde Damink SWM, Schouten LJ, et al. Associations of adipose and muscle tissue parameters at colorectal cancer diagnosis with long-term health-related quality of life. Quality of Life Research. 2017;26(7):1745-59.

10. Caan BJ, Meyerhardt JA, Kroenke CH, Alexeeff S, Xiao J, Weltzien E, et al. Explaining the Obesity Paradox: The Association between Body Composition and Colorectal Cancer Survival (C-SCANS Study). Cancer Epidemiology and Prevention Biomarkers. 2017;26(7):1008-15.

11. Miyamoto Y, Baba Y, Sakamoto Y, Ohuchi M, Tokunaga R, Kurashige J, et al. Sarcopenia is a negative prognostic factor after curative resection of colorectal cancer. Annals of surgical oncology. 2015;22(8):2663-8.

12. Black D, Mackay C, Ramsay G, Hamoodi Z, Nanthakumaran S, Park KG, et al. Prognostic Value of Computed Tomography: Measured Parameters of Body Composition in Primary Operable Gastrointestinal Cancers. Annals of Surgical Oncology. 2017:1-11.

6

13. Lavie CJ, McAuley PA, Church TS, Milani RV, Blair SN. Obesity and cardiovascular diseases: implications regarding fitness, fatness, and severity in the obesity paradox. Journal of the American College of Cardiology. 2014;63(14):1345-54.

14. Wang B, Thapa S, Zhou T, Liu H, Li L, Peng G, et al. Cancer-related fatigue and biochemical parameters among cancer patients with different stages of sarcopenia. Supportive Care in Cancer. 2020;28(2):581-8.

15. Kilgour RD, Vigano A, Trutschnigg B, Hornby L, Lucar E, Bacon SL, et al. Cancer-related fatigue: the impact of skeletal muscle mass and strength in patients with advanced cancer. Journal of Cachexia, Sarcopenia and Muscle. 2010;1(2):177-85.

16. Yang S, Chu S, Gao Y, Ai Q, Liu Y, Li X, et al. A narrative review of Cancer-Related Fatigue (CRF) and its possible pathogenesis. Cells. 2019;8(7):738.

17. Fabi A, Bhargava R, Fatigoni S, Guglielmo M, Horneber M, Roila F, et al. Cancer-related fatigue: ESMO Clinical Practice Guidelines for diagnosis and treatment. Annals of Oncology. 2020;31(6):713-23.

18. Brown JC, Caan BJ, Prado CM, Cespedes Feliciano EM, Xiao J, Kroenke CH, et al. The Association of Abdominal Adiposity With Mortality in Patients With Stage I–III Colorectal Cancer. JNCI: Journal of the National Cancer Institute. 2019.

19. National Institute of Public Health and the Environment. Obesity-current situation in the Netherlands 2020 [Available from: https://www.volksgezondheidenzorg.info/onderwerp/overgewicht/cijfers-context/huidige-situatie#definities.

20. Hales CM, Carroll MD, Fryar CD, Ogden CL. Prevalence of obesity and severe obesity among adults: United States, 2017–2018. 2020.

21. Breedveld-Peters JJ, Koole JL, Müller-Schulte E, van der Linden BW, Windhausen C, Bours MJ, et al. Colorectal cancers survivors' adherence to lifestyle recommendations and cross-sectional associations with health-related quality of life. British Journal of Nutrition. 2018:1-10.

22. van Veen MR, Mols F, Bours MJ, Weijenberg MP, Kampman E, Beijer S. Adherence to the World Cancer Research Fund/American Institute for Cancer Research recommendations for cancer prevention is associated with better health–related quality of life among long-term colorectal cancer survivors: results of the PROFILES registry. Supportive Care in Cancer. 2019;27(12):4565-74.

23. Kessels E, Husson O, Van der Feltz-Cornelis CM. The effect of exercise on cancer-related fatigue in cancer survivors: a systematic review and meta-analysis. Neuropsychiatric disease and treatment. 2018;14:479.

24. Baguley BJ, Skinner TL, Wright OR. Nutrition therapy for the management of cancer-related fatigue and quality of life: a systematic review and meta-analysis. British Journal of Nutrition. 2019;122(5):527-41.

25. Meneses-Echávez JF, Correa-Bautista JE, González-Jiménez E, Río-Valle JS, Elkins MR, Lobelo F, et al. The effect of exercise training on mediators of inflammation in breast cancer survivors: a systematic review with meta-analysis. Cancer Epidemiology and Prevention Biomarkers. 2016;25(7):1009-17.

