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Relationship of Mitochondrial Enzymes to Fatigue Intensity and Health-Related Quality of Life in Men with Prostate Cancer Receiving External Beam Radiation Therapy

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A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at Virginia Commonwealth University.

by

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Abstract

RELATIONSHIP OF MITOCHONDRIAL ENZYMES TO FATIGUE INTENSITY AND HEALTH-RELATED QUALITY OF LIFE IN MEN WITH PROSTATE CANCER RECEIVING EXTERNAL BEAM RADIATION THERAPY

By Kristin A. Filler, PhD, RN

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at Virginia Commonwealth University.

Virginia Commonwealth University, 2014.

Major Director: Nancy L. McCain, PhD, RN, FAAN
Nursing Alumni Distinguished Professor, Adult Health and Nursing Systems, School of Nursing

Introduction: Cancer-related fatigue is often described by patients as a lack of energy, mental or physical tiredness, diminished endurance, and prolonged recovery after activity. Etiologic mechanisms underlying CRF are not well understood.

Methods: A literature review was conducted to examine studies that had investigated the association of mitochondrial dysfunction with fatigue. The major conclusion from this review was that alterations in energy metabolism may contribute to fatigue. Therefore, the dissertation study focused on laboratory techniques for measuring mitochondrial oxidative phosphorylation enzymes (complexes I-V) and a mitochondrial-specific oxidative stress marker (superoxide dismutase 2 [SOD2]). The primary aim of the dissertation research was to describe levels of biomarkers of mitochondrial function, fatigue, and health-related quality of life (HRQOL) before and at the completion of external beam radiation therapy (EBRT) in men with nonmetastatic

prostate cancer (NM-PC). To achieve this aim a secondary analysis of a descriptive, longitudinal study was conducted (#10-NR-0128).

Results: A total of n = 22 men with NM-PC were included in this study. There were significant increases in fatigue and a significant decrease HRQOL from baseline to the completion of EBRT. However, there was no significant change in the biomarkers of mitochondrial function from baseline to EBRT completion. Given the exploratory nature of the study, it was decided to further investigate the patient sample to understand the relationship of fatigue and mitochondrial function in a well-characterized fatigue phenotype. There was preliminary evidence to support the possibility of distinct patterns of mitochondrial enzyme levels between those with a high intensification of fatigue and those with a low intensification of fatigue during EBRT; however, these differences were not statistically significant.

Discussion: To our knowledge, this is the first study to describe the relationship between mitochondrial enzymes and fatigue before and during EBRT in men with NM-PC. The most important preliminary finding from this study is the possibility that mitochondrial enzymes might be related to fatigue intensification during EBRT. Future studies will be critical to determine if these preliminary findings are replicable, and if so, whether there are potential therapeutic targets in individuals at highest risk for fatigue intensification during EBRT.

Chapter One

My experience as an oncology nurse has exposed me to the clinical issues surrounding quality of life in patients with cancer, especially those issues surrounding symptom management. My experiences as both a doctoral student and research assistant have provided me with the knowledge and theoretical foundation in which to conduct symptom mechanism and biobehavioral research in the oncology population. An enhanced understanding of the etiology of symptoms can lead to the development of more targeted and individualized management strategies to improve health-related quality of life in this population.

My dissertation research focused on understanding the role that mitochondria might play in the development of cancer-related fatigue during external beam radiation therapy. To identify areas needing further research, a review of the literature was conducted to examine markers of mitochondrial function that may have an association with fatigue in order. Dysfunctions in the mitochondrial structure, mitochondrial function (as indicated by mitochondrial enzymes and oxidative/nitrosative stress), mitochondrial energy metabolism (ATP production and fatty acid metabolism), immune response, and genetics were investigated as potential contributors to fatigue. A detailed description of this literature review was published in the journal *BBA Clinical* and is presented as Chapter Two.

The findings from this literature review guided the development of the dissertation proposal. The primary aim of the dissertation research was to describe levels of biomarkers of

mitochondrial function, fatigue, and health-related quality of life (HRQOL) before and at the completion of EBRT in men with nonmetastatic prostate cancer. The specific aims were to: (1) describe levels of biomarkers of mitochondrial function (mitochondrial oxidative phosphorylation enzymes [complexes I-V] and the antioxidant manganese superoxide dismutase 2 [MnSOD2]), fatigue and HRQL before and at completion of EBRT, and (2) examine relationships over time in levels of biomarkers of mitochondrial function, fatigue, and HRQOL. Following the initial analyses, an exploratory aim evolved to compare levels of biomarkers of mitochondrial function in men with little increase in fatigue from baseline to completion of EBRT to those with greater increases in fatigue from baseline to completion of EBRT.

There were significant increases in fatigue and a significant decrease in HRQOL from baseline to the completion of EBRT; however, there were no significant differences in mitochondrial enzymes from baseline to completion of EBRT. Due to the exploratory nature of this study, the participant sample was then categorized based upon their fatigue intensification during EBRT. Once categorized, we found preliminary evidence to support the possibility of patterns of mitochondrial enzyme levels between the two fatigue groups; however, these differences were not statistically significant. A detailed description of this dissertation research is presented as Chapter Three.

To our knowledge, this is the first study that explored measures of mitochondrial enzyme function in relationship to fatigue prior to and at the completion of EBRT in men with prostate cancer. Future studies will be critical to determine if these preliminary findings are replicable, and if so, whether there are potential therapeutic targets in individuals at highest risk for fatigue intensification during EBRT, in order to optimize fatigue management and improve HRQOL. Future directions for this line of research are discussed in Chapter Four.

Chapter Two

1. Introduction

Mitochondria are increasingly recognized as major contributors to human health and disease because of their location and influence (Cohen & Gold, 2001). Mitochondria have an essential role in energy production through the process of oxidative phosphorylation where nutrients are converted into adenosine triphosphate (ATP), which powers many of the cells' activities. In addition to energy production, mitochondria have been implicated in various physiologic processes including the production of reactive oxygen species (ROS), pyrimidine and lipid biosynthesis, regulation of cellular levels of substrates (amino acids, metabolites, enzyme cofactors), apoptosis, metal (Fe-S cluster and heme) metabolism, calcium homeostasis and flux, neurotransmitter synthesis, heat production, and insulin secretion (Duchen, 2004; Nunnari & Suomalainen, 2012; Pieczenik & Neustadt, 2007). Therefore, damage to mitochondria can have widespread consequences (Duchen, 2004).

Health conditions such as cancer, diabetes, fibromyalgia, and serious mental disorders such as schizophrenia and bipolar disease are also proposed to result from mitochondrial dysfunction, though the links are less clear (Pieczenik & Neustadt, 2007; Reynolds, 2007). Mechanisms underlying mitochondria–related disease states have predominantly focused on DNA damage and ROS generation (Cohen & Gold, 2001; Pieczenik & Neustadt, 2007). Mitochondrial dysfunction can be of primary (inherent) or secondary (acquired dysfunction)

origin (Cohen & Gold, 2001; Read & Calnan, 2000). Primary dysfunction results from mitochondrial DNA (mtDNA) mutations inherited from mothers, who are the sole contributors of mitochondria to their offspring (Cohen & Gold, 2001). Mitochondrial DNA has a much higher mutation rate than nuclear DNA because it lacks protective histones (Read & Calnan, 2000), is readily exposed to damage from ROS production (Alexander et al., 2010; Pieczenik & Neustadt, 2007; Reynolds, 2007), and lacks certain DNA repair mechanisms (Cohen & Gold, 2001). Secondary mitochondrial dysfunction results from the influence of external mechanisms such as environmental or pharmacologic toxins that can damage the mtDNA (Cohen & Gold, 2001). Mitochondria can protect themselves from the accumulation of damage through various quality control mechanisms (e.g., fission and fusion) (Nunnari & Suomalainen, 2012; Youle & van der Bliek, 2012); however, if these mechanisms are altered, mitochondrial dysfunction can lead to disease (Blackstone & Chang, 2011; Duchen, 2004). Past research has predominantly focused on the role of mitochondrial dysfunction on disease pathology. However, some studies have investigated how mitochondrial dysfunction is associated with the development of distressing symptoms such as fatigue, neuropathic pain, weakness, and depression (Pieczenik & Neustadt, 2007); however, these investigations are still in their infancy. This paper reviewed studies that investigated the association of mitochondrial dysfunction with fatigue.

Fatigue is a hallmark symptom of mitochondrial disease. Fatigue is often described by patients as a lack of energy, mental or physical tiredness, diminished endurance, and the need for a prolonged recovery after physical activity (Rosenthal, Majeroni, Pretorius, & Malik, 2007). Fatigue is reported by patients to be unrelieved by rest (Jason, Evans, Brown, & Porter, 2010; Rosenthal et al., 2008). As pervasive and debilitating as fatigue is, the etiology of fatigue remains poorly understood. Without a known pathophysiological mechanism for fatigue, there is

minimal consistency in its clinical definition (Alexander et al., 2010; Hardy & Studenski, 2010; Jason et al., 2010; Swain, 2000). Furthermore, the lack of a proper clinical definition of fatigue contributes to its underdiagnosis and poor management, which in turn contributes to increased symptom burden and poorer quality of life in patients with fatigue (Norheim, Jonsson, & Omdal, 2011). The purpose of this systematic review was to examine markers of mitochondrial function (in adults) that have evidence of an association with fatigue in order to identify areas needed for further research. In this paper markers of mitochondrial function that associate with fatigue in adult patients were reviewed in order to describe empirical evidence of a relationship between mitochondrial dysfunction and fatigue and to propose possible research directions that would enhance understanding of the role of mitochondrial dysfunction in fatigue.

