Received: 4 March 2022 | Revised: 8 July 2022 | Accepted: 25 July 2022

DOI: 10.1002/ijc.34252

CANCER EPIDEMIOLOGY

IJC INTERNATIONAL JOURNAL of CANCER

Longitudinal associations of plasma metabolites with persistent fatigue among colorectal cancer survivors up to 2 years after treatment

Eline H. van Roekel[1] | Martijn J. L. Bours[1] | Stéphanie O. Breukink[2] | Michèl Aquarius[3] | Eric T. P. Keulen[4] | Audrey Gicquiau[5] | Sabina Rinaldi[5] | Paolo Vineis[6,7] | Ilja C. W. Arts[8] | Marc J. Gunter[5] | Michael F. Leitzmann[9] | Augustin Scalbert[5] | Matty P. Weijenberg[1]

[1]Department of Epidemiology, GROW School for Oncology and Developmental Biology, Maastricht University, Maastricht, The Netherlands

[2]Department of Surgery, GROW School for Oncology and Developmental Biology, School of Nutrition and Translational Research in Metabolism, Maastricht University Medical Center+, Maastricht, The Netherlands

[3]Department of Gastroenterology, VieCuri Medical Center, Venlo, The Netherlands

[4]Department of Internal Medicine and Gastroenterology, Zuyderland Medical Centre, Sittard-Geleen, The Netherlands

[5]Nutrition and Metabolism Branch, International Agency for Research on Cancer (IARC-WHO), Lyon, France

[6]MRC Centre for Environment and Health, School of Public Health, Imperial College, London, UK

[7]Italian Institute of Technology, Genoa, Italy

[8]Maastricht Centre for Systems Biology (MaCSBio), Maastricht University, Maastricht, The Netherlands

[9]Department of Epidemiology and Preventive Medicine, University of Regensburg, Regensburg, Germany

Correspondence
Eline H. van Roekel, Department of Epidemiology, Maastricht University, GROW School for Oncology and Developmental Biology, P.O. Box 616, 6200 MD Maastricht, The Netherlands.
Email: eline.vanroekel@maastrichtuniversity.nl

Funding information
Kankeronderzoekfonds Limburg as part of Health Foundation Limburg, Grant/Award Number: 00005739; KWF Kankerbestrijding, Grant/Award Numbers: UM-2010-4867, UM-2012-5653; Wereld Kanker Onderzoek Fonds, Grant/Award Numbers: 2016/1620, 2017/1619

Abstract

The underlying biological mechanisms causing persistent fatigue complaints after colorectal cancer treatment need further investigation. We investigated longitudinal associations of circulating concentrations of 138 metabolites with total fatigue and subdomains of fatigue between 6 weeks and 2 years after colorectal cancer treatment. Among stage I-III colorectal cancer survivors (n = 252), blood samples were obtained at 6 weeks, and 6, 12 and 24 months posttreatment. Total fatigue and fatigue subdomains were measured using a validated questionnaire. Tandem mass spectrometry was applied to measure metabolite concentrations (BIOCRATES Absolute*IDQ*p180 kit). Confounder-adjusted longitudinal associations were analyzed using linear mixed models, with false discovery rate (FDR) correction. We assessed interindividual (between-participant differences) and intraindividual longitudinal associations (within-participant changes over time). In the overall longitudinal analysis, statistically significant associations were observed for 12, 32, 17 and three

Abbreviations: BMI, body mass index; CFS, chronic fatigue syndrome; CIS, Checklist Individual Strength; FDR, false discovery rate; IARC, International Agency for Research on Cancer; MVPA, moderate-to-vigorous physical activity; PCs, phosphatidylcholines; SMs, sphingomyelins; SQUASH, Short QUestionnaire to ASsess Health-enhancing physical activity.

Where authors are identified as personnel of the International Agency for Research on Cancer/World Health Organization, the authors alone are responsible for the views expressed in this article and they do not necessarily represent the decisions, policy or views of the International Agency for Research on Cancer/World Health Organization.

metabolites with total fatigue and the subscales "fatigue severity," "reduced motivation" and "reduced activity," respectively. Specifically, higher concentrations of several amino acids, lysophosphatidylcholines, diacylphosphatidylcholines, acyl-alkylphosphatidylcholines and sphingomyelins were associated with less fatigue, while higher concentrations of acylcarnitines were associated with more fatigue. For "fatigue severity," associations appeared mainly driven by intraindividual associations, while for "reduced motivation" stronger interindividual associations were found. We observed longitudinal associations of several metabolites with total fatigue and fatigue subscales, and that intraindividual changes in metabolites over time were associated with fatigue severity. These findings point toward inflammation and an impaired energy metabolism due to mitochondrial dysfunction as underlying mechanisms. Mechanistic studies are necessary to determine whether these metabolites could be targets for intervention.

KEYWORDS
amino acids, cancer-related fatigue, colorectal cancer, phospholipids, targeted metabolomics

What's new?

The biological mechanisms underlying persistent fatigue after colorectal cancer treatment remain to be better understood. Here, the authors observed longitudinal associations of several plasma metabolites including amino acids, phospholipids, and acylcarnitines with total fatigue and fatigue subdomains among colorectal cancer survivors. Moreover, within-individual changes in these metabolites over time were associated with changes in fatigue severity. The findings suggest that low-grade inflammation and an impaired energy metabolism due to mitochondrial dysfunction may be underlying mechanisms and point to these metabolites as potential targets for interventions to prevent and/or reduce fatigue in colorectal cancer survivors.

1 | INTRODUCTION

Persistent fatigue is one of the most common and distressing long-term symptoms after cancer.[1,2] Among the global population of 4.8 million colorectal cancer survivors,[3] more than one-third still suffer from fatigue ≥1 year after treatment.[4,5] Cancer-related fatigue is a complex symptom which may include mental, physical, emotional and motivational dimensions.[6] The fatigue complaints are more severe than among healthy individuals and often cannot be relieved by adequate sleep or rest.[6] Therefore, these complaints severely affect daily functioning and quality of life.[1,7-9] Although nonpharmacological interventions such as exercise and cognitive behavioral therapy can relieve fatigue complaints after cancer, effects are only small to modest.[10-12] Therefore, it is imperative to develop novel strategies to reduce the distressing burden of long-term fatigue among the increasing population of colorectal cancer survivors.[13]

To develop more effective interventions, there is a need for a better understanding of the underlying biological mechanisms leading to cancer-related fatigue.[9,14] Current evidence suggests that cancer-related fatigue results from disturbances in immune, neuroendocrine, and neural systems, with the role of inflammation having received most empirical attention and support.[15] However, other involved mechanisms including alteration of specific metabolic pathways that may influence fatigue such as cellular energy production in mitochondria, which can be negatively affected by inflammatory processes, need further investigation.[14-16] Metabolomics is a powerful approach to improve understanding of the etiology of health-related conditions, by allowing the simultaneous investigation of how hundreds of metabolites from key metabolic pathways, involving amino acids, phospholipids, sugars and other metabolites are related to these conditions.[17,18]

A recent pilot study where a nontargeted metabolomics analysis was conducted among 16 fatigued and 40 nonfatigued Taiwanese colorectal cancer survivors (men and women) observed significant differences in circulating concentrations of several metabolites between both groups, such as lower levels of carnitine and higher levels of L-norleucine among fatigued survivors.[19] However, these results were not adjusted for potential confounding factors, and this was a cross-sectional analysis in a small number of patients.[19] To our knowledge, no other study has been conducted among colorectal cancer survivors. Associations have been observed between fatigue complaints and circulating metabolite concentrations among early-stage breast cancer[20] and prostate cancer patients,[21] and among patients with other fatigue-related conditions such as chronic fatigue syndrome.[22]

Altogether, current evidence suggests that perturbation of certain metabolic pathways may be involved in the development of fatigue after cancer. There is a need for confounder-adjusted longitudinal studies to investigate how changes in metabolite concentrations are

associated with changes in fatigue in the years after colorectal cancer treatment. We recently published results of a longitudinal analysis of associations of self-reported physical activity in relation to targeted metabolite data among colorectal cancer survivors up to 2 years post-treatment, using data of the Energy for Life after ColoRectal Cancer (EnCoRe) study.[23] In the current analysis, we investigated longitudinal associations of plasma concentrations of 138 targeted metabolites with total fatigue and subdomains of fatigue up to 2 years after colorectal cancer treatment in the same study sample.