26. Sturgeon KM, Foo W, Heroux M, Schmitz K. Change in inflammatory biomarkers and adipose tissue in BRCA1/2+ breast cancer survivors following a yearlong lifestyle modification program. Cancer prevention research. 2018;11(9):545-50.

27. van't Klooster C, van der Graaf Y, Ridker P, Westerink J, Hjortnaes J, Sluijs I, et al. The relation between healthy lifestyle changes and decrease in systemic inflammation in patients with stable cardiovascular disease. Atherosclerosis. 2020;301:37-43.

28. Williams BA. Finding optimal cutpoints for continuous covariates with binary and time-to-event outcomes. 2006.

29. Daly LE, Ryan AM, Power DG. Response to "Loss of Muscle Mass During Chemotherapy Is Predictive for Poor Survival of Patients With Metastatic Colorectal Cancer". Journal of Clinical Oncology. 2016;34(31):3816-7.

30. Blauwhoff-Buskermolen S, Schueren MAEdvd, Langius JAE, Verheul HMW. Reply to L.E. Daly et al. Journal of Clinical Oncology. 2016;34(31):3817.

31. Sun G, Li Y, Peng Y, Lu D, Zhang F, Cui X, et al. Can sarcopenia be a predictor of prognosis for patients with non-metastatic colorectal cancer? A systematic review and meta-analysis. International journal of colorectal disease. 2018;33(10):1419-27.

32. Lee CM, Kang J. Prognostic impact of myosteatosis in patients with colorectal cancer: a systematic review and meta-analysis. Journal of Cachexia, Sarcopenia and Muscle. 2020.

33. Aleixo G, Shachar S, Nyrop K, Muss H, Malpica L, Williams G. Myosteatosis and prognosis in cancer: Systematic review and meta-analysis. Critical Reviews in Oncology/Hematology. 2020;145:102839.

34. Moreau J, Ordan M-A, Barbe C, Mazza C, Perrier M, Botsen D, et al. Correlation between muscle mass and handgrip strength in digestive cancer patients undergoing chemotherapy. Cancer medicine. 2019;8(8):3677-84.

35. Goodpaster BH, Carlson CL, Visser M, Kelley DE, Scherzinger A, Harris TB, et al. Attenuation of skeletal muscle and strength in the elderly: The Health ABC Study. Journal of Applied Physiology. 2001;90(6):2157-65.

36. Volaklis KA, Halle M, Meisinger C. Muscular strength as a strong predictor of mortality: a narrative review. European journal of Internal medicine. 2015;26(5):303-10.

37. Taaffe DR, Henwood TR, Nalls MA, Walker DG, Lang TF, Harris TB. Alterations in muscle attenuation following detraining and retrainingin resistance trained older adults. Gerontology. 2009;55(2):217.

38. Poehlman ET, Dvorak RV, DeNino WF, Brochu M, Ades PA. Effects of Resistance Training and Endurance Training on Insulin Sensitivity in Nonobese, Young Women: A Controlled Randomized Trial 1. The Journal of Clinical Endocrinology & Metabolism. 2000;85(7):2463-8.

39. Lee S, Kuk JL, Davidson LE, Hudson R, Kilpatrick K, Graham TE, et al. Exercise without weight loss is an effective strategy for obesity reduction in obese individuals with and without Type 2 diabetes. Journal of Applied Physiology. 2005;99(3):1220-5.

6

40. Brown JC, Caan BJ, Meyerhardt JA, Weltzien E, Xiao J, Cespedes Feliciano EM, et al. The deterioration of muscle mass and radiodensity is prognostic of poor survival in stage I–III colorectal cancer: a population-based cohort study (C-SCANS). Journal of cachexia, sarcopenia and muscle. 2018;9(4):664-72.

41. Hopkins JJ, Reif R, Bigam D, Baracos VE, Eurich DT, Sawyer MM. Change in Skeletal Muscle Following Resection of Stage I–III Colorectal Cancer is Predictive of Poor Survival: A Cohort Study. World Journal of Surgery. 2019:1-9.

42. Morsbach F, Zhang Y-H, Martin L, Lindqvist C, Brismar T. Body composition evaluation with computed tomography: Contrast media and slice thickness cause methodological errors. Nutrition. 2019;59:50-5.

43. van Vugt JL, van den Braak RRC, Schippers HJ, Veen KM, Levolger S, de Bruin RW, et al. Contrast-enhancement influences skeletal muscle density, but not skeletal muscle mass, measurements on computed tomography. Clinical Nutrition. 2018;37(5):1707-14.