2. Methods

An initial literature query was conducted in the following four reference databases using search strategies as summarized in Table 1. The initial search resulted in 2,055 articles. After removing duplicates, the abstracts of 1,220 articles were assessed for relevance to the area of interest. Abstracts that discussed the association of markers of mitochondrial function and fatigue were selected to be included in this review. In addition, studies were excluded if they were more than 20 years old, were not original research, were animal or cell-based studies, investigated the effect of medication or treatment on fatigue, mitochondrial markers or both, or measured induced fatigue through the use of exercise or electric stimulation. Also excluded were letters, literature reviews, notes, conference or meeting abstracts, book chapters, editorials, dissertations, case reports or series, short reports, workshop reports, and practice guidelines. A total of 54 articles were selected for full-text review. Of these, 25 were excluded based upon the

aforementioned criteria, 3 were excluded because they focused on neuroimaging, and 1 was excluded because it included children. A total of 25 articles were selected for full review.

Table 1. Search Criteria

Database	Search Terms	Filters	Yield
PubMed	mitochondria OR mitochondrial AND fatigue	Humans English	N=358
Scopus	(TITLE(mitochondria OR mitochondrial) AND TITLE(fatigue)) (TITLE(mitochondria OR mitochondrial) AND TITLE(fatigue))	English	N=519
Web of Science	Topic=(mitochondria OR mitochondrial) AND Topic=(fatigue)	English	N=624
Embase	'fatigue'/exp AND ('mitochondrion'/exp OR 'mitochondrial respiration'/exp OR 'mitophagy'/exp OR 'mitochondrial dna'/exp OR 'disorders of mitochondrial functions'/exp OR 'mitochondrial dynamics'/exp OR 'mitochondrial energy transfer'/exp OR 'mitochondrial enzyme'/exp OR 'mitochondrial gene'/exp OR 'mitochondrial genome'/exp OR 'mitochondrial membrane potential'/exp OR 'mitochondrial protein'/exp OR 'mitochondrion swelling'/exp)	Humans English	N=554

3. Results

Twelve of the 25 articles (48%) were published within the last five years. Twenty-two (88%) of the studies used a cross-sectional design; three used a repeated-measures design. Eighteen studies investigated only patients with chronic fatigue syndrome (CFS); remaining studies investigated a combination of myalgic encephalomyelitis (ME) and CFS (n=2), ME (n=1), multiple sclerosis (n=1), HIV-related fatigue (HRF) (n=1), systemic lupus erythematosus (SLE) (n=1), and cancer-related fatigue (CRF) (n=1). One study restricted participant gender and included only males (Hsiao, Wang, Kaushal, & Saligan, 2013). A complete description of study attributes are found in Tables 2 and 3.

Table 2. Studies Investigating Mitochondrial Dysfunction in CFS and/or ME

Authors	Study Design	Sample Characteristics	Fatigue Definition	Fatigue Measurement	Mitochondrial Marker Assessed	Sample Source	Association to Fatigue
Edwards, et al. (1993).	cross-sectional, descriptive	n= 74 CFS patients *Controls*: a. n= 34 patients with myalgia b. n= 22 asymptomatic controls	not specified	CFS diagnosis (criteria/guidelines not specified)	1. mitochondrial hyperplasia 2. cytochrome c oxidase 3. myoadenylate deaminase	muscle biopsy from either: a. tibialis anterior b. quadriceps c. gastrocne-mius, medial head	No significant differences between CFS patients and controls.
Kuratsune, et al. (1994).	cross-sectional, descriptive	n= 38 CFS patients *Controls*: n= 308 healthy volunteers	CDC 1988 criteria	CFS diagnosis according to CDC criteria	1. free L-carnitine 2. acylcarnitine	serum	Free L-carnitine levels lower in male CFS patients and higher in female CFS compared to controls; however, not statistically significant. Acylcarnitine levels lower ($p < .001$) in CFS patients compared to controls. In CFS patients, lower acylcarnitine levels associated with worse performance and increased symptom burden at initial exam. Upon symptom improvement, acylcarnitine levels increased ($p < .02$).
Behan et al. (1995).	cross-sectional, descriptive	n= 31 CFS patients *Controls*: n= 20 volunteers with no muscle disease	CDC 1988 criteria	CFS diagnosis according to CDC criteria	1. histological 2. histochemical 3. ultrastructural	vastus lateralis muscle biopsy	Size and morphology of mitochondria showed differences between CFS and controls. No statistical data provided.
Plioplys et al. (1995a).	cross-sectional	n= 15 CFS patients *Controls*: n= 15; age and sex-matched healthy volunteers	1. CDC 1988 & 1994 criteria 2. British & Australian definitions for CFS	1. Fatigue Severity Scale (FSS) 2. Beck Depression Inventory (BDI) 3. Symptom Checklist 90-R 4. CFS Impairment Index (CFS-II). 5. Structural Clinical Interview for the DSM III-R-Nonpatient Edition	ultrastructural exam of mitochondria	right vastus lateralis muscle biopsy	No significant differences upon structural exam between CFS patients and controls.
					carnitine levels	serum	Negative association between acylcarnitine levels and CFS-II mental index score ($r = -0.761$, $p < 0.01$) and total score ($r = -0.634$, $p < 0.05$).

Plioplys et al. (1995b).	cross-sectional	n= 35 CFS patients *Controls*: a. Mayo clinic normative data (n=85) b. historical study (Kuratsune, et al. 1994.)	CDC 1988 criteria	1. FSS 2. BDI 3. Symptom Checklist 90-R 4. CFS-II.	carnitine levels: total, free, aceylcarnitine	serum	Total carnitine lower in female ($p < .001$) and male ($p < .05$) CFS patients compared to Mayo clinic data. Free carnitine lower in female ($p < .01$) and male ($p < .05$) CFS patients compared to Mayo clinic data. Acylcarnitine lower in CFS patients compared to historical study controls ($p < .00001$). Free carnitine lower in CFS patients compared to historical controls ($p < .00001$). Total carnitine lower in CFS patients compared to historical controls ($p < .00001$). Free carnitine correlated with CFS-II physical impairment subset ($r = .412$, $p < .05$). Negative correlation between FSS and free carnitine ($r = -.496$, $p = .02$), and total carnitine and FSS ($r = -.473$, $p = .02$).
McArdle et al. (1996).	cross-sectional	n= 54 CFS patients only included n= 34 for viral analysis *Controls*: a. for enzyme comparison n = 16 from a previous study b. for RNA detection n= 10 patients undergoing orthopedic surgery; no muscle damage or fatigue.	1. diagnosis with CFS on the basis of complaints of muscle pain and fatigue 2. diagnosis conformed to the Oxford Consensus Criteria	Not mentioned	1. mitochondrial enzymes: a. citrate synthase b. succinate reductase c. cytochrome-c oxidase 2. presence of enteroviral RNA	anterior tibialis muscle biopsy	Reduction in all 3 mitochondrial enzyme activities ($p < .05$) in CFS patients compared to control values from a previous study. Failed to detect evidence of enteroviral RNA.
Behan et al. (1999).	cross-sectional	n= 16 CFS patients *Controls*: n =10 healthy volunteers	CDC 1994 criteria.	CFS diagnosis according to CDC criteria	1. Aerobic capacity a. pyruvate b. lactate c. L/P ratio d.	Right or left vastus lateralis muscle biopsy	Increased pyruvate levels in CFS patients ($p = .053$). All other biological parameters showed no

					respiratory capacity e. cytochrome-c oxidase f. LDH 2. DNA analysis a. total mtDNA volume b. mtDNA rearrangements c. point mutation at nucleotide pair 3243 d. two deletions: mtDNA 4977 mtDNA 7436 3. Histological, Histochemical, and Ultrastructural examination		significant findings between the groups.
Soetekouw et al. (2000).	cross-sectional	n= 25 Caucasian, female CFS patients *Controls*: n= 25 age- and sex- matched healthy volunteers	1. CDC 1994 criteria. 2. Fatigue with substantial ADL impairment: a. a score of 35+ on the Checklist Individual Strength subjective fatigue subscale b. a score of 750+ on the weighted total score of the Sickness Impact Profile.	1. Checklist Individual Strength (CIS) 2. Sickness Impact Profile (SIP)	1. carnitine levels: total, free, & acylcarnitine 2. carnitine ester profiles	serum	CFS patients were more fatigued ($p < .001$) and had more functional impairment ($p < .001$) as determined by questionnaires. No significant differences with any of the biologic markers between the groups.
Kurup et al. (2003b).	cross-sectional	n= 15 CFS patients *Controls*:	CDC criteria	structured clinical interview to assess CFS and comorbid conditions	Mitochondrial markers: 1. ubiquinone 2. ROS/RNS	RBCs and plasma/serum	1. Ubiquinone lower in CFS patients ($F = 259.36$, $p < .01$)

