Relationship of Mitochondrial Enzymes to Fatigue Intensity in Men With Prostate Cancer Receiving External Beam Radiation Therapy

Kristin Filler, PhD, RN, OCN¹, Debra Lyon, PhD, RN, FAAN²,
 Nancy McCain, PhD, RN, FAAN³, James Bennett Jr., MD, PhD⁴,
 Juan Luis Fernández-Martínez, PhD⁵, Enrique Juan deAndrés-Galiana, PhD⁵,
 R. K. Elswick Jr., PhD⁶, Nada Lukkahatai, PhD, RN⁷,
 and Leorey Saligan, PhD, RN, CRNP, FAAN¹

Abstract

Purpose: Mitochondrial dysfunction is a plausible biological mechanism for cancer-related fatigue. Specific aims of this study were to (1) describe the levels of mitochondrial oxidative phosphorylation complex (MOPC) enzymes, fatigue, and health-related quality of life (HRQOL) before and at completion of external beam radiation therapy (EBRT) in men with nonmetastatic prostate cancer (PC); (2) examine relationships over time among levels of MOPC enzymes, fatigue, and HRQOL; and (3) compare levels of MOPC enzymes in men with clinically significant and nonsignificant fatigue intensification during EBRT. **Methods:** Fatigue was measured by the revised Piper Fatigue Scale and the Functional Assessment of Cancer Therapy–Fatigue subscale (FACT-F). MOPC enzymes (Complexes I–V) and mitochondrial antioxidant superoxide dismutase 2 were measured in peripheral blood using enzyme-linked immunosorbent assay at baseline and completion of EBRT. Participants were categorized into high or low fatigue (HF vs. LF) intensification groups based on amount of change in FACT-F scores during EBRT. **Results:** Fatigue reported by the 22 participants with PC significantly worsened and HRQOL significantly declined from baseline to EBRT completion. The HF group comprised 12 men with clinically significant change in fatigue (HF) during EBRT. Although no significant changes were observed in MOPC enzymes from baseline to EBRT completion, there were important differences in the patterns in the levels of MOPC enzymes between HF and LF groups. **Conclusion:** Distinct patterns of changes in the absorbance of MOPC enzymes delineated fatigue intensification among participants. Further investigation using a larger sample is warranted.

Keywords

fatigue, mitochondria, radiation therapy

Prostate cancer (PC) is the most common type of cancer in men, outside of skin cancer, and the second leading cause of cancer death among American men (American Cancer Society [ACS], 2014). In the United States, there were about 2.5 million men with PC in 2014, and 233,000 of them were newly diagnosed, indicating that PC affects a significant number of men: About one in seven men will be diagnosed during their lifetime. Fortunately, advances in diagnosis and treatment have greatly improved the prognosis and survival of men with PC.

Treatment for PC depends on patient and disease characteristics. If the cancer is localized or has only progressed into the nearby tissue, then various forms of radiation therapy (RT) may be implemented (ACS, 2014). Although external beam radiation therapy (EBRT), for example, can be an effective treatment option, it can result in numerous side effects including

¹ National Institute of Nursing Research (NINR), National Institutes of Health (NIH), Bethesda, MD, USA

² University of Florida, Gainesville, FL, USA

³ Virginia Commonwealth University, Richmond, VA, USA

⁴ Department of Neurology, Virginia Commonwealth University, Richmond, VA, USA

⁵ Department of Mathematics, Universidad de Oviedo, Oviedo, Spain

⁶ Virginia Commonwealth University, Richmond, VA, USA

⁷ University of Nevada Las Vegas, Las Vegas, NV, USA

Corresponding Author:

Leorey N. Saligan, PhD, RN, CRNP, FAAN, National Institute of Nursing Research (NINR), National Institutes of Health (NIH), 9000 Rockville Pike, Building 3, Room 5E14, Bethesda, MD 20892, USA.

Email: saliganl@mail.nih.gov

urinary, bowel, and sexual dysfunctions, as well as fatigue, all of which can greatly impair the individual's health-related quality of life (HRQOL; Budäus et al., 2011). In a systematic review comparing fatigue prevalence among different treatment modes for PC, authors found that chronic fatigue or clinically significant fatigue, as determined through scores on various self-report assessments, was present in 13–22% of men who had radical prostatectomy and a similar rate was present in patients on active surveillance (Langston, Armes, Levy, Tidey, & Ream, 2013). However, authors also observed that 71% of men reported clinically significant fatigue while receiving RT. Additionally, 24–33% of men experienced persistent fatigue more than 1 year post-RT.