44. Boutin RD, Kaptuch JM, Bateni CP, Chalfant JS, Yao L. Influence of IV contrast administration on CT measures of muscle and bone attenuation: implications for sarcopenia and osteoporosis evaluation. American Journal of Roentgenology. 2016;207(5):1046-54.

45. Fuchs G, Chretien YR, Mario J, Do S, Eikermann M, Liu B, et al. Quantifying the effect of slice thickness, intravenous contrast and tube current on muscle segmentation: Implications for body composition analysis. European radiology. 2018;28(6):2455-63.

46. Brouwer NP, Bos AC, Lemmens VE, Tanis PJ, Hugen N, Nagtegaal ID, et al. An overview of 25 years of incidence, treatment and outcome of colorectal cancer patients. International journal of cancer. 2018;143(11):2758-66.

47. Deurenberg P, Deurenberg-Yap M. Differences in body-composition assumptions across ethnic groups: practical consequences. Current Opinion in Clinical Nutrition & Metabolic Care. 2001;4(5):377-83.

48. Popuri K, Cobzas D, Esfandiari N, Baracos V, Jägersand M. Body composition assessment in axial CT images using FEM-based automatic segmentation of skeletal muscle. IEEE transactions on medical imaging. 2015;35(2):512-20.

49. Gigic B, Boeing H, Toth R, Bohm J, Habermann N, Scherer D, et al. Associations Between Dietary Patterns and Longitudinal Quality of Life Changes in Colorectal Cancer Patients: The ColoCare Study. Nutrition and Cancer-an International Journal. 2018;70(1):51-60.

50. Feng Y, Jin H, Guo K, Wasan HS, Ruan S, Chen C. Causes of Death After Colorectal Cancer Diagnosis: A Population-Based Study. Front Oncol. 2021;11:647179.

51. Treanor C, Kyaw T, Donnelly M. An international review and meta-analysis of prehabilitation compared to usual care for cancer patients. Journal of Cancer Survivorship. 2018;12(1):64-73.

52. Durrand J, Singh SJ, Danjoux G. Prehabilitation. Clinical Medicine. 2019;19(6):458-64.

53. Correa-de-Araujo R, Harris-Love MO, Miljkovic I, Fragala MS, Anthony BW, Manini TM. The Need for Standardized Assessment of Muscle Quality in Skeletal Muscle Function Deficit and Other Aging-Related Muscle Dysfunctions: A Symposium Report. Frontiers in Physiology. 2017;8(87).

54. Price KL, Earthman CP. Update on body composition tools in clinical settings: Computed tomography, ultrasound, and bioimpedance applications for assessment and monitoring. Eur J Clin Nutr. 2018.

55. Correa-de-Araujo R, Addison O, Miljkovic I, Goodpaster BH, Bergman BC, Clark RV, et al. Myosteatosis in the Context of Skeletal Muscle Function Deficit: An Interdisciplinary Workshop at the National Institute on Aging. Frontiers in Physiology. 2020;11:963.

56. Beckwée D, Delaere A, Aelbrecht S, Baert V, Beaudart C, Bruyère O, et al. Exercise interventions for the prevention and treatment of sarcopenia. A systematic umbrella review. The journal of nutrition, health & aging. 2019;23(6):494-502.

57. Abbass T, Dolan RD, Laird BJ, McMillan DC. The relationship between imaging-based body composition analysis and the systemic inflammatory response in patients with cancer: a systematic review. Cancers. 2019;11(9):1304.

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Summary

Colorectal cancer (CRC) is the third most common cancer worldwide. Mainly due to the implementation of screening practices and improved treatment, survival rates for stage I-III CRC patients have increased for several decades. Identifying modifiable factors associated with better prognosis could contribute to further improvement of cancer outcomes such as better survival, good quality of life (QoL) and less recurrence in patients with stage I-III CRC. One potential modifiable factor might be body composition.

Over the last decade, the relationship between skeletal muscle mass index (SMI, skeletal muscle cross-sectional surface area divided by height squared in meters), skeletal muscle radiodensity (SMR, which reflects the lipid content of the muscle cells), visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT) at time of diagnosis, and outcomes including mortality, complications after surgery, length of hospital stay, QoL and fatigue has received much attention. At the start of this PhD project, no studies had reported on these associations in exclusively stage I-III CRC patients.

Furthermore, an association between higher adherence to the World Cancer Research Fund / American Institute for Cancer Research (WCRF/AICR) lifestyle recommendations and less fatigue had been observed among stage I-III CRC patients who had been diagnosed with CRC more than 5 years ago. If better adherence to the recommendations is also associated with less fatigue experienced during or shortly after treatment, was still unknown. Additionally, the potential mediating role of inflammation in this association needed to be confirmed.