		n= 45 age and sex-matched healthy volunteers			a. MDA b. Hydroperoxide c. conjugated dienes d. NO 3. antioxidants a. glutathione b. SD c. catalase d. GSH peroxidase e. GSH reductase		2. ROS markers higher in CFS patients a. MDA ($F= 4.56$, $p < 0.05$) b. Hydroperoxide ($F= 3.25$, $p < 0.05$) c. conjugated dienes ($F= 16.21$, $p < 0.01$) d. NO ($F= 6.54$, $p < 0.05$) 3. Antioxidants lower in ME patients a. glutathione ($F= 8.36$, $p < 0.05$) b. SD ($F= 7.56$, $p < 0.05$) c. catalase ($F= 3.98$, $p < 0.05$) d. GSH peroxidase ($F= 11.26$, $p < 0.01$) e. GSH reductase ($F= 4.26$, $p < 0.05$)
Kaushik et al. (2005).	repeated measures: two time points 6 months apart during which symptoms did not vary significantly	n= 25 CFS patients *Controls*: 1. Microarray, n= 25 age- and sex- matched normal blood donors 2. Real-time PCR, n =21 age- and sex-matched normal blood donors	CDC 1994 criteria.	1. diagnosis of CFS according to CDC criteria 2. Chalder Fatigue Scale	Real-time PCR	Peripheral blood mononuclear cells (PBMCs)	16 genes differentially expressed in CFS patients (15 genes up-regulated, 1 down-regulated). 3 up-regulated genes are located in the mitochondria: a. *EIF2B4* ($p = 1.8 \times 10^{-5}$) b. *EIF4G1* ($p = 7.63 \times 10^{-13}$) c. *MRPL23* ($p = 1.25 \times 10^{-6}$) 2 up-regulated genes for peroxisomal function, *ABCD4* ($p = .00190$ and *PEX16* ($p = .0126$), suggesting enhanced defense to oxidative damage.
Vernon et al. (2006).	repeated measures: baseline, 2-3 weeks, 4-6 weeks, 3 months, 6 months (in those with symptoms), & 12 months.	n= 5 with symptoms suggestive of infectious mononucleosis with provisional lab confirmation *Controls*: n= 5 controls that recovered promptly from infectious mononucleosis; HLA –A and -B, sex, and age-matched.	CDC 1994 criteria	1. diagnosis of CFS according to CDC criteria 2. self-report and interview assessment of psychological and physical health.	gene transcription patterns	PBMCs	Due to small *n* in each group, data was categorized by time periods: early (baseline-3 months) and late (> 6 months following disease onset). *Early Phase*: 23 genes differentially expressed between cases and controls; 8 expressed in cases and involved binding and metabolism ontologies. *Early & Late Phase*: 24 genes significantly different between cases and controls; 12 genes are associated with mitochondrial function.

Hokama et al. (2008).	cross-sectional	n= 328 CFS patients n= 18 CCFP patients n= 8 GWVs n= 15 PC patients n= 49 normal, healthy controls	CDC 1994 criteria	CFS diagnosis according to CDC criteria	1. Phospholipids 2. Anti-cardiolipin (aCL) antibodies	serum	CFS, CCCP, GWV, and PC patients have cardiolipin associated with mitochondrial membrane. The presence of aCL was also detected.
Hokama et al. (2009).	cross-sectional	n= 40 CFS patients	CDC 1994 criteria	CFS diagnosis according to CDC criteria	Anti-cardiolipin antibodies	serum	IgM isotope present in 95% of CFS patients. IgG isotype present in 10% and the IgA isotype present in 5% of CFS patients.
Maes et al. (2009b).	cross-sectional	n= 35 major depressed patients; n= 17 patients had a diagnosis with CFS *Controls*: n= 22 healthy volunteers	1. 1994 CDC criteria 2. Criteria: a. severe chronic fatigue for at least 6 months b. at least 4 additional symptoms from a checklist	1. CFS diagnosis according to CDC criteria 2. Fibromyalgia and CFS Rating Scale (FF scale)	CoQ10 levels	plasma	Depressed patients with CFS had lower plasma CoQ10 than depressed patients without CFS ($F = 8.7$, $df = 1/33$, $p = .006$). CFS independently predicted low CoQ10 values ($F = 4.3$, $df = 1/31$, $p = .04$).
Myhill, et al. (2009)	cross-sectional	n= 71 CFS patients *Controls*: n= 53 normal, healthy volunteers	CDC 1994 criteria	1. CFS diagnosis according to CDC criteria 2. CFS Ability Scale	1. "ATP Profile" test: a. ATP concentration and ATP ratio b. The efficiency of ox-phos process c. Translocator (TL) protein function (TL-OUT and TL-IN) 2. Mitochondrial Energy Score (MES)	neutrophils	Patients grouped into 3 groups based on CFS Ability Scale scores: very severe (VS), severe (S), and moderate (M). For most of the 5 factors of the "ATP Profile Test" the percentage of patients who are in the normal region increases from VS, S, to M. The MES is highly correlated with the CFS Ability scale ($R^2 = .645$, $p < .001$).
Pietrangelo et al. (2009).	cross-sectional	n= 4 CFS patients n= 2 female patients, meeting CDC criteria n= 2 male patients	CDC 1994 criteria	1. CFS diagnosis according to CDC criteria 2. skeletal muscle membrane testing	Global transcriptome analysis	aaRNA obtained from vastus lateralis muscle biopsy	47 genes significantly altered in CFS patients: 2 up-regulated, 38 down-regulated and 7 up-regulated in females, but down-regulated in males. *Gene Pathways*:

		meeting CDC criteria for CFS *Controls*: n= 9 healthy volunteers					Control of Ox-Phos; 3 mitochondrial genes were down-regulated: *SOD2*, *FDX1*, and *NQO1*. Energy Balance; depressed transcription of several genes implicated in the energy metabolism: *PFKFB3*, *PDK4*, *GOT1*, *AMPD3*, and *ATP-binding cassette member* 5. One gene, *VLDLR* was up-regulated Apoptosis; *FOS*, *MYC*, *SOX17*, *AATF*, *CEBPD* were all down-regulated.
Reuter, et al. (2011).	cross-sectional	n= 44 CFS patients *Controls*: n= 49 age and gender-matched healthy subjects	Royal Australasian College of Physicians CFS clinical practice guidelines	1. medical diagnosis of CFS using the Royal Australasian College of Physicians CFS clinical practice guidelines 2. FSS	1. Endogenous carnitine: total, L-, and acylcarnitine 2. 35 individual carnitines	plasma	CFS patients had lower individual carnitines: C8:1 (p = .0201), C14 (p = .0023), C16:1 (p = .0383), C18 (p = .0104), C18:1 (p < .0001) , and C18:2 (p < .0001). CFS patients had higher C12DC (p < .0001) and C18:1-OH (p = .0191). Negative correlation between FSS scores and C8:1, C14, C16:1, specifically with C18:1 (R = -.3547, p = .0009) and C18:2 (R = -.4191, p < .0001). Significant positive correlations between fatigue severity and C12DC, C16, and C18:1-OH.
Smits et al. (2011).	cross-sectional	n= 16 CFS patients *Controls*: n= 11 male healthy volunteers	1. CDC 1994 criteria 2. Severe fatigue determined as > 35 on the fatigue subscale of the CIS. 3. Fatigue longer than 6 months 4. Fatigue not explained by somatic or psychiatric illness or ongoing exertion and	1. diagnosis of CFS according to CDC criteria 2. CIS 3. SIP-8.	1. ATP production rate 2. Respiratory chain complexes activity (Complex I, II+III, II, and IV) 3. Citrate Synthase levels	right quadriceps muscle biopsies	No significant differences in ATP production or respiratory chain complex activity in CFS patients. Citrate synthase levels were lower in CFS patients (p < .001).

			is not relieved by rest. 5. myalgia and/or exercise intolerance. 6. Substantial functional impairment determined by a score of 700+ on the SIP-8.				
Maes et al. (2009a).	cross-sectional	n= 58 ME/CFS patients *Controls*: n= 22 healthy volunteers	1994 CDC criteria	1. CFS diagnosis 2. FF scale	CoQ10 levels	plasma	Plasma CoQ10 lower in ME/CFS patients ($F = 31.0$, $df = 1/78$, $p = .00001$) Negative association between CoQ10 and total FF scale score ($r = .28$, $p = .03$). Negative correlation between CoQ10 and fatigue ($r = -.86$, $p < 10^{-5}$).
Booth et al. (2012).	cross-sectional	n= 61 ME/CFS patients (Cohort 1; still ill after interventions; from previous study) n= 138 ME/CFS patients (Cohort 2; no prior interventions) *Controls*: n= 53 normal, healthy volunteers (Myhill et al., 2012)	1. CDC 1994 criteria 2. ICCME (most, if not all were met)	1. CFS diagnosis according to CDC criteria 2. the Bell CFS Ability Scale	1. 5 parameters of the ATP Profile test: a. ATP^{mg} b. ATP_{end} c. Ox-Phos d. TL OUT e. TL IN 2. MES 3. Nfn 4. MESinh	neutrophils	ME/CFS patients had reduced ATP production Mitochondrial dysfunction, mainly through partial blockage of the TL was demonstrated in both cohorts. It was observed that neutrophils used at least two different pathways to compensate for mitochondrial dysfunction.