2 | METHODS

2.1 | Study design and participants

The EnCoRe study is an ongoing prospective cohort study, which was initiated in 2012 (Netherlands Trial Register no. NL6904). Stage I-III colorectal cancer patients are recruited at diagnosis (response ~45%) in three participating hospitals in the southeastern part of The Netherlands (Maastricht University Medical Center+, VieCuri Medical Center, and Zuyderland Medical Centre).[24] Eligible participants include men and women aged 18 years and older, while individuals with Stage IV colorectal cancer and comorbidities obstructing successful study participation (eg, Alzheimer's disease) are excluded. Repeated blood samples and measurements (including self-reported fatigue) are obtained at diagnosis, and at 6 weeks, 6 months, and 1, 2 and 5 years after the end of treatment. Data was collected by trained research dieticians during home visits.

For the current analysis, we used data collected up until November 1, 2016 as described in a previous publication on self-reported physical activity in relation to these metabolite data.[23] In the current analysis on targeted metabolite data and fatigue, individuals with at least one posttreatment measurement of targeted metabolomics, self-reported fatigue, and covariates were included (n = 252, 69% men). Of these, n = 241 had data at 6 weeks, n = 192 at 6 months, n = 152 at 12 months and n = 67 at 24 months posttreatment (Figure S1). At posttreatment measurements, response rates were > 90% and mortality during follow-up was low (n = 17, 7%). The declining numbers of participants at subsequent time points and the lack of 5 year posttreatment measurements are (predominantly) due to the fact that not all participants included at diagnosis from April 2012 onwards had reached these time points in November 2016.

2.2 | Fatigue

The Checklist Individual Strength (CIS) was used to measure total fatigue and subdomains of fatigue.[25-27] The questionnaire was originally developed and validated in patients with CFS,[25,27] but has also been applied and validated in cancer survivors.[26,28] The CIS provides a score for total fatigue based on all 20 items (range: 20-140), as well as separate scores for subdomains of fatigue including "fatigue severity" (subjective experience of fatigue; 8 items, range: 8-56), "concentration problems" (5 items, range: 5-35), "reduced motivation" (4 items, range: 4-28) and "reduced activity" (3 items, range: 3-21). A higher score indicates more fatigue. According to developed cut-offs for the "fatigue severity" subscale, participants were classified as having severe (score ≥35), elevated (score 27-34) or normal levels of fatigue (score <27).[25,26] A high test-retest reliability has been observed for the total score and all subscales (Spearman's ρ: 0.7-0.9).[28] In addition, validation studies have found that the total fatigue and subdomain scores are able to discriminate between groups with known different levels of fatigue (eg, healthy individuals, CFS patients, fatigued cancer survivors), and modest to high correlations have been observed with other instruments measuring similar constructs including the EORTC QLQ-C30 fatigue subscale among cancer survivors (Spearman's ρ: 0.3-0.8).[28,29]

2.3 | Targeted metabolomics

Blood samples were drawn from fasting participants according to standardized protocols. Samples were collected in 6 mL EDTA plasma tubes, and then centrifuged, aliquoted and stored at −80°C within 4 hours after blood draw.

The Absolute*IDQ* p180 kit (BIOCRATES Life Sciences AG, Austria) was applied for targeted metabolomics analysis at the International Agency for Research on Cancer (IARC), Lyon, France, as previously described.[23] Briefly, the kit measures a panel of up to 188 metabolites, including acylcarnitines, amino acids, biogenic amines, a sum of hexoses, phosphatidylcholines (PCs), including lysoPCs, diacylPCs, and acyl-alkylPCs, and sphingomyelins (SMs). A good to excellent reliability for the majority of metabolites measured using this kit has been observed when comparing fasting samples collected in the same individuals over a period of 4 months[30] and 2 years[31] (intraclass correlation coefficients >0.50 for most metabolites). Samples collected at diagnosis had been previously analyzed according to the same protocol.[32] Metabolites with interbatch or intrabatch coefficients of variation (CVs) >20% for IARC technical replicates were excluded, as well as metabolites with >20% of missing values and/or measurements outside the measurable range (ie, below the limit of detection/quantification or above highest calibration standards).[33] A total of 138 metabolites were included in the current analysis, including 13 acylcarnitines, 21 amino acids, 9 biogenic amines, 80 PCs (11 lysoPCs, 34 diacylPCs, and 35 acyl-alkylPCs), 14 SMs, and a sum of hexoses. Detailed measurement information of all metabolites in the kit have been previously reported.[23] A log-transformation was applied (natural logarithm) to metabolite concentrations (μM) to reduce right skewness of distributions.

2.4 | Covariates

Highest attained education level was self-reported by participants at diagnosis. Several lifestyle-related variables were assessed at each posttreatment time point, including self-reported smoking status (never, former or current), body mass index (BMI; kg/m^2) based on body weight and height measured by trained dieticians, alcohol intake

(g/day) assessed through 7-day dietary records,[34] self-reported hours/week of moderate-to-vigorous physical activity (MVPA) assessed using the Short QUestionnaire to ASsess Health-enhancing physical activity (SQUASH),[35] and hours/day of prolonged sedentary time (ie, time accrued in sedentary bouts with a duration of at least 30 minutes) measured using the validated tri-axial MOX activity monitor that was worn 24 hours/day by participants for 7 consecutive days.[36-38] The number of comorbidities at each posttreatment time point was determined using the 13-item Self-Administered Comorbidity Questionnaire.[39] Clinical characteristics including tumor site (colon/rectum), cancer stage and treatment were retrieved from clinical records.

2.5 | Statistical analysis

Descriptives were obtained for sociodemographic, lifestyle and clinical variables and metabolite data, by categories of fatigue severity. Correlation coefficients between fatigue variables were assessed and visualized in heat maps. The development of fatigue during posttreatment measurements was assessed using linear mixed models with random intercepts for individuals and time since treatment included as independent continuous variable (per 6 months).

To be able to adjust for sex and analytical batch in the main analysis, residuals of each of the ln-transformed metabolite concentrations were computed from linear mixed models with sex as independent variable and random intercepts for individuals nested within analytical batches.[23] The residuals were summed with individual random effects (to retain individual variation in metabolites over time, while excluding batch effects) to be used as dependent variables in further analyses.

In the main analysis, longitudinal associations of circulating concentrations of each of the metabolites (batch and sex-adjusted residuals) with total fatigue and each of the fatigue subdomains, between 6 weeks up to 2 years posttreatment, were analyzed. We applied linear mixed regression with random intercepts for individuals to analyze these longitudinal associations. Obtained regression coefficients of the overall longitudinal relationship are a weighted average of a between-subject component (ie, how differences in metabolite concentrations *between* participants are associated with fatigue variables over time: interindividual associations) and a within-subject component (ie, how changes in metabolite concentrations *within* participants over time are associated with fatigue variables over time: intraindividual associations). We also applied a previously described hybrid modeling method,[40] where the between-subject component was modeled as the mean metabolite concentration for each participant across time points. In addition, the within-subject component was modeled as the difference between the metabolite concentration at each time point and the mean across time points.[40,41]

Models were adjusted for fixed variables including age at 6 weeks posttreatment (continuous), sex, hospital, neo-adjuvant treatment (yes/no), adjuvant treatment (yes/no), and for time-varying variables including time since end of treatment (continuous), BMI (continuous), smoking status (current/former/never), alcohol intake (continuous), hours/week of MVPA and number of comorbidities (0/1/≥2 comorbidities) at posttreatment measurements. Potential confounders were identified a priori based on literature and theoretical considerations. We considered other covariates in sensitivity analyses as described below. To adjust for multiple testing, we used false discovery rate (FDR) adjustment of *P*-values (Benjamini-Hochberg method) and *q*-values <.05 were considered significant.[42]

2.5.1 | Heterogeneity analyses

To explore potential heterogeneity by sex, chemotherapy (yes/no), tumor site, number of comorbidities (≥2 vs <2) and time since end of treatment (continuous, per 6 months), interaction terms were tested with FDR-adjustment.

2.5.2 | Sensitivity analyses

To assess potential confounding by cancer stage (I/II/III) and prolonged sedentary time (continuous), sensitivity analyses were performed including these covariates. Further, we ran models with additional adjustment for metabolite concentrations at diagnosis, using batch-adjusted residuals (calculated similarly as for posttreatment data).

All analyses were conducted using R (version 3.6.2).