The overall objectives of this thesis were 1) to increase knowledge about the association of body composition in stage I-III CRC patients with both mortality and fatigue by using CT images to quantify skeletal muscle mass, skeletal muscle radiodensity and adipose tissue, and 2) to increase knowledge about the association of adherence to the WCRF/AICR lifestyle recommendations with fatigue among stage I-III CRC and to elucidate the underlying mechanism.

Body composition and mortality

In **Chapter 2** the association between low SMR and mortality was investigated among 1,681
stage I-III CRC patients. The average age of the study population was 67.7 ± 10.3 years, 41% were women and 414 patients (25%) died. Thirty-nine percent of the patients had low SMR. Low SMR was significantly associated with higher overall mortality (low vs. normal: adjusted HR 1.91, 95% CI 1.53–2.38).

The association of SMI, VAT and SAT with mortality among 2,016 men and among women with stage I-III CRC was investigated in **Chapter 3,** using restricted cubic splines analyses. The average age of the patients was 67.9 ± 10.6 years, 42% were women and 558 (28%) patients died. Among men, the association of SMI and mortality was statistically significant in a non-linear way, with lower SMI levels associated with higher mortality, but there was no association among women. SAT was non-linearly associated with mortality for both men and women, with lower SAT levels being associated with higher mortality. VAT was not significantly associated with mortality.

Body composition and fatigue

In **Chapter 4** we studied how SMR, SMI, VAT and SAT at diagnosis are associated with fatigue at diagnosis, at 6 and 24 months post-diagnosis in 646 stage I-III CRC patients. The association of SMI, SMR, VAT, and SAT with fatigue (yes/no) was assessed using confounder-adjusted restricted cubic spline analyses. At diagnosis, a significant non-linear association of higher levels of SAT with higher prevalence of fatigue was observed. Lower SMR was linearly associated with higher prevalence of fatigue at 6 months post-diagnosis. None of the body composition parameters were significantly associated with fatigue at 24 months.

Adherence to the WCRF/AICR lifestyle recommendations and fatigue

We examined the association between adherence to the WCRF/AICR lifestyle recommendations and fatigue among 1,417 stage I-III CRC patients in **Chapter 5**. Within a subpopulation we investigated whether inflammation mediated this association. Adherence to the WCRF/AICR recommendations was assessed shortly after diagnosis. Six months post-diagnosis fatigue and plasma levels of inflammation markers (IL6, IL8, TNFα, and hsCRP) in non-fasting blood samples were assessed.

A higher WCRF/AICR adherence score at diagnosis was associated with less fatigue six months after diagnosis. A statistically significant indirect association via inflammation was explaining 45% of the total association between lifestyle and fatigue. Thus, inflammation is probably one of the underlying mechanisms linking lifestyle to fatigue.

General conclusion and recommendations

The results presented in this thesis show that low SMR, low SAT and, among men, low SMI are associated with higher mortality risk among stage I-III CRC patients. SMI in women and

VAT in men and women were not statistically significantly associated with mortality risk. Low SMR was associated with more fatigue at six months post-diagnosis and high levels of SAT were associated with more fatigue at diagnosis. Furthermore, a higher WCRF/AICR adherence score at diagnosis was associated with less fatigue six months after diagnosis among stage I-III CRC patients. This association was observed to be partly mediated by inflammation. The next step will be investigating the effect of improving pre-treatment body composition in a controlled setting (i.e. prehabilitation) on fatigue, QoL and surgical outcomes; and investigating the effect of improving body composition by healthy lifestyle changes on fatigue and other QoL components after treatment.

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About the author

Curriculum Vitae

Harm van Baar was born on the 18th of March 1981 in Alkmaar. In 1998, he completed secondary school at the Jac. P. Thijsse College in Castricum. After working for several years and studying Nutrition and Dietetics for one year in Amsterdam, Harm moved to Wageningen to study Nutrition and Health at Wageningen University in 2008. After obtaining his BSc degree, he continued with the MSc Nutrition and Health where he specialized in Nutritional Physiology and Health Status. During his MSc Harm worked full-time as a laboratory analyst in Ede. For his MSc thesis he developed a new prediction equation for the assessment of appendicular skeletal muscle mass in (pre-) frail elderly people aged 65 and older. The results of this thesis were published in Clinical Nutrition Espen. After obtaining his master in 2014, he started his PhD at Wageningen University studying the association between body composition and colorectal prognosis in stage I-III colorectal cancer patients. The results of his PhD project are found in this thesis. In 2019 he co-wrote a proposal for a multi-year grant from the World Cancer Research Fund International and its Dutch foundation Wereld Kanker Onderzoek Fonds that was funded. In 2020 he started working as a manager Nutrition and Health at Kenniscentrum suiker & voeding.