Table 3. Studies Investigating Mitochondrial Dysfunction in Other Fatigued Populations

Authors	Study Design	Sample Characteristics	Fatigue Definition	Fatigue Measurement Technique	Mitochondrial Dynamic Assessed	Sample Source	Findings
Fukazawa et al. (1996).	cross-sectional	n= 25 MS patients; n= 11 with disabling fatigue and n= 14 without fatigue *Controls*: n= 25 age- and sex-matched healthy volunteers	1. debilitating, persistent, or relapsing fatigue noted after the onset of MS 2. lack of other causes of fatigue based on a history, physical examination, and laboratory tests.	Medical diagnosis of fatigue	carnitine levels: total, free, and acylcarnitine	serum	No significant differences in carnitine levels between the groups.
Kurup et al. (2003a).	cross-sectional	n= 15 ME patients *Controls*: n= 45 age and sex-matched healthy volunteers	CDC criteria	structured clinical interview to assess CFS and comorbid conditions	Mitochondrial markers: 1. ubiquinone 2. ROS/RNS a. MDA b. Hydroperoxide c. conjugated dienes d. NO 3. antioxidants a. glutathione b. SD c. catalase d. GSH peroxidase e. GSH reductase	RBCs and plasma/serum	1. Ubiquinone lower in ME patients ($F = 259.36$, $p < .01$) 2. ROS markers higher in ME patients a. MDA ($F= 4.56$, $p < 0.05$) b. Hydroperoxide ($F= 3.25$, $p < 0.05$) c. conjugated dienes ($F= 16.21$, $p < 0.01$) d. NO ($F= 6.54$, $p < 0.05$) 3. antioxidants lower in ME patients a. glutathione ($F= 8.36$, $p < 0.05$) b. SD ($F= 7.56$, $p < 0.05$) c. catalase ($F= 3.98$, $p < 0.05$) d. GSH peroxidase ($F= 11.26$, $p < 0.01$) e. GSH reductase ($F= 4.26$, $p < 0.05$)
Segal et al. (2012).	cross-sectional	n= 71 SLE patients *Controls*: n= 51 healthy volunteers	not specified	1. Visual Analogue Scale-fatigue 2. FSS 3. Profile of Fatigue (ProF)	F_2-isoprostane	Plasma	SLE patients with fatigue had higher levels of F_2-isoprostane than non-fatigued SLE patients ($p = .0076$). Positive correlation between F_2-isoprostane and fatigue in SLE patients. F_2-isoprostane predicts higher FSS scores in SLE patients ($p = .0002$).
Hsiao et al. (2013).	repeated measures; Baseline Day 1,	n= 15 men with non-metastatic prostate cancer receiving ADT	not specified	revised Piper Fatigue Sale	radiation-induced changes in mitochondria-related gene	WBCs-RNA	Eleven genes related to mitochondrial function were differentially expressed over time

	Day 7, Day 14, Day 19/21, Day 38-42 of EBRT, and Day 68-72 after EBRT	and scheduled to receive EBRT. *Controls*: n= 15 age, gender, and race matched controls.			expression		during EBRT ($p < .05$). After Bonferroni, only *SLC25A23* was significantly down-regulated post-EBRT ($p = .008 - .02$). Eight of the 11 differentially expressed genes were significantly associated with fatigue scores ($p = .012 - .0003$).
Voss et al. (2013).	cross-sectional	n= 5 HIV patients with high fatigue n=5 HIV patients with low fatigue *Controls*: n= 5 healthy controls	HIV-related fatigue	revised 26-Item Piper Fatigue Scale	genomic (mitochondrial and nuclear) expression markers of mitochondrial dysfunction	CD14+ cells	Genes pertaining to mitochondrial function include: *CHD1L* (τ = -.49) and *ALDOB* (τ = -.62), *TIMM17B* (τ = .62), *GSR* (τ = .62), *IMMT* (τ = .57), and *SLC25A26* (τ = .62). 2 HIV-associated genes related to mitochondrial function, fatty acid metabolism: *ACAD9* (τ = .20) and *PPAR-alpha* (τ = -.44).

CFS= chronic fatigue syndrome; ME= myalgic encephalomyelitis; CDC= Centers for Disease Control and Prevention; DSM III-R = Diagnostic & Statistical Manual of Mental Disorders 3rd Edition Revised RNA = ribonucleic acid; L/P ratio = lactate/pyruvate ratio; DNA= deoxyribonucleic acid; mtDNA= mitochondrial DNA; ADLs= activities of daily living; ROS= reactive oxygen species; RNS= reactive nitrogen species; MDA= malondialdehyde; NO= nitric oxide; SD= superoxide dismutase; GSH= glutathione; RBCs= red blood cells; PCR= polymerase chain reaction; PBMCs= peripheral blood mononuclear cells; *EIF2B4*= eukaryotic translation initiation factor 2B, subunit 4δ, tv-1 ; *EIF4G1*= eukaryotic translation initiation factor 4γ, 1, tv-5; *MRPL23* = mitochondrial ribosomal protein L23; *ABCD4*= ATP binding cassette, subfamily D (ALD), member 4, tv-4 ; *PEX16*=peroxisomal biogenesis factor 16, tv-1; HLA = human leukocyte antigen; CCFP = chronic ciguatera fish poisoning; GWVs = Gulf War Veterans; PC = prostate cancer; IgM= immunoglobulin subtype M ; IgG= immunoglobulin subtype G ; CoQ10= Coenzyme Q10; ATP= adenosine triphosphate; TL-IN= transports ATP to cytosol; TL-OUT= transports ADP from cytosol to mitochondria; aaRNA = amino allyl RNA ; Ox-Phos= oxidative phosphorylation; *SOD2*= superoxide dismutase 2, mitochondrial; *FDX1*= ferredoxin 1; NQO1= nicotinamide adenine dinucleotide phosphate dehydrogenase quinone 1; *PFKFB3*= 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3(allosteric enzyme);; *PDK4* = pyruvate dehydrogenase kinase, isoenzyme 4 ; *GOT1*= glutamic-oxaloacetic transaminase 1; *AMPD3*= adenosine monophosphate deaminse (isoform E); *VLDLR* = very low density lipoprotein receptor; *FOS*= V-fos FBJ murine osteosarcoma viral oncogene homolog; *MYC* = v-myc myelocytomatosis viral oncogene homolog; *SOX17*= SRY-related HMG-box transcription factor;*AATF*= apoptosis antagonizing transcription factor; *CEBPD* = nuclear factor-IL6-beta; ICCME= International Consensus Criteria Myalgic Encephalomyelitis; ATP^{mg}= whole cell ATP measured by adding excess Mg; ATP_{end}= ATP measured with endogenous Mg only; Nfn= number of factors with normal; values; MES_{inh}= revised MES using the % ATP inhibited instead of TL IN; MS= multiple sclerosis; EBRT= external beam radiation therapy; WBCs= white blood cells; *SLC25A23* = solute carrier family 25, member 23; HIV= human immunodeficiency virus; CD14 = monocyte; LDH = lactate dehydrogenase.

CFS patients were the most studied (72% of all articles reviewed). CFS subjects were phenotyped by clinical diagnosis using the 1988 or 1994 Center for Disease Control (CDC) diagnostic criteria alone (n=17) or in combination with other developed criteria (n=3), such as the Australian definition for CFS (n= 1), the British definition for CFS (n=1), and the International Consensus Criteria for Myalgic Encephalomyelitis (ICCME) (n=1). Two studies

used other diagnostic guidelines; one used the Oxford Consensus criteria and one group used the diagnostic criteria established by the Royal Australasian College of Physicians Working Group.

Fatigue was defined either through clinical diagnosis alone or in combination with self-report questionnaires. Most articles (n=16) quantified fatigue using a variety of questionnaires such as the Fatigue Severity Scale (n=4), CFS Impairment Index (CFS-II) (n=2), Checklist Individual Strength (CIS) fatigue subscale (n=2), Sickness Impact Profile (n=2), Chalder Fatigue Scale (n=1), the Fibromyalgia and the CFS Rating Scale (FF Scale) (n=2), Symptom Checklist 90-R (n=2), CFS Ability Scale (n=2), the revised Piper Fatigue Scale (n=2), Visual Analogue Scale-fatigue (VAS) (n=1), the Profile of Fatigue (ProF) (n=1), and structured interviews (n=3).

A number of mitochondrial parameters were investigated as potential markers for the fatigue conditions. In about 70% (n=18) of the studies, mitochondrial biomarkers were obtained from peripheral blood samples; in the remaining 7 studies, lower extremity skeletal muscle biopsy specimens were used. Dysfunctions in the mitochondrial structure, mitochondrial function, mitochondrial energy metabolism, immune/inflammatory response, and genetics were investigated as potential contributors to fatigue. Studies are grouped into these four areas for review.

3. 1 Mitochondrial Structure

Four studies investigated the association of fatigue with mitochondrial number, size, and/or shape. All four studies were cross-sectional in design, included patients with CFS, and used muscle biopsy specimens to determine the potential mitochondrial markers. One study quantified fatigue through the use of a self-report questionnaire; whereas, three studies assumed fatigue through medical diagnosis. In one study about 7% of tibialis anterior (n=69), quadriceps (n=4), or medial head gastrocnemius (n=1) muscle biopsies from CFS subjects had

mitochondrial hyperplasia; however, because there were no structural abnormalities noted, the authors concluded that mitochondrial structural abnormalities were not a feature of CFS (Edwards, Gibson, Clague, & Helliwell, 1993). Findings were similar in another study in which no significant mitochondrial structural differences were found in the right leg vastus lateralis muscle biopsies of CFS patients versus controls (Plioplys & Plioplys, 1995a).