3 | RESULTS

3.1 | Participant characteristics

Table 1 shows the characteristics of included study participants (n = 252) at 6 weeks posttreatment, by categories of fatigue severity. Mean age at 6 weeks posttreatment was 66.7 years (SD: 9.2), and about two-third of participants were men (68.7%). Mean BMI was 27.8 kg/m^2 (SD: 4.4), and participants reported a median of 6.4 hours/week of MVPA (interquartile range: 2.5, 13.5) and spent on average 4.8 hours/day in prolonged sedentary time, that is, in bouts with a duration of ≥30 minutes (SD: 2.4). More than half of participants reported at least two comorbidities (53.6%). The majority of participants were colon cancer survivors (60.7%), while 39.3% were rectum cancer survivors. Nearly all participants received surgery (89.7%), 27.4% received neo-adjuvant treatment (19.0% radiotherapy and chemotherapy, 8.3% only radiotherapy) and 28.6% received adjuvant chemotherapy. Participants who reported more severe fatigue levels were more often women, reported a lower alcohol intake and less MVPA, more often had at least two comorbidities, were more often rectum cancer survivors and more frequently received neo-adjuvant treatment. Descriptive statistics of metabolite concentrations at 6 weeks posttreatment, by categories of fatigue severity, are reported in Table S1. Other descriptive information of metabolites

TABLE 1 Sociodemographic, lifestyle, and clinical characteristics of study participants at 6 weeks posttreatment, by self-reported severity of fatigue categories

	Total group of participants (n = 252, 100%)[a]		Severe levels of fatigue (n = 74, 29%)		Elevated levels of fatigue (n = 55, 22%)		Normal levels of fatigue (n = 123, 49%)	
Age, mean (SD)	66.7	(9.2)	67.2	(10.3)	64.8	(9.4)	67.2	(8.2)
Sex, n (%)								
Men	173	(68.7)	45	(60.8)	38	(69.1)	90	(73.2)
Women	79	(31.3)	29	(39.2)	17	(30.9)	33	(26.8)
Highest attained education level, n (%)[b]								
Low	64	(25.4)	23	(31.1)	13	(23.6)	28	(22.8)
Medium	102	(40.5)	25	(33.8)	26	(47.3)	51	(41.5)
High	86	(34.1)	26	(35.1)	16	(29.1)	44	(35.8)
BMI (kg/m^2), mean (SD)	27.8	(4.4)	28.9	(4.7)	26.9	(4.0)	27.6	(4.3)
Smoking status, n (%)								
Current smoker	23	(9.1)	9	(12.2)	4	(7.3)	10	(8.1)
Former smoker	145	(57.5)	40	(54.1)	35	(63.6)	70	(56.9)
Never smoker	84	(33.3)	25	(33.8)	16	(29.1)	43	(35.0)
Alcohol intake in men (g/d), median (25th, 75th perc)	10.9	(0.2, 24.4)	0.5	(0.0, 15.7)	12.8	(0.6, 21.4)	15.5	(2.8, 27.8)
Alcohol intake in women (g/d), median (25th, 75th perc)	1.5	(0.0, 9.7)	0.1	(0.0, 6.5)	0.9	(0.0, 3.1)	4.3	(0.0, 16.9)
MVPA (h/wk), median (25th, 75th perc)	6.4	(2.5, 13.5)	3.5	(1.1, 7.7)	5.3	(2.1, 12.4)	8.5	(4.4, 16.0)
Prolonged sedentary time (h/d), mean (SD)[c]	4.8	(2.4)	5.3	(2.6)	4.5	(2.0)	4.8	(2.4)
Number of comorbid conditions, n (%)								
0	53	(21.0)	14	(18.9)	13	(23.6)	26	(21.1)
1	64	(25.4)	13	(17.6)	9	(16.4)	42	(34.1)
≥2	135	(53.6)	47	(63.5)	33	(60.0)	55	(44.7)
Tumor site, n (%)								
Colon	153	(60.7)	41	(55.4)	32	(58.2)	80	(65.0)
Rectum	99	(39.3)	33	(44.6)	23	(41.8)	43	(35.0)
Colorectal cancer stage, n (%)[d]								
I	75	(31.0)	22	(30.6)	21	(38.9)	32	(27.6)
II	57	(23.6)	18	(25.0)	10	(18.5)	29	(25.0)
III	110	(45.5)	32	(44.4)	23	(42.6)	55	(47.4)
Received surgery, n (%)	226	(89.7)	68	(91.9)	48	(87.3)	110	(89.4)
Received neo-adjuvant treatment, n (%)	69	(27.4)	25	(33.8)	16	(29.1)	28	(22.8)
Received adjuvant treatment, n (%)	72	(28.6)	20	(27.0)	16	(29.1)	36	(29.3)
Hospital, n (%)								
Maastricht UMC+	153	(60.7)	47	(63.5)	36	(65.5)	70	(56.9)
VieCuri Medical Center	68	(27.0)	22	(29.7)	9	(16.4)	37	(30.1)
Zuyderland Medical Centre	31	(12.3)	5	(6.8)	10	(18.2)	16	(13.0)

Abbreviations: BMI, body mass index; MVPA, moderate-to-vigorous physical activity; n, number; perc, percentile; SD, SD.

[a]Of included participants, a total of 10 participants had missing data on blood metabolites and/or fatigue and/or covariates at 6 weeks posttreatment and 1 participant had missing data on blood metabolites and/or fatigue and/or covariates at 6 weeks and 6 months posttreatment, but all of these participants had available data at later time points and were therefore included in the analysis.

[b]Education level was categorized as low (none/primary education/lower vocational training), medium (lower general secondary education/intermediate vocational education), or high (higher general secondary education/higher vocational education/university).

[c]Total daily time spent in bouts of sedentary behavior of at least 30 minutes, based on accelerometer data. Data on prolonged sedentary time was missing for n = 38 participants because no accelerometer data were available (due to participants refusing to wear accelerometer or invalid data), including n = 18 participants with severe levels of fatigue, n = 6 with elevated levels of fatigue and n = 14 with elevated levels of fatigue.

[d]Data missing for n = 10 participants, including n = 2 participants with severe levels of fatigue, n = 1 with elevated levels of fatigue and n = 7 with normal levels of fatigue.

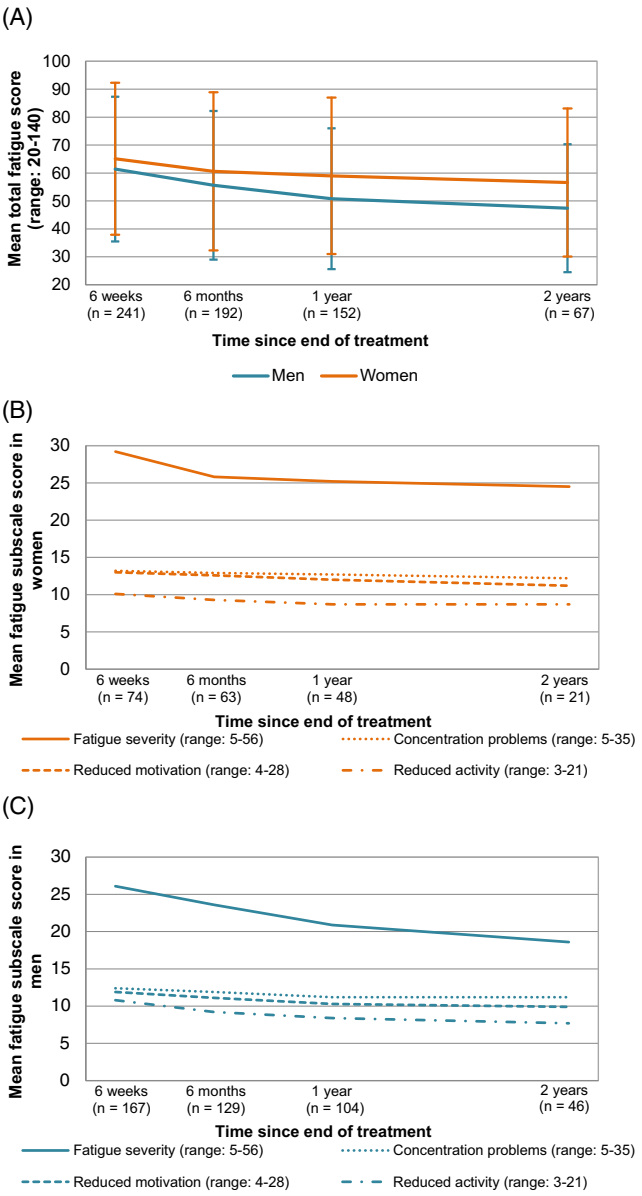

FIGURE 1 Self-reported (A) mean and SD of sex-stratified total fatigue scores, (B) mean fatigue subscale scores in women and (C) mean fatigue subscale scores in men, at posttreatment time points among colorectal cancer survivors included in the current analysis (n = 252). The total ranges in scores of fatigue variables are depicted in the y-axis of the title (A) and figure legends (B and C), the total ranges reported by the study population were 20-134 for total fatigue, 8-56 for "fatigue severity", 5-34 for "concentration problems," 4-28 for "reduced motivation" and 3–21 for "reduced activity"

including heat maps of correlations and the longitudinal development over time have previously been reported.[23]