Fatigue related to RT is a type of cancer-related fatigue (CRF). CRF is one of the most commonly reported side effects of cancer and its treatment, affecting about 80% of people receiving chemotherapy or RT (Hofman, Ryan, Figueroa-Moseley, Jean-Pierre, & Morrow, 2007; National Comprehensive Cancer Network, 2012; Piper & Cella, 2010). People with cancer often characterize CRF as a lack of energy, weakness, muscle heaviness, inability to recover from physical activity in a timely manner, the need for exaggerated effort to complete a task, or the need for greater rest periods once a task is complete (Cheville, 2009; Hofman et al., 2007; Mitchell & Berger, 2011). Not only is CRF one of the most prevalent of cancer symptoms, it is also one of the most distressing, often negatively affecting multiple HRQOL domains (Barsevick, Frost, Zwinderman, Hall, & Halyard, 2010; El Tazi & Errihani, 2011; Ryan et al., 2007). CRF is poorly understood, having an unknown etiology and lacking a clear, single, clinical definition. This gap in knowledge makes it a challenging symptom for health care providers to diagnose, resulting in increased symptom burden and decreased HRQOL.

The biological basis for fatigue in individuals with cancer is an area of great research interest. Authors have theorized that many different biological mechanisms play a role in the etiology of CRF, such as proinflammatory cytokines, serotonin dysregulation, and hypothalamic–pituitary–adrenal axis dysfunction (Wang, 2008). An alternative plausible biological mechanism for fatigue is mitochondrial dysfunction. Self-reported descriptions of reduced energy and muscle weakness lend support for a possible relationship of CRF to mitochondrial dysfunction in that these symptoms are similar to those that might be present with mitochondrial disease (such as exercise intolerance and weakness, among other skeletal muscle and energy or metabolic manifestations; Haas et al., 2007).

Given the paucity of research in this area, we designed the current study to expand knowledge of the relationship of mitochondrial dysfunction to fatigue. Specific aims of the study were to (1) describe levels of biomarkers of mitochondrial function, fatigue, and HRQOL before and at the completion of EBRT; (2) examine relationships over time among levels of mitochondrial oxidative phosphorylation complex (MOPC) enzymes, fatigue, and HRQOL; and (3) compare levels of MOPC enzymes in men with nonclinically significant fatigue intensification from baseline to completion of

EBRT to levels in men with clinically significant fatigue intensification.

Method

For this study, we used data collected from a descriptive longitudinal study approved by the National Institutes of Health (NIH) institutional review board (#09-NR-0088; Molecular-Genetic Correlates of Fatigue in Cancer Patients Receiving Localized External Beam Radiation Therapy). Men were enrolled in that study if they (1) had nonmetastatic PC, (2) were scheduled to receive EBRT, and (3) were 18 years of age or older. Patients were excluded if they (1) had any inflammatory or infectious condition such as rheumatoid arthritis, lupus, or cirrhosis or an infectious disease such as human immunodeficiency virus (HIV), tuberculosis, or hepatitis; (2) had other types of cancer; (3) had a major psychiatric disorder or alcohol or drug abuse within the past 5 years; (4) were receiving or scheduled to receive chemotherapy; or (5) were taking steroids, nonsteroidal anti-inflammatories, or tranquilizers. All study participants were seen in the outpatient clinics of the Radiation Oncology Department of the National Cancer Institute at the NIH, Bethesda, MD, from August 2010 to August 2012. Informed consents were obtained prior to data collection.

Measures and Data Collection

Data for this study were collected at two time points: baseline (before the start of EBRT) and on the last day (completion) of EBRT. Study data were collected using medical records, self-report questionnaires, and blood samples.

Medical records. We reviewed medical records to obtain demographic data, including age, race, and socioeconomic status, and disease-related factors, including tumor characteristics and tumor-related laboratory markers.

Questionnaires. CRF is a complex symptom because of its multidimensional nature (Given, 2008). Therefore, we used two previously validated self-report fatigue questionnaires to quantify various dimensions of participants' fatigue.

The revised Piper Fatigue Scale (rPFS) is a 22-item paper/pencil questionnaire that measures multiple dimensions of fatigue: behavioral/severity (6 items), sensory (5 items), cognitive/mood (6 items), and affective meaning (5 items) using a 0–10 intensity rating scale (0 = *none*; 10 = *worst intensity*). Psychometric properties of the rPFS from previous studies have included excellent reliability and validity estimates when used in cancer patients receiving radiotherapy; internal consistency has ranged from 0.83 to 0.97 for the total instrument and its subscales (Borneman et al., 2012).