One study investigating the vastus lateralis muscle biopsies of CFS subjects compared to normal controls found a significantly larger (hypertrophic) mitochondrial size and shape (3-8 times larger), often with noticeable branching of the cristae, termed compartmentalization, (Behan, More, Downie, & Gow, 1995). In another study of CFS patients with decreased energy metabolism, the vastus lateralis muscle biopsy specimens, as categorized by observed lactate to pyruvate production in the biopsy samples, were found to have minor, nonspecific changes to mitochondrial shape and structure (paracrystalline inclusions and increased numbers of pleomorphic mitochondria with proliferation of cristae) (Behan, Holt, Kay, & Moonie, 1999). However, a vastus lateralis muscle specimen from a CFS patient with normal energy metabolism showed abundant pleomorphic mitochondria, but these structural changes were not observed in the vastus lateralis muscle specimens from CFS subjects with increased energy metabolism (Behan et al., 1999).

In summary, three of these four cross-sectional studies investigated the relationship between fatigue and mitochondrial number, shape, and/or size and found no significant differences with mitochondrial shape and structure among CFS patients and controls. Only one study observed significant mitochondrial hypertrophy in the CFS patients compared to healthy controls using skeletal muscle specimens.

3.2 Mitochondrial Function

Eleven studies investigated aspects of mitochondrial function; all were cross-sectional in design. Most studies (n=8) focused on mitochondrial enzymes, while the rest (n=3) focused on oxidative and/or nitrosative stress. Most studies looked at CFS or ME/CFS patients, while one study investigated fatigue in SLE patients.

3.2.1 Mitochondrial enzymes.

Four studies measured mitochondrial enzymes using muscle biopsy specimens and the remaining ones used peripheral blood specimens. Six studies only enrolled CFS patients, one included ME/CFS patients, and the remaining study enrolled only ME patients. Almost all participants were enrolled based on their medical diagnosis, except for one study which used a self-report questionnaire to measure fatigue. In four studies fatigue was further assessed by structured clinical interviews (n=2) and self-report questionnaires (n=2).

A significant reduction in citrate synthase, succinate reductase, and cytochrome-c oxidase was observed in the anterior tibialis muscle biopsy samples of CFS patients compared to healthy controls, which was attributed to the reduction in physical activity commonly present in CFS subjects (McArdle et al., 1996). A significant reduction in citrate synthase in the right quadriceps muscle biopsy samples from CFS subjects was also confirmed recently (Smits et al., 2011). Citrate synthase is located in the mitochondrial matrix and is an essential enzyme in the citric acid cycle (Tymoczko, Berg, & Stryer, 2010). Succinate reductase (Complex II) and cytochrome-c oxidase (Complex IV) are two of the four mitochondrial transmembrane enzyme complexes of the electron transport chain (Tymoczko et al., 2010).

In contrast, one study observed no significant difference with the skeletal muscle biopsies of partial cytochrome-c oxidase and myoadenylate deaminase (MAD) between CFS patients and

healthy controls; MAD was more associated with the symptom of myalgia than fatigue (Edwards et al., 1993). MAD is the muscle-specific subtype of adenosine monophosphate (AMP) deaminase and is involved in nucleotide metabolism (Tymoczko et al., 2010; Verzijl et al., 1998). Another study observed no difference in the vastus lateralis muscle biopsy levels of lactate dehydrogenase and cytochrome-c oxidase activity in CFS patients and controls (Behan et al., 1999). Lactate dehydrogenase is an enzyme located in the cytoplasm of cells and contributes to the formation of lactate from pyruvate (Tymoczko et al., 2010).

Four articles examined the levels of Coenzyme Q10 (CoQ10) in fatigued patients with either ME or CFS compared to healthy controls (Kurup & Kurup, 2003a, 2003b; Maes et al., 2009a, 2009b). ME and CFS patients had significantly lower plasma levels of CoQ10 compared to healthy controls (Kurup & Kurup, 2003a, 2003b). Plasma CoQ10 was also observed to be significantly lower in ME/CFS and depressed patients with CFS compared to controls (Maes et al., 2009a, 2009b). Significant, inverse relationships were observed with plasma CoQ10 levels and severity of illness scores of ME/CFS patients, specifically, greater fatigue and autonomic symptoms were associated with lower levels of CoQ10 (Maes et al., 2009a). However, in patients with depression there was no correlation observed with severity of illness total or individual scores and plasma CoQ10 levels (Maes et al., 2009b). The presence of CFS independently predicted low plasma CoQ10 levels (Maes et al., 2009b; Kurup & Kurup, 2003a, 2003b).

No longitudinal studies investigated the association between mitochondrial enzymes and fatigue. The cross-sectional studies reviewed reported conflicting associations between mitochondrial enzymes and fatigue. CoQ10 was the most studied mitochondrial enzyme, where

reduced plasma levels of CoQ10 were found in the fatigued populations compared to healthy controls.

3.2.2 Oxidative/Nitrosative stress.

Three cross-sectional studies, two from the same research group, investigated oxidative and nitrosative stress in CFS and ME patients compared to healthy controls. All three used peripheral blood specimens for biologic analyses, one study investigated patients with CFS, one study investigated ME, and one study investigated fatigue in SLE. Two of the studies complemented medical diagnosis with a structured clinical interview while one study defined fatigue through the use of self-report questionnaires.

Antioxidants (Glutathione, superoxide dismutase, catalase, GSH peroxidase, and GSH reductase) were significantly decreased, while ROS and reactive nitrogen species (RNS) (malondialdehyde [MDA], conjugated dienes, hydroperoxides, and nitric oxide) were significantly increased in the plasma/serum samples of CFS patients compared to healthy controls (Kurup & Kurup, 2003a). Similar results were observed in subjects with ME (Kurup & Kurup, 2003b). It is hypothesized that the aforementioned disruption in the oxidative stress pathway is the downstream result of an imbalance in intracellular calcium and magnesium, which results in high intracellular calcium and low intracellular magnesium (Kurup & Kurup, 2003a, 2003b).

Another study investigated the association between F_2-isoprostance levels and fatigue in patients with SLE. They observed that fatigued SLE patients had higher levels of F_2-isoprostane than non-fatigued SLE patients (Segal et al., 2012). A positive correlation between F_2-isoprostane and fatigue was observed in SLE patients and it was observed that F_2-isoprostane predicts higher FSS scores in SLE patients. F_2-isoprostane is currently acknowledged as the

most reliable measure of in vivo oxidative stress (Segal, 2012). The results from this study provide further evidence of an association between oxidative stress and the development of fatigue.

3.3 Mitochondrial Energy Metabolism

Ten studies investigated aspects of mitochondrial energy metabolism; all were cross-sectional in design. Most studies (n=6) focused on fatty acid metabolism, while the rest (n=4) focused on ATP production. Most studies looked at CFS or ME/CFS patients, while one study investigated fatigue in MS patients.

3.3.1 ATP production.

Four cross-sectional studies investigated mitochondrial energy metabolism as a potential marker in fatiguing conditions. Mitochondrial energy metabolism was assessed from muscle biopsies in two of the studies and from peripheral blood in the remaining studies. Two studies only enrolled CFS patients, while the remaining two studies included both ME and CFS patients.

The mitochondrial energy metabolism was measured using the ATP profile test in two studies (Myhill, Booth, & McLaren-Howard, 2009; Booth, Myhill, & McLaren-Howard, 2012). The ATP profile tests measures three parameters of mitochondrial function in neutrophils extracted from peripheral blood, (1) ATP concentration (how much ATP is present) and ATP ratio (what fraction of ATP is available for energy supply), (2) the efficiency of oxidative phosphorylation (ADP to ATP recycling efficiency), and (3) TL OUT (ADP out of cytosol into mitochondria) and TL IN (ATP from mitochondria into the cytosol). A Mitochondrial Energy Score (MES) was calculated by multiplying all five factors (ATP, ATP ratio, Ox Phos, TL OUT, TL IN, TL OUT x TL IN). One of the two studies that used this ATP Profile test observed that the percentage of participants with normal values of mitochondrial function increased as fatigue

symptoms decreased (Myhill et al., 2009). In addition, the MES was positively correlated with scores on the CFS Ability scale (Myhill et al., 2009) indicating that greater mitochondrial efficiency was associated with higher levels of activity and function in those with CFS/ME.

A second study by the same group confirmed the presence of mitochondrial dysfunction in patients with ME/CFS by observing partial blockage of the ADP-ATP translocator protein, adenosine nucleotide translocase (TL) (Booth et al., 2012). The TL protein functions to transfer ATP out of the mitochondria into the cell cytoplasm as well as transferring ADP from the cell cytoplasm into the mitochondria to generate more ATP (Booth, 2012). Cells can compensate for some of the dysfunction in ATP production through two alternative pathways: by increased glyocolysis and the use of adenlyate kinase pathway of ATP formation (Booth et al., 2012). Therefore, partial blockage of the TL protein can lead to impaired energy production.

Another study measured two aspects of energy metabolism in the vastus lateralis muscle biopsy samples: aerobic respiration and respiratory chain function, which showed no difference between CFS patients and healthy controls for either parameter (Behan et al., 1999). In addition, there were no differences in ATP production rate or respiratory chain complex activity found in the right quadriceps muscle biopsy samples of CFS patients compared to healthy controls (Smits et al., 2011). Although ME/CFS patients had impaired energy production as determined by the ATP profile test, no differences in either aerobic respiration or respiratory chain complex activity were found in CFS patients compared to healthy controls.