During the posttreatment period, reported mean levels of fatigue, including the total fatigue score and all subdomains, decreased over time (Figure 1). In linear mixed models with only time since treatment as independent variable and using data for men and women combined, statistically significant negative changes over time were observed for the "total fatigue" score (β, that is, change in fatigue score per 6 months: −3.0; 95% CI: −4.1, −2.0), "fatigue severity" (β: −1.7, 95% CI: −2.2, −1.1), "reduced motivation" (β: −0.5; 95% CI: −0.8, −0.2) and "reduced activity" (β: −0.7; 95% CI: −1.0, −0.5), while a nonsignificant and small negative change was found for "concentration problems" (β: −0.1; 95% CI: −0.4, 0.2). At each posttreatment time point, the mean total fatigue and fatigue subscale scores were higher among women than men (Figure 1). Moderate to strong correlations were observed for all fatigue variables across posttreatment time points (range in Pearson's r: 0.41, 0.81; heat maps in Figure S2). The total fatigue score showed strong correlations with fatigue subscales at each time point (r: 0.72, 0.93), while moderate to strong correlations were found across fatigue subscales (r: 0.38, 0.75).

3.2 | Associations of metabolites with fatigue

In confounder-adjusted analyses assessing longitudinal associations, statistically significant associations were observed of metabolites with total fatigue and the subscales "fatigue severity," "reduced motivation" and "reduced activity" (Figure 2, full results in Table S2). In the overall analysis, higher concentrations of three amino acids, PC aa C36:0, five acyl-alkylPCs and two SMs were associated with lower total fatigue, while a higher concentration of acylcarnitine C18:1 was associated with more total fatigue (Figure 2A). In the hybrid model, a significant interindividual association was only observed for histidine being associated with lower total fatigue. For "fatigue severity," higher concentrations of six amino acids, six diacylPCs, nine acyl-alkylPCs and seven SMs were significantly associated with a lower fatigue severity, while higher concentrations of four acylcarnitines were related to a higher fatigue severity in the overall analysis (Figure 2B). Significant intraindividual associations were found for higher concentrations of arginine, PC aa C36:0, seven acyl-alkylPCs, and six SMs with a lower fatigue severity and for three acylcarnitines with a higher fatigue severity, in the hybrid model. Similar as for total fatigue, a significant interindividual association was only found for histidine, which was associated with a lower fatigue severity. In the overall analysis of "reduced motivation," higher concentrations of five amino acids, two lysoPCs, three diacylPCs and six acyl-alkyl PCs were associated with less fatigue (ie, more motivation), while a higher concentration of acylcarnitine C18:1 was associated with more fatigue (ie, less motivation; Figure 2C). Associations appeared mostly driven by interindividual associations with higher concentrations of histidine, two lysoPCs, six diacylPCs and two acyl-alkylPCs associated with less fatigue, while an intraindividual association was only observed for arginine with less fatigue. Finally, in the overall analysis of "reduced activity," higher concentrations of histidine, lysine and SM C24:0 were associated with less fatigue (ie, more activity); of these metabolites only histidine showed a significant interindividual association (Figure 2D). A total of 19 metabolites were associated with multiple fatigue outcomes with a similar direction of association (Table 2).

3.3 | Heterogeneity analyses

No statistically significant interactions were observed by sex, chemotherapy (yes/no), tumor site and time since end of treatment (results not shown). Interactions between the number of comorbidities (≥2 vs <2) and 43 metabolites were found for intraindividual associations with "reduced activity" (Table S3). Results of stratified analyses by number of comorbidities indicated that among participants with <2 comorbidities a higher concentration of these metabolites was associated with less reduced activity (ie, less fatigue), while among participants with ≥2 comorbidities a higher metabolite concentration was associated with more reduced activity (ie, more fatigue; Figure S3).

3.4 | Sensitivity analyses

Results of the analysis with additional adjustment for cancer stage and prolonged sedentary time indicated a slight attenuation of results compared to those obtained in the main analysis (Figure S4). Adjustment for metabolite concentrations at diagnosis produced results similar to those found in the main analysis, although for "reduced motivation" a slight attenuation of results was observed (Figure S5).

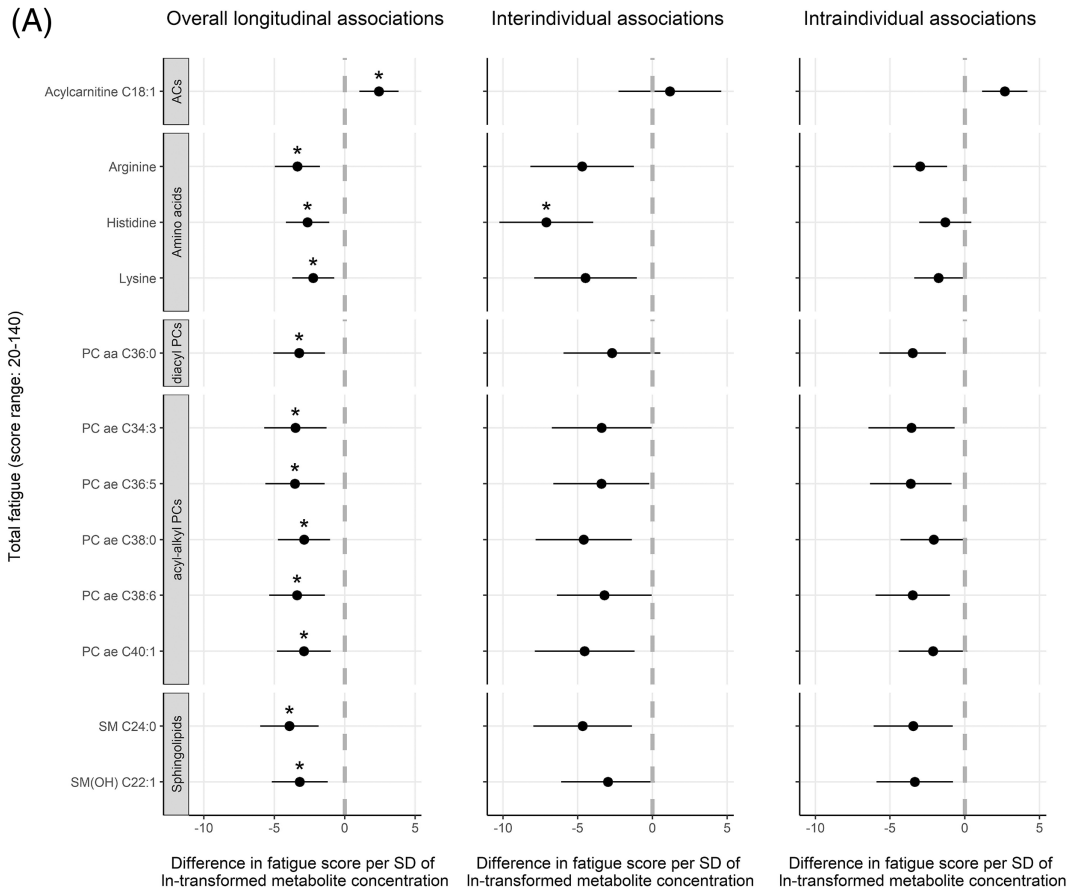

FIGURE 2 Forest plots showing effect estimates and 95% confidence intervals of metabolites that were statistically significantly (FDR q-value <0.05) related, in the overall, interindividual and/or intraindividual longitudinal analysis, to (A) total fatigue and the subscales (B) "fatigue severity," (C) "reduced motivation" and (D) "reduced activity" among colorectal cancer survivors, between 6 weeks and 2 years posttreatment. Asterisk (*) denotes statistical significance after FDR-adjustment. Since the subscale "concentration problems" was not statistically significantly associated with any of the metabolites, these results are not shown. Full results for all fatigue variables and metabolites are included in Table S2 Analyzed with multivariable linear mixed regression models analyzing associations of the batch-adjusted ln-transformed metabolite residuals (see Section 2,2.5) as the main independent variables and as dependent variables the fatigue variables, with a separate model for each metabolite and each fatigue variable. Models were adjusted for: sex; age at 6 weeks posttreatment (y; continuous), time since treatment (per 6 months; continuous), center (Maastricht UMC+; VieCuri Medical Center; Zuyderland Medical Centre), body mass index (kg/m^2; continuous), smoking status (current; former; never), self-reported alcohol consumption (grams/day), self-reported hours/week of moderate-to-vigorous physical activity, number of comorbidities (no comorbidity; 1 comorbidity; ≥2 comorbidities), neo-adjuvant treatment (yes/no) and adjuvant treatment (yes/no). ACs, acylcarnitines; PCs, phosphatidylcholines; SMs, sphingomyelins