The Functional Assessment of Cancer Therapy–Fatigue (FACT-F) subscale is a validated 13-item questionnaire that explores fatigue symptoms in various populations, including cancer patients and healthy participants. This questionnaire has

shown good test–retest reliability ($r = .90$) and internal consistency ($\alpha = .93$ and $.95$) on initial and test–retest administration, suggesting that it can be administered as an independent, unidimensional measure of fatigue (Yellen, Cella, Webster, Blendowski, & Kaplan, 1997). A decline of more than three points in the FACT-F score is considered to be the threshold denoting a minimally important difference (MID) that is clinically relevant (Cella, Eton, Lai, Peterman, & Merkel, 2002). This MID method was used to categorize study participants into either a high fatigue (HF) intensification group or a low fatigue (LF) intensification group during EBRT.

In addition, we measured HRQOL using the FACT-Prostate (FACT-P) questionnaire. This questionnaire assesses HRQOL in five different domains: physical well-being (7 items), social/family well-being (7 items), emotional well-being (6 items), functional well-being (7 items), and the PC-specific assessment of functional status (12 items). The instrument has been validated for use in men with PC (across two samples, internal consistency $\alpha = .87$ – $.89$; Cella, Nichol, Eton, Nelson, & Mulani, 2009; Esper et al., 1997).

Blood samples. We used peripheral blood, collected at both study time points immediately after questionnaires were administered, to obtain protein markers of mitochondrial function. Peripheral whole blood samples collected using ethylenediaminetetraacetic acid (EDTA) tubes were centrifuged immediately after collection at 3,000 rpm and 4°C for 10 min. After the plasma was extracted from the tube, the remaining cell sample was divided into 500- μ l aliquots and stored in –80°C freezers until batch analysis. The cells from the whole blood samples were lysed with a protein extraction buffer (10 mM Tris, pH 7.4, 100 mM NaCl, 1 mM EDTA, 1% Triton X-100, 10% glycerol, and 0.1% sodium dodecyl sulfate). A protease inhibitor was added to the buffer to prevent the degradation of enzymes. Protein concentration was quantified using the Pierce™ BCA™ Protein Assay (Thermo Fisher Scientific™, Rockford, IL) per manufacturer's protocol. After protein quantification, the cell lysate samples were diluted with incubation buffer per assay protocol and stored at –20°C overnight.

Peripheral whole blood collected using serum-separating tubes (Becton, Dickinson, and Company, Franklin Lakes, NJ) was centrifuged immediately after collection at 3,000 rpm and 4°C for 10 min, then the serum was divided into 250- μ l aliquots and stored in –80°C freezers until batch analysis. We investigated two areas of mitochondrial function in this study: (a) energy metabolism and (b) oxidative stress.

Energy metabolism: Mitochondrial oxidative phosphorylation. Mitochondrial oxidative phosphorylation is comprised of five enzyme complexes: Complexes I–IV, also known as the electron transport chain, and Complex V (Kim, Wei, & Sowers, 2008). Dysfunction in any complex could interrupt the transfer of electrons, thereby disrupting efficient and effective energy metabolism. We used the Human Profiling ELISA kits (Abcam®, Cambridge, MA) for quantitative detection of the MOPC enzymes (Complexes I–V; one kit for each complex)

from cell lysates. We loaded 50 μ l of cell lysates into each well and performed enzyme-linked immunosorbent assays (ELISAs) according to manufacturer's instructions. The absorbance (600 nm; wavelength used to measure ELISA optical density) was measured and analyzed for the ELISA results. All samples were tested in duplicate and assays were performed by one technical staff member experienced in conducting ELISAs.

Oxidative stress: Antioxidants. Manganese superoxide dismutase (MnSOD) provides the first line of defense against reactive oxygen species (ROS) in the mitochondria, countering oxidative stress (Lustgarten et al., 2011). We loaded 50 μ l of undiluted serum samples into each well and performed ELISAs according to manufacturer's instructions. Serum MnSOD concentrations were assessed by the MnSOD ELISA kit (Abcam, Cambridge, MA). All samples were tested in duplicate and assays were performed by one technical staff member experienced in conducting ELISAs.

Statistical Analyses

Descriptive statistics were used to illustrate the demographic characteristics of participants. Medians and ranges were calculated for fatigue questionnaire scores, whereas means and standard deviations were calculated for HRQOL questionnaire scores. Nonparametric Mann–Whitney U tests were used to analyze changes in the levels of fatigue from baseline to completion of EBRT, and paired t -tests were used to analyze changes in HRQOL scores from baseline to completion of EBRT.

Geometric means were used to describe the levels of MOPC enzymes. Geometric means are a better measure of central tendency when data follow a lognormal distribution. In addition, geometric means are useful for biologic data analysis to assess for biological significance in the absence of statistical significance. Estimated correlations among mitochondrial biomarkers, fatigue (as assessed by rPFS and FACT-F scores), and HRQOL were calculated using Spearman's rank correlation coefficients. Participants in this study were then grouped into the HF group or the LF group based upon changes in FACT-F scores from baseline to completion of EBRT. Those in the HF group had a decrease (worsening fatigue) of three or more points in FACT-F scores. Mann–Whitney U tests for equal medians were used to examine differences in the levels of MOPC enzymes between LF and HF groups at baseline and at treatment completion.