3.3.2 Fatty acid metabolism.

Six cross-sectional articles investigated carnitine levels in fatiguing conditions. Five studies included CFS patients and one study included MS patients. Four studies used peripheral blood specimens for biologic analyses and two studies used muscle biopsies. Four studies

complimented medical diagnosis with self-report questionnaires, while two studies assumed fatigue through medical diagnosis.

Acylcarnitine serum levels were significantly lower in CFS patients compared to healthy controls; however, free L-carnitine serum levels were not significantly different between the two groups (Kuratsune et al., 1994). Another study observed significantly lower serum levels of total and free carnitine in CFS patients of both genders when compared to healthy controls, as well as lower serum levels of acylcarnitine in CFS patients compared to controls, using historical data (Plioplys & Plioplys, 1995b). However, no difference between total, free, and acylcarnitine serum levels were found in a more recent study of CFS patients versus healthy controls (Soetekouw et al., 2000), as well as in patients with MS (with and without fatigue) compared to healthy controls (Fukazawa, Sasaki, Kikuchi, Hamada, & Tashiro, 1996). Another study also showed no significant difference in L-carnitine, total carnitine, or total acylcarnitine plasma levels between CFS patients and healthy controls; however, when individual acylcarnitine subtypes were investigated in the CFS sample, 6 acylcarnitine subtypes (C8:1, C14, C16:1, C18, C18:1, and C18:2) were significantly lower, while 2 acylcarnitine subtypes (C12DC and C18:1-OH) were significantly higher in plasma of CFS participants compared to the matched controls (Reuter & Evans, 2011).

Higher acylcarnitine serum levels were inversely correlated with the Chronic Fatigue Syndrome Impairment Index (CFS-II) mental index score and CFS-II total score (Plioplys & Plioplys, 1995a). Lower serum acylcarnitine was associated with worse activity levels and symptom presentation, but these relationships were not observed with free L-carnitine serum levels (Kuratsune et al., 1994). A later study, however, showed that higher free carnitine serum levels were significantly associated with better physical abilities, and higher free and total

carnitine serum levels were significantly associated with lower fatigue severity (Plioplys & Plioplys, 1995b).

4. Immune Response

Four studies investigated dysfunctional immune responses to mitochondria in various fatiguing conditions. Three of these studies were cross-sectional and one study used repeated measures. Three studies only enrolled CFS patients and one study included fatigued patients with various diagnoses. Three studies used peripheral blood specimens for biologic analyses, while one study used muscle biopsies. In one study clinical diagnosis was complemented with self-report questionnaires and interview assessments, while three studies assumed fatigue through medical diagnosis.

Two studies from the same research group investigated autoimmune responses to acute phase phospholipids in patients with fatiguing illnesses (Hokama et al., 2008, 2009). CFS subjects had serum lipid fractions that resembled those commonly found in patients poisoned with ciguatoxin, a marine toxin (Hokama et al., 2008). Sera from patients with CFS, chronic ciguartera fish poisoning (CCFP), gulf war veterans (GWV), and prostate cancer patients contained antibodies to cardiolipin (aCL), a phospholipid of the mitochondrial membrane (Hokama et al., 2008). Further study found that 95% of CFS patients had anticardiolipin antibody (ACA) of the IgM subtype, 10% showed an IgG response, 2.5% had an IgA response; 4 patients were positive for IgG and IgM, and one patient was positive for all three antibody subtypes (Hokama et al., 2009).

Two studies investigated the role of enteroviral infection with the onset of CFS (McArdle et al., 1996; Vernon et al., 2006). One study examined anterior tibialis muscle biopsy specimens for the presence of enteroviral RNA. However, they failed to detect the presence of a persistent

enteroviral infection in patients with CFS (McArdle et al., 1996). Another study observed 23 differentially expressed genes from peripheral blood samples of patients with persistent post-Epstein-Barr (EBV) fatigue compared to controls (those who recovered without persistent fatigue) in the early phase (0-3 months) of infection. Of the 23 differentially expressed genes, 8 were found in subjects with persistent fatigue post-EBV and were involved in binding and metabolism ontologies (Vernon et al., 2006). When exploring both early and late (>6 months) phases of infection, 24 genes were differentially expressed between cases and controls. Half of the 24 differentially expressed genes were associated with mitochondrial functions such as fatty acid oxidation (CRAT, carnitine acetyltransferase; APOA2, apolipoprotein A-II), apoptosis (BTG1, B-cell translocation gene 1; FOLR1, folate receptor 1; CTRL, chymotrypsin-like), DNA repair, and mitochondrial membrane (COX8A, cytochrome c oxidase subunit VIII; COX11, cytochrome c oxidase assembly protein; KCNA10, potassium voltage-gated channel; MGP, matrix G1a protein; ATP5L, ATP synthase) (Vernon et al., 2006).

In the four studies reviewed an autoimmune response was found as evidenced by the presence of mitochondrial phospholipids in the sera of CFS patients. Although there was no evidence of persistent enteroviral infection found in muscle biopsies of CFS patients, differential expression of genes associated with mitochondrial function was noted in patients post EBV infection. Comparing the results among these studies is challenging. Only one research group investigated autoimmune responses to acute phase phospholipids, publishing two different studies. Both of the studies investigating post-infective fatigue investigated different viral infections (enterovirus vs. EBV) and the two studies employed different study designs, cross-sectional (McArdle et al., 1996) and repeated measures (Vernon et al., 2006).

5. Genetics

Five articles explored the association between gene expression profiles in fatigue conditions versus controls. Three studies were cross-sectional and two studies used repeated measures designs. Three studies only included CFS patients, one study included men with prostate cancer, and one study included patients with Human Immunodeficiency Virus (HIV). Three studies used peripheral blood specimens for genomic analyses, while two studies used muscle biopsies. One study complemented clinical diagnosis with self-report questionnaires, two studies used self-report questionnaires alone to measure fatigue, and two assumed fatigue through medical diagnosis.

The first genomic study found no significant differences between CFS patients and healthy controls in the total volume of mitochondrial DNA present, two mtDNA rearrangements, and the presence of one point mutation (Behan et al., 1999). A real-time PCR (qPCR) study found 11 mitochondrial function-related genes to be differentially expressed during radiation therapy for prostate cancer and 8 of the 11 genes were significantly associated with fatigue intensification during radiation therapy (Hsiao et al., 2013). These 8 genes are involved in mitochondrial apoptosis and signaling, mitochondrial membrane polarity and potential, mitochondrial morphology and fission/fusion, and mitochondrial and small molecule transport (Hsiao et al., 2013).

One microarray analysis found 35 differentially expressed genes in the peripheral blood mononuclear cells (PBMCs) from CFS patients, where 3 up-regulated genes had activities specific to mitochondrial function: EIF2B4 (eukaryotic translation initiation factor 2B, subunit 4δ, tv-1), EIF4G1 (eukaryotic translation initiation factor, 4γ, 1, tv-5), and MRPL23 (mitochondrial ribosomal protein L23) (Kaushik et al., 2005). Another microarray study found

47 differentially expressed genes (2 up-regulated and 38 down-regulated in both genders; 7 up-regulated in females, yet down-regulated in males) from vastus lateralis muscle biopsies of CSF patients compared to healthy controls. The down-regulated genes were associated with impairment of antioxidant mechanisms, aerobic energy production, and metabolism (Pietrangelo et al., 2009). Another study investigated gene networks in CD14+ cells from HIV-infected patients who reported high fatigue, versus those who reported low fatigue or healthy controls, where 6 mitochondrial-related genes (CHD1L and ALDOB genes were negatively associated with fatigue; TIMM17B, GSR, IMMT, and SLC25A26 were positively associated with fatigue) are implicated in protein translocation into the mitochondrial matrix, cristae morphology, ATP-binding and protein binding, metabolism, oxidation and reduction processes, and energy production were identified (Voss et al., 2013).

Five articles explored the association between gene expression profiles in fatiguing conditions versus controls. Common mitochondria-specific functional pathways were reported from the results of the gene expression studies included in review, to include pathways related to metabolism, energy production, protein transport, mitochondrial morphology, central nervous system dysfunction and post-viral infection. The pathways identified in these studies were similar across three different patient populations and supported areas of dysfunction identified in the previous sections.

6. Discussion

The purpose of this systematic review was to examine markers of mitochondrial function that have evidence of an association with fatigue in order to identify areas needed for further research. This review included studies focusing on markers of mitochondrial function in relation to fatigue. Dysfunctions in the mitochondrial structure, mitochondrial function (mitochondrial

enzymes and oxidative/nitrosative stress), mitochondrial energy metabolism (ATP production and fatty acid metabolism), immune response, and genetics were investigated as potential contributors to fatigue.

Carnitine was the most investigated mitochondrial function marker reported in this review (n=6). Dysfunctional carnitine levels were reported in all six studies that investigated the biomarker; however, the specifics of the dysfunction varied among the studies. Genetic profiles were the second most studied mitochondrial parameter. Even though different genes were reported across the studies, common pathways (metabolism, energy production, protein transport, mitochondrial morphology, central nervous system dysfunction, and post-viral infection) were identified among the articles. The most commonly investigated mitochondrial enzyme was CoQ10. It was the only mitochondrial biomarker found to have a consistent association with fatigue identified in this review (Kurup & Kurup, 2003a, 2003b; Maes et al., 2009a, 2009b). CoQ10 is an essential enzyme in the electron transport chain, responsible for shuttling electrons and protons (Tymoczko et al., 2010). Further investigation is needed to understand the role of CoQ10 in fatigue.