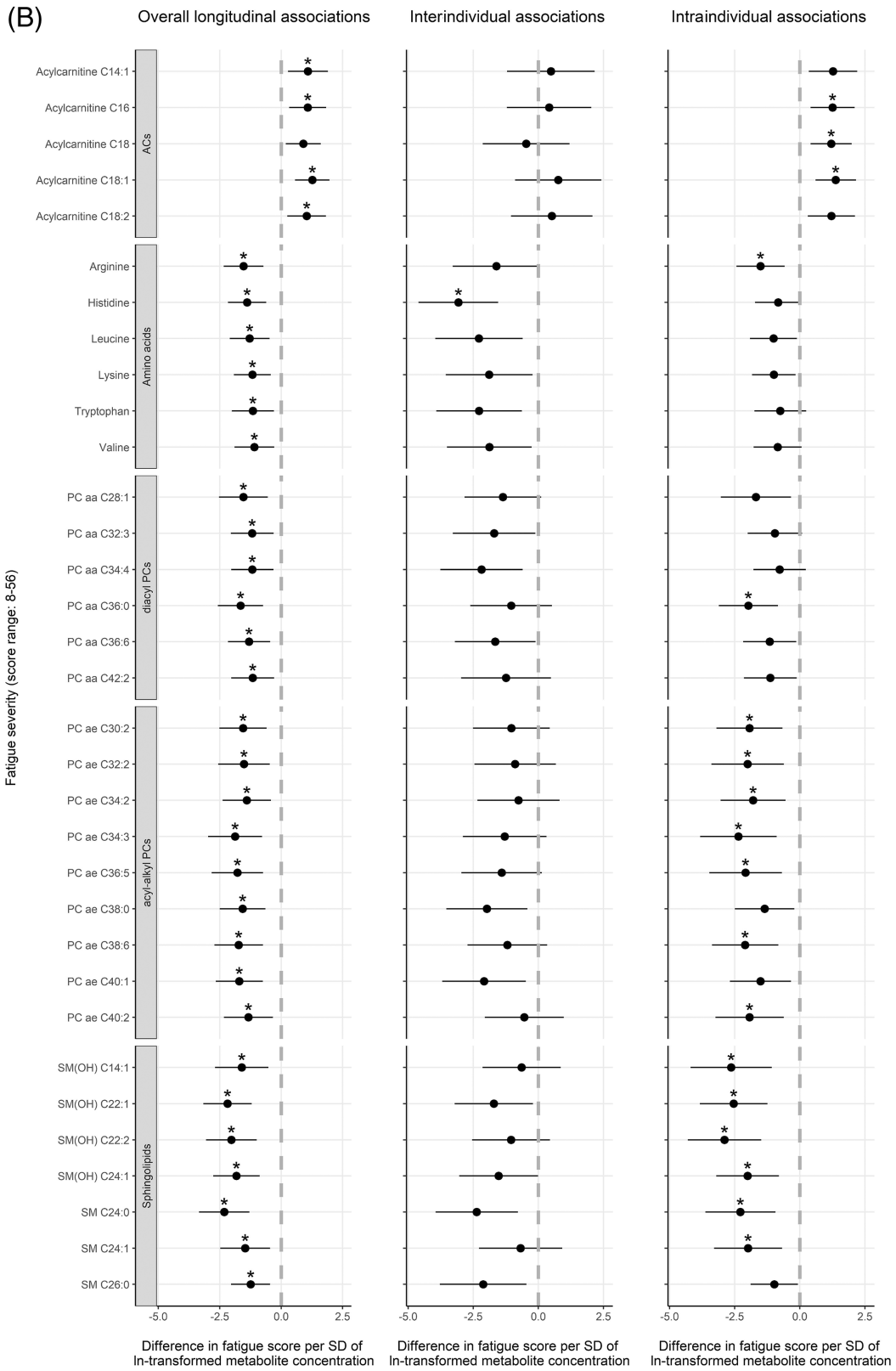

FIGURE 2 (Continued)

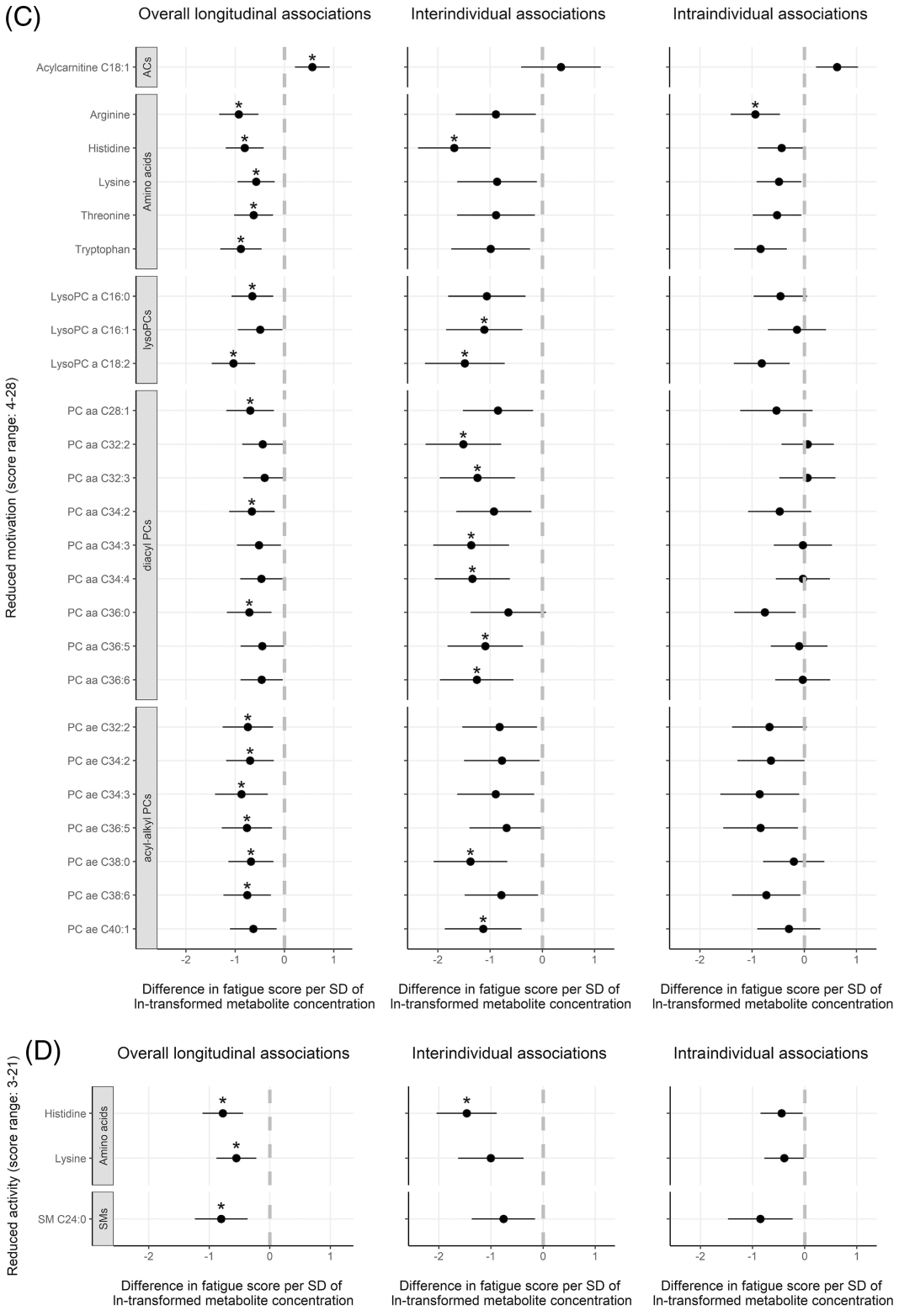

FIGURE 2 (Continued)