Results

Sample Demographics

The 22 men enrolled in the study were all able to carry on all predisease activities without restrictions based on their Karnofsky performance scores (Johnson et al., 2014). Table 1 describes the demographic and clinical characteristics of the sample. About 70% of participants had Gleason scores of 7 (31.82%) or 8 (36.36%). About 70% of participants had a

Table 1. Demographic and Clinical Characteristics of Sample.

Variable	Value
Demographic	
Age, years, mean (SD)	65.86 (6.87)
Race, <i>n</i> (%)	
White	16 (72.73)
African American/Black	4 (18.18)
Hispanic	2 (9.09)
Ethnicity, <i>n</i> (%)	
Hispanic/Latino	3 (13.64)
Not Hispanic/Latino	17 (77.27)
Not documented	2 (9.09)
Disease characteristics	
Gleason, mean (SD)	7.59 (0.91)
Clinical T-stage, <i>n</i> (%)	
T1c	5 (22.72)
T2a	6 (27.27)
T2b	2 (9.09)
T2c	5 (22.72)
T3a	1 (4.55)
T3b	1 (4.55)
Not documented	2 (9.09)
Pre-EBRT clinical markers	
PSA (N° = 0–3.99 ng/ml), mean (SD)	3.69 (5.24)
Testosterone (N° = 181–758 ng/dl), mean (SD)	220.37 (166.02)
WBC (N° = 4.23–9.07 K/ μ l), mean (SD)	6.13 (1.67)
RBC (N° = 4.63–6.08 M/ μ l), mean (SD)	4.61 (0.44)
Hemoglobin (N° = 13.70–17.50 g/dl), median (range)	13.95 (12.80–15.60)
Albumin (N° = 3.70–4.70 g/dl), median (range)	4.10 (2.70–4.40)
BMI (N° = 18.50–24.90 kg/m ²), median (range)	29.85 (22.90–40.70)

Note. *N* = 22. BMI = body mass index; EBRT = external beam radiation therapy; N° = normal ranges; PSA = prostate-specific antigen; RBC = red blood cell count; T-stage = tumor stage (TNM = primary tumor, lymph node, metastases, classification for tumor staging); WBC = white blood cell count.

clinical T-stage of T1c, T2a, or T2c, indicating that most participants had disease that had not spread outside the prostate gland (Cheng, Montironi, Bostwick, Lopez-Beltran, & Berney, 2012). Participants received EBRT 5 days a week, with 17 participants receiving daily equal fractionation doses for a total dose of 75.50 Gy (Grays) for 42 days, while 5 participants who had prior prostatectomies received daily equal fractionation doses for a total dose of 68.40 Gy for 38 days.

Fatigue. The median score on the FACT-F was 47.50 at baseline and 43.00 at treatment completion; lower scores on the FACT-F indicate worsening fatigue. The median score on the rPFS was 0.93 at baseline and was 3.68 at treatment completion; higher scores on the rPFS indicate worsening fatigue.

There was a significant change in fatigue scores detected on both fatigue instruments from baseline to the completion of EBRT for the total sample (FACT-F p = .05; rPFS p = .02). There was no significant difference in the change in FACT-F scores from baseline to completion of EBRT between

Table 2. Fatigue and Health-Related Quality of Life (HRQOL) Scores for All Study Participants.

Measure	<i>n</i>	Score at Baseline	Score on Last Day of Treatment	Possible Range	<i>p</i> Value
Fatigue, median (range)					
rPFS	22	0.93 (0–4.41)	3.68 (0–6.73)	0–10	.02
FACT-F	22	47.50 (28–52)	43.00 (20–52)	0–52	.05
HRQOL (FACT-P), mean (SD)	19 ^a	132.79 (12.85)	123.74 (18.24)	0–156	.003

Note. *N* = 22. FACT-F = Functional Assessment of Cancer Therapy–Fatigue subscale; FACT-P = Functional Assessment of Cancer Therapy–Prostate; rPFS = revised Piper Fatigue Scale.

^aThree men had missing FACT-P scores at treatment completion and hence were not included in the analysis.

Table 3. Fatigue Scores, Median (Range), by Fatigue Group (High Vs. Low).

Fatigue Group	<i>n</i>	Score at Baseline	Score on Last Day of Treatment	<i>p</i> Value
High	12	48 (28–52)	35.5 (20–46)	<.001
Low	10	44 (30–52)	47.50 (41–52)	.47

participants who received 38 versus 42 days of treatment (p = .24). Although the change in rPFS scores over time was significant, the actual change in the level of fatigue would not be categorized as clinically relevant using that instrument's guidelines, given that significant fatigue for the rPFS is defined as a score of ≥ 6 (Piper et al., 1998). In contrast to scores on the rPFS, the change in FACT-F scores over time indicated both statistically and clinically significant change in fatigue symptoms.