CoQ10 deficiency can be either primary or secondary in nature. Primary deficiency stems from dysfunction with the genes coding for the synthesis of CoQ10, whereas secondary deficiency results from anything that is not primary in nature (Horvath, 2012; Littarru & Tiano, 2010); Potgeiter, Pretorius, and Pepper, 2013). CoQ10 is endogenously produced and therefore, dietary intake has minimal influence on the CoQ10 concentration in the body. However, if CoQ10 is found to be depleted, especially with primary deficiency, supplementation is a therapeutic option (Potgeiter, Pretorius, and Pepper, 2013). In clinical practice, vitamins such as riboflavin B2, niacin B3, vitamin E and other mitochondrial cofactors including levo-carnitine,

lipoic acid, acetyl-l-carnitine are used as supplemental treatment for mitochondrial disorders in order to either enhance ETC enzyme activity or antioxidant defenses (Cohen, 2000). The efficacy of these vitamins and mitochondrial cofactors as treatments for mitochondrial disorders remain controversial (Cohen, 2000).

CoQ10 has been shown to have clinical benefits attributed to its antioxidant properties and its role in cellular bioenergetics (Littarru & Tiano, 2010); hence, it is being used as a therapeutic option for a number of mitochondria-related clinical conditions including those with cardiovascular disease, reproductive issues, and neurodegenerative diseases (Littarru & Tiano, 2010). In this review, there are only two research teams that investigated the relationship between CoQ10 and fatigue. More clinical studies are needed to confirm the role of vitamins and mitochondrial cofactors in alleviating fatigue symptoms (Horvath, 2012; Potgeiter, Pretorius, and Pepper, 2013).

Based on the findings of this review, alterations in energy metabolism may contribute to fatigue. Support for this conclusion was evident from dysfunctions reported with both oxidative phosphorylation and ATP production and recycling. Impaired oxidative phosphorylation was noted through reduced citrate synthase activity, reduced levels of succinate reductase (Complex I), cytochrome-c oxidase (Complex IV), and CoQ10 (electron shuttle from Complex I and II to Complex III), as well as evidenced through disrupted oxidative stress (decreased levels of antioxidants and increased levels of ROS and F_2-isoprostance) (McArdle et al., 1996; Kurup & Kurup, 2003a, 2003b; Maes et al., 2009a, 2009b; Segal et al., 2012; Smits et al., 2011). ATP concentration, recycling, and the efficiency of oxidative phosphorylation were found to be disrupted through the ATP Profile Test (Myhill et al., 2009; Booth et al., 2012). Fatty acid metabolism was also impaired as evidenced by abnormal carnitine levels (Kuratsune et al., 1994;

Plioplys & Plioplys, 1995a, 1995b; Reuter & Evans, 2011). Genetic investigations found abnormal gene transcription pathways related to metabolism, energy production, protein transport (Hsiao et al., 2013; Kaushik et al., 2005; Pietrangelo et al., 2009; Voss et al., 2013). These genetic pathways support and the areas of dysfunction found in the aforementioned studies.

The limitations identified in this review are: 83% of the studies were cross-sectional, 79% of the studies enrolled only CFS patients, and there were inconsistent associations found between mitochondrial biomarkers and fatigue. Future studies utilizing longitudinal designs need to be conducted to establish associations between mitochondrial dysfunction and fatigue development. Additionally, if mitochondrial dysfunction and fatigue are observed, longitudinal studies can provide more evidence about the characteristics of the association.

The inclusion of diverse patient populations in future studies would provide evidence regarding common mitochondrial mechanisms as an etiology for fatigue. The predominant patient population included in the reviewed studies was CFS. Knowledge gained from studies of the CFS population needs to be translated to other fatigued populations, such as those with cancer or other chronic diseases. Understanding the biological mechanisms underlying fatigue development is important to enhance clinical evaluation and treatment.

Furthermore, fatigue was defined differently among the reviewed studies and different diagnostic criteria and self-report instruments were used. This variability limited the ability to compare findings across studies. Furthermore, the self-report questionnaires included in the reviewed studies are valid measures of fatigue, but not necessarily for the populations included in the studies, namely CFS. Future research needs to work towards establishing a global agreement on the clinical definition of fatigue. Once a clinical definition of fatigue is established,

research will need to focus on developing a valid and reliable tool for measurement of fatigue in the clinical setting. Until a global definition is developed, researchers need to ensure that the existing fatigue tools are validated in their clinical populations of interest.

The literature review identified some potential relationships between mitochondrial dysfunction and fatigue; however, the findings were limited to predominantly one patient population, were from mostly cross-sectional studies, and results were confounded by the use of multiple definitions of fatigue. These limitations culminated in inconsistent findings across studies. Therefore, the results from the review suggest further investigation to address the gaps in the current literature. Once the underlying mechanisms of fatigue are better understood, individualized and tailored therapies can be developed to improve quality of life of patients.

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Chapter Three

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). It is estimated that in 2014, there will be 2.5 million men in the United States with PC, and 233,000 of them will be newly diagnosed. These statistics indicate that PC is a condition that affects a significant number of men; about 1 in 7 men will be diagnosed during their lifetime. Fortunately, advances in diagnosis and treatment have greatly improved the prognosis and survival of men with PC (ACS, 2014).

Treatment for PC depends on patient and disease characteristics. If the cancer is localized or has progressed into the nearby tissue, then various forms of radiation therapy (RT) may be implemented (ACS, 2014). External beam radiation therapy (EBRT), for example, can be an effective treatment option, but it can result in numerous side effects including urinary, bowel, and sexual dysfunctions, as well as fatigue, which can greatly impair the individual's health-related quality of life (HRQOL) (Budäus et al., 2012). A systematic review comparing fatigue prevalence among different treatment modes for PC 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 found in patients on active surveillance (Langston, Armes, Levy, Tidey, & Ream, 2013). However, it was observed that 71% of men reported clinically significant fatigue while receiving radiation therapy.

Additionally, 24-33% of men experienced persistent fatigue more than one year post-RT (Langston et al., 2013).

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 [NCCN], 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 once the task is complete, the need for greater rest periods (Cheville, 2009; Hofman, et al., 2007; Mitchell & Berger, 2011). Not only is CRF one of the most prevalent symptoms, it is also reported to be one of the most distressing, often negatively affecting multiple HRQOL domains (Barsevick, Frost, Zwinderman, Hall, & Halyard, 2010; Ryan, et al., 2007; Tazi & Errihani, 2011). CRF is poorly understood and lacks a clear, single, clinical definition or etiology. This makes it a challenging symptom for healthcare 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. Many different biological mechanisms have been theorized to 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). Therefore, mitochondrial dysfunction is a plausible mechanism of CRF.

Mitochondria have an essential role in energy production through the process of oxidative phosphorylation whereby nutrients are converted into adenosine triphosphate (ATP), which powers many of the cells' activities. In addition to energy production, mitochondria have been implicated in various physiologic processes including the production of reactive oxygen species (ROS), pyrimidine and lipid biosynthesis, regulation of cellular levels of substrates (amino acids, metabolites, enzyme cofactors), apoptosis, metal (Fe-S cluster and heme) metabolism, calcium homeostasis and flux, neurotransmitter synthesis, heat production, and insulin secretion (Duchen, 2004; Nunnari & Suomalainen, 2012; Pieczenik & Neustadt, 2007). Therefore, damage to mitochondria can have widespread health outcomes (Duchen, 2004). Mitochondria are becoming increasingly recognized as major contributors to human health and disease because of their widespread influences (Cohen & Gold, 2001).

Radiation therapy has been observed to cause mitochondrial dysfunction through two primary insults. First, radiation can induce a decline in mitochondrial electron transport chain (ETC) enzyme function (Yoshida, Goto, Kawakatsu, Urata, & Li, 2012). Secondly, with the decline in ETC function, there is a resulting increase in the production of ROS (Yoshida et al., 2012). ROS production under normal conditions is beneficial for cellular signaling; however, during exposure to radiation, ROS production increases to toxic levels resulting in damage to mitochondrial and nuclear DNA, thereby disrupting normal cell metabolic and physiologic activities (Azzam, Jay-Gerin, & Pain, 2012). While mitochondrial dysfunction has been suggested as a possible mechanism underlying CRF (Wang, 2008), only one reported study has investigated the potential role of mitochondrial dysfunction in CRF (Hsiao, Wang, Kaushal, &

Saligan, 2013). The investigators examined changes in fatigue scores (measured by the revised Piper Fatigue Scale) and mitochondria-related gene expression (measured from RNA in peripheral blood) at seven time points (baseline, day 1, day 7, day 14, treatment midpoint, treatment completion, and 30-days post treatment) during EBRT in men with nonmetastatic prostate cancer. The investigators observed that differential expression of genes related to mitochondrial metabolism, energy production, and protein transport were associated with self-reported fatigue in their participants (Hsiao et al., 2013).