4 | DISCUSSION

To our knowledge, this is the first study to analyze longitudinal associations of metabolites with fatigue among colorectal cancer survivors. We found that higher concentrations of several amino acids, diacylPCs, acyl-alkylPCs and SMs were longitudinally associated with less total fatigue and a lower fatigue severity, while several acylcarnitines were associated with more total fatigue and a higher fatigue severity. For "fatigue severity," associations appeared mostly driven by intraindividual associations indicating that changes in metabolite

TABLE 2 Overall, inter- and intraindividual longitudinal associations with total fatigue and fatigue subscales of metabolites that were statistically significantly (FDR *q*-value <0.05) related to at least two fatigue outcomes, between 6 weeks and 2 years posttreatment

	Total fatigue			Fatigue severity			Reduced motivation			Reduced activity		
Metabolites	Overall	Inter-indiv.	Intra-indiv.	Overall	Inter-indiv.	Intra-indiv.	Overall	Inter-indiv.	Intra-indiv.	Overall	Inter-indiv.	Intra-indiv.
Acylcarnitine C18:1	+			+			+					
Arginine	–			–		–			–			
Histidine	–	–		–	–		–	–		–	–	
Lysine	–			–		–				–		
Tryptophan				–			–					
PC aa C28:1				–			–					
PC aa C32:3				–				–				
PC aa C34:4				–				–				
PC aa C36:0	–			–		–	–					
PC aa C36:6				–				–				
PC ae C32:2				–		–	–					
PC ae C34:2				–		–	–					
PC ae C34:3	–			–		–	–					
PC ae C36:5	–			–		–	–					
PC ae C38:0	–			–			–	–				
PC ae C38:6	–			–		–	–					
PC ae C40:1	–			–				–				
SM C24:0	–			–						–		
SM(OH) C22:1	–			–								

Note: A plus-sign (+) indicates a significant positive association (ie, higher metabolite concentration associated with more fatigue) and a minus-sign (–) indicates a negative association. Since the subscale concentration problems was not statistically significantly associated with any of the metabolites, these results are not shown. Analyzed with multivariable linear mixed regression models analyzing associations of the batch-adjusted ln-transformed metabolite residuals (see Section 2,2.5) as the main independent variables and as dependent variables the fatigue variables, with a separate model for each metabolite and each fatigue variable. Models were adjusted for: sex; age at 6 weeks posttreatment (y; continuous), time since treatment (per 6 months; continuous), center (Maastricht UMC+; VieCuri Medical Center; Zuyderland Medical Centre), body mass index (kg/m^2; continuous), smoking status (current; former; never), self-reported alcohol consumption (grams/day), self-reported hours/week of moderate-to-vigorous physical activity, number of comorbidities (no comorbidity; 1 comorbidity; ≥2 comorbidities), neo-adjuvant treatment (yes/no) and adjuvant treatment (yes/no).
Abbreviations: Inter-indiv., interindividual; Intra-indiv., intraindividual; PCs, phosphatidylcholines; SMs, sphingomyelins.

concentrations over time (eg, increases in concentrations of amino acids, and decreases in acylcarnitines) were related to changes (eg, decreases) in fatigue severity over time. Similar results were obtained for "reduced motivation," except that mostly interindividual associations were observed including positive associations with a number of lysoPCs, while no associations were found with SMs. These interindividual associations indicate that this fatigue subscale is mostly related to an individuals' average metabolite level across time points rather than changes in metabolite concentrations over time. Finally, higher concentrations of histidine, lysine and SM C24:0 were associated with lower "reduced activity." Many metabolites were associated with multiple fatigue outcomes, including histidine for which interindividual associations with the strongest magnitude of associations were found. None of the metabolites were associated with "concentration problems." Below, for each class of metabolites, the results are compared to previous studies and potential underlying mechanisms based on literature are described.

Some of the amino acids that were related to fatigue in our study have previously been associated with fatigue among cancer patients. Based on nontargeted metabolomics data and a pathways analysis, significant overrepresentation by metabolites involved in histidine metabolism have been found when comparing fatigued (mostly prostate) cancer patients before treatment with healthy controls.[21] However, associations with individual metabolites were not reported in that study,[21] and therefore it is unclear whether associations were found with histidine and in what direction. Lower circulating tryptophan levels have been associated with more fatigue among lymphoma survivors[43] and patients with various types of cancer.[44] In addition, inverse associations of circulating concentrations of arginine with fatigue have been reported in early-stage breast cancer patients.[20] Together, these findings may point to low grade inflammation as a mechanism underlying fatigue complaints, since experimental studies showed that histidine can have anti-inflammatory effects,[45] arginine can strengthen

immune function after colorectal cancer resection,[46] and a proinflammatory immune response can lead to increased tryptophan degradation, which has been correlated with more fatigue after cancer.[43,44] To our knowledge, associations of lysine, leucine, threonine and valine with cancer-related fatigue have not been reported. However, a previous study in CFS patients observed lower circulating levels of several amino acids compared to healthy controls, including leucine, lysine, tryptophan, valine and histidine.[47] The authors proposed an impaired energy metabolism due to mitochondrial dysfunction in skeletal muscle followed by increased consumption of amino acids to fuel oxidative metabolism and maintain ATP production as an underlying mechanism,[47] which has previously also been postulated as a mechanism underlying cancer-related fatigue.[12,48] Specifically, it is thought that a reduced energy intake in cancer patients due to alterations in appetite, in combination with adverse effects of cancer treatment such as cachexia, may lead to reduced levels of ATP in muscle which can compromise muscle function and lead to fatigue.[48] In line with this hypothesis, a pilot study among colorectal cancer survivors found significant differences in other metabolites involved in metabolic pathways regulating ATP production and cellular energy between fatigued and nonfatigued survivors.[19]

We also observed longitudinal associations with several lipid metabolites including lysoPCs, diacylPCs, acyl-alkylPCs and SMs. In line with our findings, based on nontargeted metabolomics data and a pathways analysis, significant overrepresentation by metabolites involved in sphingolipid metabolism have previously been observed when comparing fatigued (mostly prostate) cancer patients before treatment with healthy controls, although the (direction of) associations with individual metabolites were not reported.[21] Similar results have been obtained for other diseases with a high prevalence of fatigue symptoms, including decreased circulating concentrations of PCs and SMs among fatigued compared to nonfatigued patients with inflammatory bowel disease[49] and among CFS patients compared to healthy controls.[50] Decreased levels of SMs may be indicative of a prolonged inflammatory state and associated oxidative stress, which increase the activity of sphingolipid metabolizing enzymes leading to an increase in ceramide and sphingosine-1-phosphate that can promote fatigue in skeletal muscle.[21,49,51] Decreased levels of PCs may also be the result of oxidative damage, particularly to PCs in mitochondrial membranes leading to a loss of mitochondrial function and impaired energy metabolism leading to fatigue.[49]

Finally, we observed longitudinal associations of higher circulating concentrations of several acylcarnitines with more fatigue, particularly with fatigue severity. Opposite findings have been observed among CFS patients, where lower plasma levels of acylcarnitines including acylcarnitines C18, C18:1 and C18:2 were found compared to healthy controls, and an inverse association was observed with fatigue severity.[52] Acylcarnitines are used as a substrate for mitochondrial oxidation of fatty acids,[53] and the authors proposed that the observed deficiency in acylcarnitines may result in an impaired mitochondrial energy production leading to fatigue.[52] Our opposite findings may point toward differences in alteration of carnitine metabolism involved in CFS and cancer-related fatigue, which may be due to the different etiologies involved in these conditions,[48] which needs further investigation.