Based on changes in FACT-F score from baseline to completion of EBRT, we categorized 12 participants in the HF group and 10 in the LF group. Within the HF group, there was a significant decrease in FACT-F scores from baseline (median = 48.00) to completion of EBRT (median = 35.50, p < .001), while in the LF group, there was no significant change in median FACT-F score from baseline (44.00) to completion of EBRT (47.50, p = .47). Table 2 describes the fatigue and HRQOL characteristics of all participants, and Table 3 describes the fatigue characteristics for the two fatigue cohorts.

Hemoglobin (Hgb). There was a significant difference in Hgb levels between the HF and LF groups at baseline (p = .02) and at treatment completion (p = .004), with the HF participants having lower Hgb levels than the LF participants, suggesting that Hgb levels may have contributed to the fatigue experience at each study time point. However, there were no significant correlations (p > .18) between Hgb levels and fatigue scores in either HF and LF participants at each study time point. Both HF and LF participants had significant declines in Hgb levels

Table 4. Geometric Mean Levels of and Percentage Change in Mitochondrial Enzymes From Baseline to End of External Beam Radiation Treatment (EBRT) by Fatigue Group.

Mitochondrial enzyme	HF (n = 12)				LF (n = 10)			
	Baseline	Completion	Percentage Change	Direction of Change	Baseline	Completion	Percentage Change	Direction of Change
Complex I	0.892	1.016	13.87	↑	1.395	0.901	−35.44	↓
Complex II	0.004	0.010	125.68	↑	0.002	0.002	−1.17	↓
Complex III	1.371	1.993	45.39	↑	4.535	2.175	−52.05	↓
Complex IV	0.480	0.581	21.01	↑	0.705	0.598	−15.25	↓
MnSOD	1,315.1	1,276.7	−2.92	↓	1,097.6	1,110.4	1.17	↑

Note. Values for Complexes I–IV are expressed as geometric mean absorbance at 600 nm. MnSOD values are expressed as geometric mean concentration (ng/ml). HF = high fatigue; LF = low fatigue; MnSOD = manganese superoxide dismutase. *p* Values >.05 for all measures.

over time, with values lower than the normal range at treatment completion (normal range = 13.70–17.50 g/dl; HF = 13.3–12.3, $p = .002$; and LF = 14.7–13.5, $p = .01$). However, there was no significant correlation between the change in Hgb level and the change in fatigue score using fatigue questionnaire from baseline to treatment completion in either the HF or LF group ($p = .51$ and $p = .58$, respectively).

HRQOL. There was a significant decline in mean FACT-P score from baseline, at 132.79 ± 12.85 , to completion of EBRT, at 123.74 ± 18.24 ($p = .003$); lower scores on the FACT-P indicate declining HRQOL. At completion of EBRT, the mean HRQOL score of participants was below the mean score of 126.30 for the general population on the FACT-P (Wei et al., 2002). When we looked at HRQOL by fatigue group, we found that, among HF participants, there was a significant decline in HRQOL from baseline (mean = 132.78 ± 10.05) to completion of treatment (mean = 118.44 ± 17.11 , $p = .01$), but the decline among LF participants was not significant (baseline mean = 132.80 ± 15.5 ; completion mean = 128.5 ± 18.75 ; $p = .12$).

Mitochondrial measures. There were no significant changes in the absorbance of MOPC I–IV enzymes or in the concentration of MnSOD enzyme from baseline to the end of EBRT for the participants as a whole ($p \geq .55$). We observed a colorimetric change for the Complex V enzyme; however, the change was not measurable even after we attempted many different serial dilutions. We observed no significant difference in any of the MOPC enzymes between HF and LF participants at baseline ($p \geq .53$) or at treatment completion ($p \geq .06$). However, we observed patterns of changes in the levels of MOPC enzymes from baseline to completion of EBRT when participants were categorized into HF and LF groups. The HF group had substantial changes (>25% change) in the concentrations of Complex II (125% change) and Complex III (45% change) enzymes from baseline to treatment completion, while in the LF group, there were substantial changes in the concentrations of Complex I (−35% change) and Complex III (−52% change) enzymes from baseline to treatment completion. However, none of these changes were statistically significant. There was

no significant change in MnSOD concentration for either fatigue group from baseline to treatment completion. Table 4 lists each of the mitochondrial enzyme levels at baseline and at completion of EBRT for the HF and LF groups.