List of publications

van Baar H, Beijer S, Bours M, Weijenberg M, van Zutphen M, van Duijnhoven F, et al. Low radiographic muscle density is associated with lower overall and disease-free survival in early-stage colorectal cancer patients. Journal of cancer research and clinical oncology. 2018:1-9.

van Baar H, Winkels RM, Brouwer JG, Posthuma L, Bours MJ, Weijenberg MP, et al. Associations of Abdominal Skeletal Muscle Mass, Fat Mass, and Mortality among Men and Women with Stage I–III Colorectal Cancer. Cancer Epidemiology and Prevention Biomarkers. 2020;29(5):956-65.

van Baar H, Bours M, Beijer S, van Zutphen M, van Duijnhoven F, Kok D, et al. Body composition and its association with fatigue in the first 2 years after colorectal cancer diagnosis. Journal of Cancer Survivorship. 2020:1-10.

Wesselink E[*], **van Baar H**[*], van Zutphen M, Tibosch M, Kouwenhoven EA, Keulen ET, et al. Inflammation Is a Mediating Factor in the Association between Lifestyle and Fatigue in Colorectal Cancer Patients. Cancers. 2020;12(12):3701. ([*]=Contributed equally)

Plas RLC, van Norren K, **van Baar H**, van Aller C, de Bakker M, Botros N, et al. Side-effects related to adjuvant CAPOX treatment for colorectal cancer are associated with intermuscular fat area, not with total skeletal muscle or fat, a retrospective observational study. JCSM Clinical Reports. 2018;3(1).

Wesselink E, Winkels R, **Van Baar H**, Geijsen A, Van Zutphen M, van Halteren H, et al. Dietary intake of magnesium or calcium and chemotherapy-induced peripheral neuropathy in colorectal cancer patients. Nutrients. 2018;10(4):398.

van Zutphen M, Boshuizen HC, Kok DE, **van Baar H**, Geijsen AJ, Wesselink E, et al. Colorectal cancer survivors only marginally change their overall lifestyle in the first 2 years following diagnosis. Journal of Cancer Survivorship. 2019;13(6):956-67.

Wesselink E, Balvers M, Bours MJL, de Wilt JHW, Witkamp RF, **van Baar H**, et al. The association between circulating levels of vitamin D and inflammatory markers in the first 2 years after colorectal cancer diagnosis. Therapeutic Advances in Gastroenterology. 2020;13:1756284820923922.

Overview of completed training activities

Discipline specific activities	Organiser and location	Year
Courses		
Energy metabolism and body composition in nutrition and health research	VLAG, Wageningen, NL	2016
Conferences and meetings		
Sarcopenia, Cachexia and Muscle Wasting 9th International Conference	SCWD, Berlin, DE	2016
WEON 2018	VvE, Bilthoven, NL	2018
ESPEN	ESPEN, Madrid, SP	2018
11th international SCWD conference on cachexia, sarcopenia and muscle wasting	SCWD, Maastricht, NL	2018
eICDAM 2021 (online)	WUR, Wageningen, NL	2021
General courses and activities		
PhD Workshop Carousel 2015	VLAG, Wageningen, NL	2015
VLAG PhD Week	VLAG, Wageningen, NL	2015
Project & Time Management	WGS, Wageningen, NL	2015
Data Management Planning (DMP)	WGS, Wageningen, NL	2016
Introduction to R for Statistical Analysis	PE&RC, Wageningen, NL	2016
Reviewing a Scientific Paper	WGS, Wageningen, NL	2016
PhD symposium	WPC, Wageningen, NL	2016
Teaching and supervising Thesis students	WGS, Wageningen, NL	2016
Presenting with impact	WGS, Wageningen, NL	2017
Career assessment	WGS, Wageningen, NL	2020
Optional courses and activities		
Staff seminars	WUR, Wageningen, NL	2015-2019
Journal Club	WUR, Wageningen, NL	2015-2019
MENU-D meeting	WUR, Wageningen, NL	2015-2019
Preparing PhD research proposal	WUR, Wageningen, NL	2015
MOOC: introduction-to-biomedical-imaging	UQ, Queensland, AUS	2018
Consortium Meetings	WUR, Wageningen, NL	2018
Body Composition and Cancer webinar series	KP, Oakland, USA	2019-2020

Colophon

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