Many of the metabolites identified in our study were longitudinally associated with at least two fatigue subscales. Some differences were observed between subscales in terms of the number or classes of associated metabolites, as well as intra- vs interindividual associations. These differences may point toward different underlying mechanisms. The highest number of metabolites were associated with fatigue severity and results were mostly driven by intraindividual associations, indicating that changes in this subscale may particularly be associated with changes in metabolite concentrations over time. This finding may also be related to the fact that the highest intraindividual changes in this subscale were observed over time, compared to other subscales. Nevertheless, these intraindividual associations may be relevant in light of future intervention development, as it indicates that targeting these metabolites through intervention programs (eg, physical activity) may be particularly promising to prevent and/or reduce fatigue after colorectal cancer treatment.

Strengths of our study include the prospective design including repeated measurements of fatigue, metabolomics, and potential confounders up to 2 years posttreatment. Further, we were able to disentangle intra- and interindividual associations using a hybrid model. Finally, fatigue was assessed in a comprehensive manner by using the validated CIS to determine total fatigue and subdomains of fatigue, including fatigue severity, concentration problems, reduced motivation and reduced activity. There are also limitations to consider. Our observational design does not allow drawing conclusions regarding causality and we cannot exclude the possibility of reverse causation. Nevertheless, the identified metabolites point toward underlying metabolic pathways, as described in previous paragraphs, that may be associated with the development of persistent fatigue complaints. Although we included a comprehensive set of potential confounders, we cannot rule out the possibility of residual confounding. In particular, we had no information available on prediagnosis fatigue and therefore could not adjust our analysis for fatigue levels before colorectal cancer diagnosis, which may be related to both metabolites and fatigue after treatment. Finally, even though the samples in our study were only stored up to 4.5 years at −80°C and Wagner-Golbs et al. observed that the majority of metabolites are stable during 7-year storage at −80°C,[54] we cannot exclude the possibility that storage effects may have occurred for some metabolites of the BIOCRATES p180 kit.[55]

In conclusion, we observed that plasma concentrations of several amino acids, diacylPCs, acyl-alkylPCs, lysoPCs and SMs were longitudinally associated with less fatigue, while several acylcarnitines were associated with more fatigue. In addition, we found that intraindividual changes in these metabolites over time were associated with fatigue severity. These findings point toward underlying biological mechanisms of fatigue, including low grade inflammation and an impaired energy metabolism due to mitochondrial dysfunction in skeletal muscle. These mechanisms may be shared with the occurrence of fatigue among survivors of other types of cancer as well as fatigue-related diseases such as CFS. Further mechanistic research is

necessary to determine whether and how these metabolites may be targets for intervention to prevent and/or reduce persistent fatigue complaints after colorectal cancer treatment.

AUTHOR CONTRIBUTIONS

All authors contributed to the conception and design of the study; Eline H. van Roekel, Martijn J. L. Bours, Audrey Gicquiau, Augustin Scalbert and Matty P. Weijenberg acquired the data; Eline H. van Roekel, Martijn J. L. Bours and Matty P. Weijenberg analyzed the data; all authors contributed to the interpretation of data; Eline H. van Roekel drafted the manuscript; all authors contributed to the advanced draft of the manuscript; all authors read and approved the final manuscript. The work reported in the paper has been performed by the authors, unless clearly specified in the text.

ACKNOWLEDGEMENTS

We would like to thank all participants of the EnCoRe study and the health professionals in the three hospitals involved in the recruitment of participants of the study: Maastricht University Medical Centre+, VieCuri Medical Center, and Zuyderland Medical Centre. We would also like to thank the MEMIC center for data and information management for facilitating the logistic processes and data management of our study. Finally, we would like to thank the research dieticians and research assistant who are responsible for patient inclusion and follow-up, performing home visits, as well as data collection and processing.

FUNDING INFORMATION

Our study was funded by the Wereld Kanker Onderzoek Fonds (WKOF), as part of the World Cancer Research Fund International grant program (Grant No. 2016/1620). The EnCoRe study is also funded by grants from the Stichting Alpe d'HuZes within the research program "Leven met kanker" of the Dutch Cancer Society (Grant Nos. UM-2010-4867 and UM-2012-5653), by grants from Kankeronderzoekfonds Limburg as part of Health Foundation Limburg (Grant No. 00005739) and by a grant of the Wereld Kanker Onderzoek Fonds (WKOF), as part of the World Cancer Research Fund International grant program (Grant No. 2017/1619). Eline H. van Roekel is funded by the Wereld Kanker Onderzoek Fonds (WKOF), as part of the World Cancer Research Fund International grant program (Grant No. 2016/1620). These funding agencies had no role in the collection, analysis, and interpretation of data.

CONFLICT OF INTEREST

The authors declare no potential conflicts of interest.

DATA AVAILABILITY STATEMENT

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

ETHICS STATEMENT

The EnCoRe study has been approved by the Medical Ethics Committee of the Academic Hospital Maastricht and Maastricht University, The Netherlands. The study is being conducted in accordance with the Declaration of Helsinki and all participants provide written informed consent.

ORCID

Eline H. van Roekel https://orcid.org/0000-0002-9402-2029
Sabina Rinaldi https://orcid.org/0000-0002-6846-1204

REFERENCES

1. Hofman M, Ryan JL, Figueroa-Moseley CD, Jean-Pierre P, Morrow GR. Cancer-related fatigue: the scale of the problem. *Oncologist*. 2007;12(Suppl 1):4-10.
2. Minton O, Berger A, Barsevick A, et al. Cancer-related fatigue and its impact on functioning. *Cancer*. 2013;119(Suppl 11):2124-2130.
3. GLOBOCAN project: CANCER TODAY. http://gco.iarc.fr/today/data/factsheets/cancers/10_8_9-Colorectum-fact-sheet.pdf. Accessed October 21, 2020.
4. Koole JL, Bours MJL, Breedveld-Peters JJL, et al. Is dietary supplement use longitudinally associated with fatigue in Stage I-III colorectal cancer survivors? *Clin Nutr*. 2020;39:234-241.
5. Husson O, Mols F, van de Poll-Franse L, de Vries J, Schep G, Thong MS. Variation in fatigue among 6011 (long-term) cancer survivors and a normative population: a study from the population-based PROFILES registry. *Support Care Cancer*. 2015;23:2165-2174.
6. Berger AM, Mooney K, Alvarez-Perez A, et al. Cancer-Related Fatigue, version 2.2015. *J Natl Compr Canc Netw*. 2015;13:1012-1039.
7. Jones JM, Olson K, Catton P, et al. Cancer-related fatigue and associated disability in post-treatment cancer survivors. *J Cancer Surviv*. 2016;10:51-61.
8. Curt GA, Breitbart W, Cella D, et al. Impact of cancer-related fatigue on the lives of patients: new findings from the Fatigue Coalition. *Oncologist*. 2000;5:353-360.
9. Barsevick AM, Irwin MR, Hinds P, et al. Recommendations for high-priority research on cancer-related fatigue in children and adults. *J Natl Cancer Inst*. 2013;105:1432-1440.
10. Mustian KM, Alfano CM, Heckler C, et al. Comparison of pharmaceutical, psychological, and exercise treatments for cancer-related fatigue: a meta-analysis. *JAMA Oncol*. 2017;3:961-968.
11. van Vulpen JK, Sweegers MG, Peeters PHM, et al. Moderators of exercise effects on cancer-related fatigue: a meta-analysis of individual patient data. *Med Sci Sports Exerc*. 2020;52:303-314.
12. Serdà IFBC, van Roekel E, Lynch BM. The role of physical activity in managing fatigue in cancer survivors. *Curr Nutr Rep*. 2018;7:59-69.
13. Arnold M, Sierra MS, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global patterns and trends in colorectal cancer incidence and mortality. *Gut*. 2017;66:683-691.
14. Saligan LN, Olson K, Filler K, et al. The biology of cancer-related fatigue: a review of the literature. *Support Care Cancer*. 2015;23:2461-2478.
15. Bower JE. The role of neuro-immune interactions in cancer-related fatigue: biobehavioral risk factors and mechanisms. *Cancer*. 2019;125:353-364.
16. Lacourt TE, Heijnen CJ. Mechanisms of neurotoxic symptoms as a result of breast cancer and its treatment: considerations on the contribution of stress, inflammation, and cellular bioenergetics. *Curr Breast Cancer Rep*. 2017;9:70-81.
17. Scalbert A, Brennan L, Fiehn O, et al. Mass-spectrometry-based metabolomics: limitations and recommendations for future progress with particular focus on nutrition research. *Metabolomics*. 2009;5:435-458.
18. Dettmer K, Aronov PA, Hammock BD. Mass spectrometry-based metabolomics. *Mass Spectrom Rev*. 2007;26:51-78.
19. Chou YJ, Kober KM, Kuo CH, et al. A pilot study of metabolomic pathways associated with fatigue in survivors of colorectal cancer. *Biol Res Nurs*. 2020;23:42-49.