Correlations. For all study participants, level of Complex II enzyme was significantly associated with FACT-F ($p = -.43$, $p = .04$), rPFS ($p = .42$, $p = .05$), and FACT-P ($p = -.47$, $p = .03$) scores at baseline, suggesting that low levels of Complex II enzymes are associated with less fatigue and high HRQOL before EBRT. At completion of EBRT, higher FACT-F scores were significantly associated with lower levels of Complex II enzymes ($p = -.50$, $p = .02$) for the total sample. For HF participants, lower levels of Complex II enzymes were associated with higher FACT-F ($p = -.71$, $p = .009$) and FACT-P ($p = -.84$, $p < .001$) scores at baseline. No significant associations were observed at completion of EBRT among HF participants. There were no significant associations between the variables at either time point for those in the LF group.

There was a significant correlation between the change in Complex III enzymes from baseline to treatment completion and the change in FACT-P from baseline to treatment completion ($p \leq .02$) among participants as a whole. There were no significant correlations ($p > .05$) between changes in any of the MOPC enzymes and changes in fatigue scores using either FACT-F or rPFS during EBRT for participants as a whole or within the fatigue groups.

Discussion

This novel, exploratory study is the first, to our knowledge, to investigate the potential association of mitochondrial function–related enzymes and fatigue during EBRT. The study findings reveal that the level of mitochondrial Complex II enzyme was associated with fatigue in all participants at both time points as well as in the HF group at baseline, suggesting that future studies should investigate the activity of succinate dehydrogenase to better understand its role in the etiology of radiation-related fatigue. Further, when we categorized participants into HF and LF groups, we observed patterns of changes in the

MOPC enzymes that could possibly delineate the fatigue phenotype in this population.

Our findings regarding the relationship between mitochondrial Complex II enzyme and fatigue may be related to a hypoxic cellular environment generated by the lack of Hgb. Authors have proposed that mitochondrial Complex II is implicated in the activation of the hypoxia/angiogenesis response (Gimenez-Roqueplo et al., 2001). Hgb levels differed significantly between the HF and LF groups at both study time points, suggesting that oxygenation status associated with Hgb concentrations contributes to, but does not fully explain, the fatigue experience during EBRT, which is consistent with prior report (Ryan et al., 2007).

The available literature explains that, during hypoxic conditions, mitochondrial ROS production is increased to regulate cellular pathways in order to adapt to the stressful state (Dehne & Brune, 2014; Sena & Chandell, 2012). The pathways regulated by ROS, such as the hypoxia inducible factors, can in turn influence the mitochondrial metabolic pathways, altering normal function. Alterations in mitochondrial function contribute to alteration in the homeostatic regulation of ROS production, resulting in an altered cellular redox state that has been implicated in various disease states (Sena & Chandell, 2012).

The present study findings suggest that similar pathways may be implicated in the fatigue experience in men undergoing EBRT for PC, where a significant decline in Hgb is observed during EBRT, creating a hypoxic cellular environment. This hypoxic state exacerbates an already increased production of ROS triggered by the RT. The inability of patients to mount an effective adaptive response to increased ROS production can result in skeletal muscle dysfunction (Westerblad & Allen, 2011).

The etiology of CRF is complex, as our findings demonstrate. There were no significant associations between mitochondrial enzymes and fatigue scores during EBRT; however, the results are limited by the small sample size and the exploratory nature of the group comparisons. Additionally, the differentiation between HF and LF scores on the FACT-F does not control for baseline fatigue levels; however, we noted no differences between groups in the baseline fatigue scores. Further investigations, using larger samples, more diverse populations, and perhaps an optimal mitochondrial-specific biologic sample, are warranted to investigate potential associations between mitochondrial function and CRF.

Conclusions

In this study, we explored the association between levels of mitochondrial enzymes and fatigue intensity in men with non-metastatic PC receiving EBRT. The study findings did not reveal an association between these two variables, suggesting that other mechanisms may be involved in the complex etiology of CRF, such as the adaptive response to oxidative stress. The importance of understanding the etiology of CRF cannot be overstated because the clinical implications of an increased ability to personalize cancer therapy and prospectively

attenuate toxicity risk are significant. Furthermore, providing this information to patients and their families would help them to make optimal treatment decisions.

Acknowledgments

The authors would like to acknowledge Dr. Dan Wang for her help in the conduct of the laboratory experiments and Dr. Rebekah Feng for her help with data interpretation.

Author Contribution

Kristin Filler contributed to conception, design, acquisition, analysis, and interpretation; drafted and critically revised manuscript; gave final approval; and agrees to be accountable for all aspects of work ensuring integrity and accuracy. Debra Lyon contributed to conception, design, and interpretation; critically revised manuscript; gave final approval; and agrees to be accountable for all aspects of work ensuring integrity and accuracy. Nancy McCain contributed to conception, design, and interpretation; critically revised manuscript; gave final approval; and agrees to be accountable for all aspects of work ensuring integrity and accuracy. James Bennett contributed to conception, design, and interpretation; critically revised manuscript; gave final approval; and agrees to be accountable for all aspects of work ensuring integrity and accuracy. R. K. Elswick contributed to conception, design, analysis, and interpretation; critically revised manuscript; gave final approval; and agrees to be accountable for all aspects of work ensuring integrity and accuracy. Nada Lukkahatai contributed to conception, design, analysis, and interpretation; critically revised manuscript; gave final approval; and agrees to be accountable for all aspects of work ensuring integrity and accuracy. Leorey Saligan contributed to conception, design, acquisition, analysis, and interpretation; drafted and critically revised manuscript; gave final approval; and agrees to be accountable for all aspects of work ensuring integrity and accuracy. Enrique Juan deAndrés-Galiana contributed to analysis and interpretation; critically revised manuscript; gave final approval; and agrees to be accountable for all aspects of work ensuring integrity and accuracy. Juan Luis Fernández-Martínez contributed to analysis and interpretation; critically revised manuscript; gave final approval; and agrees to be accountable for all aspects of work ensuring integrity and accuracy.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This research was supported by grants from the Jonas Nurse Leaders Scholar Program, the Jonas Center for Nursing Excellence/AACN, the Doctoral Degree Scholarship in Cancer Nursing (122565-DSCN-12-204-01-SCN) from the American Cancer Society, and the Graduate Partnership Program of the National Institutes of Health, National Institute of Nursing Research.

References

- American Cancer Society. (2014). *Prostate cancer*. Retrieved from <http://www.cancer.org/cancer/prostatecancer/detailedguide/index>
- Barsevick, A., Frost, M., Zwiderman, A., Hall, P., & Halyard, M. (2010). I'm so tired: Biological and genetic mechanisms of