20. Lyon DE, Starkweather A, Yao Y, et al. Pilot study of metabolomics and psychoneurological symptoms in women with early stage breast cancer. *Biol Res Nurs*. 2018;20:227-236.
21. Feng LR, Barb JJ, Regan J, Saligan LN. Plasma metabolomic profile associated with fatigue in cancer patients. *Cancer Med*. 2021;10:1623-1633.
22. Huth TK, Eaton-Fitch N, Staines D, Marshall-Gradisnik S. A systematic review of metabolomic dysregulation in Chronic Fatigue Syndrome/Myalgic Encephalomyelitis/Systemic Exertion Intolerance Disease (CFS/ME/SEID). *J Transl Med*. 2020;18:198.
23. van Roekel EH, Bours MJL, van Delden L, et al. Longitudinal associations of physical activity with plasma metabolites among colorectal cancer survivors up to 2 years after treatment. *Sci Rep*. 2021;11:13738.
24. van Roekel EH, Bours MJ, de Brouwer CP, 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 Epidemiol Biomarkers Prev*. 2014;23:1394-1405.
25. Vercoulen JH, Swanink CM, Fennis JF, Galama JM, van der Meer JW, Bleijenberg G. Dimensional assessment of chronic fatigue syndrome. *J Psychosom Res*. 1994;38:383-392.
26. Servaes P, van der Werf S, Prins J, Verhagen S, Bleijenberg G. Fatigue in disease-free cancer patients compared with fatigue in patients with chronic fatigue syndrome. *Support Care Cancer*. 2001;9:11-17.
27. Vercoulen JH, Hommes OR, Swanink CM, et al. The measurement of fatigue in patients with multiple sclerosis. A multidimensional comparison with patients with chronic fatigue syndrome and healthy subjects. *Arch Neurol*. 1996;53:642-649.
28. Worm-Smeitink M, Gielissen M, Bloot L, et al. The assessment of fatigue: psychometric qualities and norms for the Checklist Individual Strength. *J Psychosom Res*. 2017;98:40-46.
29. Beurskens AJ, Bultmann U, Kant I, Vercoulen JH, Bleijenberg G, Swaen GM. Fatigue among working people: validity of a questionnaire measure. *Occup Environ Med*. 2000;57:353-357.
30. Floegel A, Drogan D, Wang-Sattler R, et al. Reliability of serum metabolite concentrations over a 4-month period using a targeted metabolomic approach. *PLoS One*. 2011;6:e21103.
31. Carayol M, Licaj I, Achaintre D, et al. Reliability of serum metabolites over a two-year period: a targeted metabolomic approach in fasting and non-fasting samples from EPIC. *PLoS One*. 2015;10:e0135437.
32. Geijsen A, van Roekel EH, van Duijnhoven FJB, et al. Plasma metabolites associated with colorectal cancer stage: findings from an international consortium. *Int J Cancer*. 2019;145:1221-1231.
33. van Roekel EH, Trijsburg L, Assi N, et al. Circulating metabolites associated with alcohol intake in the European prospective investigation into cancer and nutrition cohort. *Nutrients*. 2018;10:654.
34. Breedveld-Peters JJL, Koole JL, Muller-Schulte E, et al. Colorectal cancers survivors' adherence to lifestyle recommendations and cross-sectional associations with health-related quality of life. *Br J Nutr*. 2018;120:188-197.
35. Wendel-Vos GC, Schuit AJ, Saris WH, Kromhout D. Reproducibility and relative validity of the short questionnaire to assess health-enhancing physical activity. *J Clin Epidemiol*. 2003;56:1163-1169.
36. Annegarn J, Spruit MA, Uszko-Lencer NH, et al. Objective physical activity assessment in patients with chronic organ failure: a validation study of a new single-unit activity monitor. *Arch Phys Med Rehabil*. 2011;92:1852-1857.e1.
37. Berendsen BA, Hendriks MR, Meijer K, Plasqui G, Schaper NC, Savelberg HH. Which activity monitor to use? Validity, reproducibility and user friendliness of three activity monitors. *BMC Public Health*. 2014;14:749.
38. van Roekel EH, Winkler EA, Bours MJ, et al. Associations of sedentary time and patterns of sedentary time accumulation with health-related quality of life in colorectal cancer survivors. *Prev Med Rep*. 2016;4:262-269.
39. Sangha O, Stucki G, Liang MH, Fossel AH, Katz JN. The Self-Administered Comorbidity Questionnaire: a new method to assess comorbidity for clinical and health services research. *Arthritis Rheum*. 2003;49:156-163.
40. Twisk JWR, de Vente W. Hybrid models were found to be very elegant to disentangle longitudinal within- and between-subject relationships. *J Clin Epidemiol*. 2019;107:66-70.
41. Neuhaus JM, Kalbfleisch JD. Between- and within-cluster covariate effects in the analysis of clustered data. *Biometrics*. 1998;54:638-645.
42. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J R Stat Soc B Methodol*. 1995;57:289-300.
43. Fosså A, Smeland KH, Fluge Ø, et al. Metabolic analysis of amino acids and vitamin B6 pathways in lymphoma survivors with cancer related chronic fatigue. *PLoS One*. 2020;15:e0227384.
44. Schroecksnadel K, Fiegl M, Prassl K, Winkler C, Denz HA, Fuchs D. Diminished quality of life in patients with cancer correlates with tryptophan degradation. *J Cancer Res Clin Oncol*. 2007;133:477-485.
45. Son DO, Satsu H, Shimizu M. Histidine inhibits oxidative stress- and TNF-alpha-induced interleukin-8 secretion in intestinal epithelial cells. *FEBS Lett*. 2005;579:4671-4677.
46. Karimian J, Hadi A, Salehi-Sahlabadi A, Kafeshani M. The effect of arginine intake on colorectal cancer: a systematic review of literatures. *Clin Nutr Res*. 2019;8:209-218.
47. Fluge Ø, Mella O, Bruland O, et al. Metabolic profiling indicates impaired pyruvate dehydrogenase function in myalgic encephalopathy/chronic fatigue syndrome. *JCI Insight*. 2016;1:e89376.
48. Ryan JL, Carroll JK, Ryan EP, Mustian KM, Fiscella K, Morrow GR. Mechanisms of cancer-related fatigue. *Oncologist*. 2007;12:22-34.
49. Horta D, Moreno-Torres M, Ramírez-Lázaro MJ, et al. Analysis of the association between fatigue and the plasma lipidomic profile of inflammatory bowel disease patients. *J Proteome Res*. 2021;20:381-392.
50. Naviaux RK, Naviaux JC, Li K, et al. Metabolic features of chronic fatigue syndrome. *Proc Natl Acad Sci U S A*. 2016;113:E5472-E5480.
51. Nikolova-Karakashian MN, Reid MB. Sphingolipid metabolism, oxidant signaling, and contractile function of skeletal muscle. *Antioxid Redox Signal*. 2011;15:2501-2517.
52. Reuter SE, Evans AM. Long-chain acylcarnitine deficiency in patients with chronic fatigue syndrome. Potential involvement of altered carnitine palmitoyltransferase-I activity. *J Intern Med*. 2011;270:76-84.
53. Indiveri C, Iacobazzi V, Tonazzi A, et al. The mitochondrial carnitine/acylcarnitine carrier: function, structure and physiopathology. *Mol Aspects Med*. 2011;32:223-233.
54. Wagner-Golbs A, Neuber S, Kamlage B, et al. Effects of long-term storage at −80°C on the human plasma metabolome. *Metabolites*. 2019;9:99.
55. Haid M, Muschet C, Wahl S, et al. Long-term stability of human plasma metabolites during storage at −80°C. *J Proteome Res*. 2018;17:203-211.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: van Roekel EH, Bours MJL, Breukink SO, et al. Longitudinal associations of plasma metabolites with persistent fatigue among colorectal cancer survivors up to 2 years after treatment. *Int J Cancer*. 2023;152(2):214-226. doi:10.1002/ijc.34252