- cancer-related fatigue. *Quality of Life Research*, 19, 1419–1427. doi:10.1007/s11136-010-9757-7
- Borneman, T., Piper, B., Koczywas, M., Munevar, C., Sun, V., Uman, G., & Ferrell, B. (2012). A qualitative analysis of cancer-related fatigue in ambulatory oncology. *Clinical Journal of Oncology Nursing*, 16, E26–E32. doi:10.1188/12.CJON.E26-E32
- Budäus, L., Bolla, M., Bossi, A., Cozzarini, C., Crook, J., Widmark, A., & Wiegel, T. (2011). Functional outcomes and complications following radiation therapy for prostate cancer: A critical analysis of the literature. *European Urology*, 61, 112–127. doi:10.1016/j.eururo.2011.09.027
- Cella, D., Eton, D. T., Lai, J. S., Peterman, A. H., & Merkel, D. E. (2002). Combining anchor and distribution based methods to derive minimal clinically important differences on the Functional Assessment of Cancer Therapy (FACT) Anemia and Fatigue scales. *Journal of Pain and Symptom Management*, 24, 547–561.
- Cella, D., Nichol, M. B., Eton, D., Nelson, J. B., & Mulani, P. (2009). Estimating clinically meaningful changes for the Functional Assessment of Cancer Therapy–Prostate: Results from a clinical trial of patients with metastatic hormone-refractory prostate cancer. *Value in Health*, 12, 124–129. doi:10.1111/j.1524-4733.2008.00409.x
- Cheng, L., Montironi, R., Bostwick, D., Lopez-Beltran, A., & Berney, D. (2012). Staging of prostate cancer. *Histopathology*, 60, 87–117. doi:10.1111/j.1365-2559.2011.04025.x
- Cheville, A. L. (2009). Cancer-related fatigue. *Physical Medicine & Rehabilitation Clinics of North America*, 20, 405–416. doi:10.1016/j.pmr.2008.12.005
- Dehne, N., & Brüne, B. (2014). Sensors, transmitters, and targets in mitochondrial oxygen shortage-A hypoxia-inducible factor relay story. *Antioxidants & Redox Signaling*, 20, 339–352. doi:10.1089/ars.2012.4776
- El Tazi, M., & Errihani, H. (2011). Evaluation and management of fatigue in oncology: A multidimensional approach. *Indian Journal of Palliative Care*, 17, 92–97. doi:10.4103/09731075.84528
- Esper, P., Mo, F., Chodak, G., Sinner, M., Cella, D., & Pienta, K. J. (1997). Measuring quality of life in men with prostate cancer using the Functional Assessment of Cancer Therapy Prostate instrument. *Urology*, 50, 920–928. doi:10.1016/S0090-4295(97)00459-7
- Gimenez-Roqueplo, A. P., Favier, J., Rustin, P., Mourad, J. J., Plouin, P. F., Corvol, P., . . . Jeunemaitre, X. (2001). The R22X mutation of the SDHD gene in hereditary paraganglioma abolishes the enzymatic activity of complex II in the mitochondrial respiratory chain and activates the hypoxia pathway. *American Journal of Human Genetics*, 69, 1186–1197.
- Given, B. (2008). Cancer-related fatigue: A brief overview of current nursing perspectives and experiences. *Clinical Journal of Oncology Nursing*, 12, 7–9. doi:10.1188/08.CJON.S2.7-9
- Haas, R., Parikh, S., Falk, M., Saneto, R., Wolf, N., Darin, N., & Cohen, B. (2007). Mitochondrial disease: A practical approach for primary care physicians. *Pediatrics*, 120, 1326–1333. doi:10.1542/peds.2007-0391
- Hofman, M., Ryan, J. L., Figueroa-Moseley, C. D., Jean-Pierre, P., & Morrow, G. R. (2007). Cancer-related fatigue: The scale of the problem. *Oncologist*, 12, 4–10. doi:10.1634/theoncologist.12-S1-4
- Johnson, M., Bland, M., Davidson, P., Newton, P., Oxberry, S., Abernethy, A., & Currow, D. (2014). The relationship between two performance scales: New York Heart Association Classification and Karnofsky Performance Status Scale. *Journal of Pain and Symptom Management*, 47, 652–658. doi:10.1016/j.jpainsymman.2013.05.006
- Kim, J., Wei, Y., & Sowers, J. (2008). Role of mitochondrial dysfunction in insulin resistance. *Circulation Research*, 102, 401–414. doi:10.1161/CIRCRESAHA.107.165472
- Langston, B., Armes, J., Levy, A., Tidey, E., & Ream, E. (2013). The prevalence and severity of fatigue in men with prostate cancer: A systematic review of the literature. *Supportive Care in Cancer*, 21, 1761–1771. doi:10.1007/s00520-013-1751-5
- Lustgarten, M. S., Jang, Y. C., Liu, Y., Qi, W., Qin, Y., Dahia, P. L., . . . van Remmen, H. (2011). MnSOD deficiency results in elevated oxidative stress and decreased mitochondrial function but does not lead to muscle atrophy during aging. *Aging Cell*, 10, 493–505. doi:10.1111/j.1474-9726.2011.00695.x
- Mitchell, S., & Berger, A. (2011). Fatigue. In V. DeVita, T. Lawrence, & S. Rosenberg (Eds.), *Cancer: Principles and practice of oncology* (pp. 2387–2392). Philadelphia, PA: Lippincott, Williams, & Wilkins.
- National Comprehensive Cancer Network. (2012). *NCNN guidelines for supportive care: Cancer-related fatigue*. Retrieved from http://www.nccn.org/professionals/physician_gls/f_guidelines.asp#fatigue
- Piper, B. F., & Cella, D. (2010). Cancer-related fatigue: Definitions and clinical subtypes. *Journal of the National Comprehensive Cancer Network*, 8, 958–966.
- Piper, B. F., Dibble, S. L., Dodd, M. J., Weiss, M. C., Slaughter, R. E., & Paul, S. M. (1998). The revised Piper Fatigue Scale: Psychometric evaluation in women with breast cancer. *Oncology Nursing Forum*, 25, 677–684.
- Ryan, J. L., Carroll, J. K., Ryan, E. P., Mustian, K. M., Fiscella, K., & Morrow, G. R. (2007). Mechanisms of cancer-related fatigue. *Oncologist*, 12, 22–34. doi:10.1634/theoncologist.12-S1-22
- Sena, L., & Chandel, N. (2012). Physiological roles of mitochondrial reactive oxygen species. *Molecular Cell*, 48, 158–167. doi:10.1016/j.molcel.2012.09.025
- Wang, X. S. (2008). Pathophysiology of cancer-related fatigue. *Clinical Journal of Oncology Nursing*, 12, 11–20. doi:10.1188/08.CJON.S2.11-20
- Wei, J., Dunn, R., Sandler, H., McLaughlin, P., Montie, J., Litwin, M., . . . Sanda, M. (2002). Comprehensive comparison of health-related quality of life after contemporary therapies for localized prostate cancer. *Journal of Clinical Oncology*, 20, 557–566.
- Westerblad, H., & Allen, D. (2011). Emerging roles of ROS/RNS in muscle function and fatigue. *Antioxidants & Redox Signaling*, 15, 2487–2499. doi:10.1089/ars.2011.3909
- Yellen, S. B., Cella, D. F., Webster, K., Blendowski, C., & Kaplan, E. (1997). Measuring fatigue and other anemia-related symptoms with the Functional Assessment of Cancer Therapy (FACT) measurement system. *Journal of Pain and Symptom Management*, 13, 63–74.