Mechanisms underlying mitochondria-related disease states have predominantly focused on DNA damage and ROS generation (Cohen & Gold, 2001; Pieczenik & Neustadt, 2007). Mitochondrial dysfunction can be categorized as primary (inherent) or secondary (acquired dysfunction) (Cohen & Gold, 2001; Read & Calnan, 2000). Primary dysfunction results from mitochondrial DNA (mtDNA) mutations inherited from mothers, who are the sole contributors of mitochondria to their offspring (Cohen & Gold, 2001). Mitochondrial DNA has a much higher mutation rate than nuclear DNA because it lacks protective histones (Read & Calnan, 2000), is readily exposed to damage from ROS production (Alexander et al., 2010; Pieczenik & Neustadt, 2007; Reynolds et al., 2007), and lacks certain DNA repair mechanisms (Cohen & Gold, 2001). Secondary mitochondrial dysfunction results from the influence of external mechanisms such as environmental or pharmacologic toxins that can damage the mtDNA (Cohen & Gold, 2001). Mitochondria can protect themselves from the accumulation of damage through the processes of fission and fusion (Chan, 2006; Nunnari & Suomalainen, 2012; Youle & van der Bliek, 2012); however, if these mechanisms are altered, mitochondrial dysfunction can contribute to diseases such as cancer, diabetes, Alzheimer's Disease, Parkinson's Disease, Fibromyalgia Syndrome,

and serious mental disorders such as schizophrenia and bipolar disease (Blackstone & Chang, 2011; Duchen, 2004; Pieczenik & Neustadt, 2007; Reynolds et al., 2007).

Mitochondrial research has predominantly focused on the role of mitochondrial dysfunction in disease pathology (Chan, 2006; Seo et al., 2010). However, there has been some work associating mitochondrial dysfunction with the development of distressing symptoms such as fatigue, neuropathic pain, weakness, and depression in addition to disease states (Pieczenik & Neustadt, 2007). Abnormal (swollen and vacuolated) mitochondria have been observed to have a relationship with chemotherapy-induced neuropathy in the absence of evidence of nerve fiber damage or dysfunction (Flatters & Bennett, 2006). Patients with depression have also been observed to have alterations in metabolism, specifically with mitochondrial ETC enzymes (Rezin, Amboni, Zugno, Quevedo, & Streck, 2009). More research is needed to understand the role of mitochondrial dysfunction in symptom onset or exacerbation.

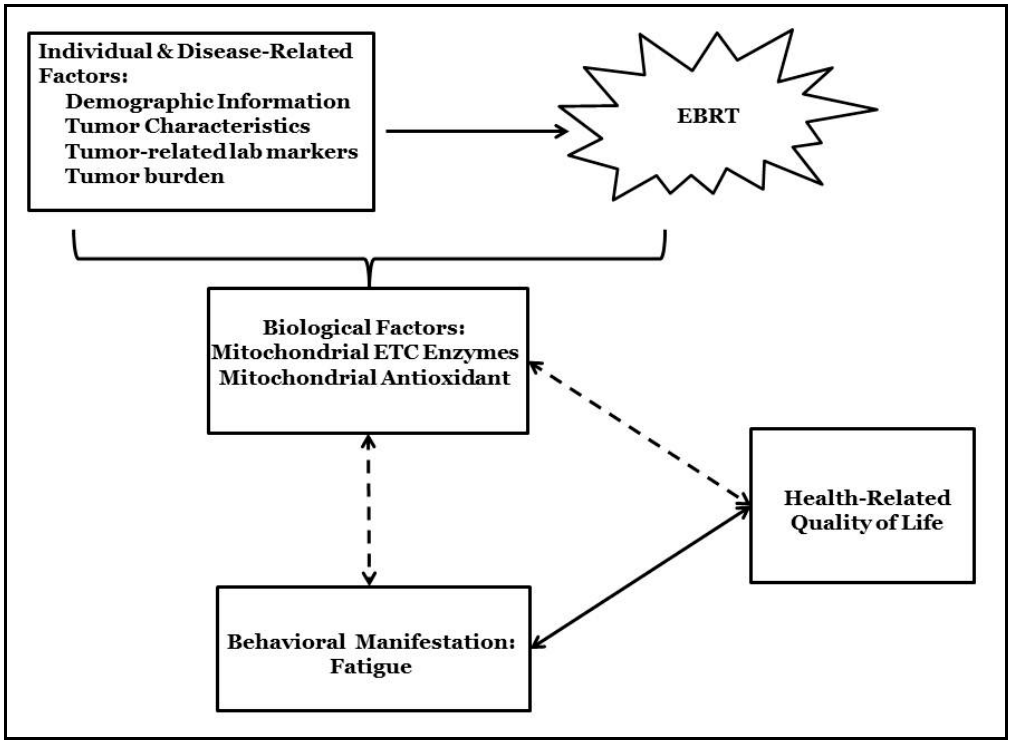
Given the paucity of research in this area, the current study was designed to expand knowledge of the relationship of mitochondrial dysfunction to fatigue and HRQOL at baseline and at completion of EBRT among men with nonmetastatic (NM)-PC. This study was a secondary analysis of existing data from a National Institutes of Health (NIH) Institutional Review Board approved study titled *Relationship between Mitochondrial Dysfunction and Fatigue in Cancer Patients Following External Beam Radiation Therapy* (#10-NR-0128). Specific aims of the secondary 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 in levels of biomarkers of mitochondrial function, fatigue, and HRQOL, and (3) compare levels of biomarkers of mitochondrial function in men with non-clinically significant

fatigue (low fatigue) to those with clinically significant fatigue (high fatigue) from baseline to completion of EBRT.

Conceptual Framework

Biobehavioral research encompasses interactions of the physiological, psychological, and social components of health (Grady, 2006). For the purposes of this research, HRQOL of men with NM-PC was conceptualized within a biobehavioral framework with individual, treatment, and disease-related factors, biological factors, behavioral manifestations, and HRQOL components (Figure 1).

Figure 1. Biobehavioral Research Model



Individual and disease-related factors. Individual factors were empirically defined as demographic data and comorbidities. Disease-related factors were defined as tumor characteristics (Gleason scores), tumor-related laboratory markers (prostate specific antigen [PSA]), and tumor burden (tumor-stage [t-stage]). Gleason scores indicate the extent of tumor

differentiation (range = 1 [well differentiated] to 5 [lack of differentiation]) (Epstein, Allsbrook, Amin, Egevad, & the ISUP Grading Committee, 2005). Higher Gleason scores indicate less prostate tissue differentiation, suggesting that the disease is more likely to spread (Epstein et al., 2005). PSA levels are used in the routine screening of men for detection of prostate cancer (Pater, Hart, Blonigen, Lindsell, & Barrett, 2012) and as a surveillance marker for disease progression or recurrence. T-staging is used to grade the severity of prostate cancer, ranging from stage 1a (early-stage) to stage IVb (metastatic cancer) (Cheng, Montironi, Bostwick, Lopez-Beltran, & Berney, 2012).

EBRT. Radiation-related factors included radiation dose and length of treatment. EBRT treatment can result in untoward side effects that could impact patient HRQOL (ACS, 2014).

Biological factors. Biological factors included biomarkers of mitochondrial function (complex I-V; SOD2) that may result from the disease and/or its treatment. Despite sharing similar disease profiles and treatment regimens, men with NM-PC can exhibit great variance in their symptom severity. The underlying mechanisms behind the symptoms and severity of symptoms are not clear. Mitochondrial dysfunction is one plausible mechanism for the presence of symptoms such as CRF.

Behavioral manifestation. The behavioral manifestation for this study was fatigue, a highly prevalent symptom in cancer patients (Hofman et al., 2007; NCCN, 2012; Piper & Cella, 2010). While fatigue has been associated with mitochondrial dysfunction in various clinical populations (Pieczenik & Neustadt, 2007), these associations have not yet been examined in men with NM-PC.

Health-related quality of life. As more treatment options are emerging for PC, HRQOL is becoming an increasingly important factor in the treatment decision-making process (Torvinen et al., 2013). Even though it is a crucial concept in health research, HRQOL has been difficult to define because of its complexity, with meaning imparted by the values of the individual. However, accurately assessing HRQOL is important for addressing the individual's well-being during and after the treatment process.

Methods

This paper reports a secondary analysis of a descriptive, longitudinal study *Relationship between Mitochondrial Dysfunction and Fatigue in Cancer Patients Following External Beam Radiation Therapy* that was approved by the NIH Institutional Review Board (#10-NR-0128). Men were enrolled if they: (a) had NM-PC, (b) were scheduled to receive EBRT, and (c) were 18 years of age or older. Patients were excluded if they: (a) had any inflammatory or infectious condition such as rheumatoid arthritis, lupus, or cirrhosis; an infectious disease such as HIV, tuberculosis, or hepatitis; (b) had other types of cancer; (c) had a major psychiatric disorder or alcohol or drug abuse within the past 5 years; (d) were receiving or scheduled to receive chemotherapy; or (e) were taking steroids, non-steroidal anti-inflammatories, or tranquilizers. All study participants were seen in the outpatient clinics of the Radiation Oncology Department of the National Cancer Institute (NCI) at NIH, Bethesda, MD from August 2010 to August 2012.

Study time points. Data for this study were collected at two time points: baseline (before EBRT) and on the last day (completion) of EBRT. Study variables were measured using demographic, questionnaire, and biologic data sources.

Demographic data. Demographic data were obtained by reviewing medical records to obtain age, race, socioeconomic status, and disease-related factors including tumor characteristics and tumor-related laboratory markers.

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

The revised Piper Fatigue Scale (rPFS). This 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 to 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; internal consistency ranged from 0.83 to 0.97 for the total instrument and its subscales (Borneman et al., 2011).

The Functional Assessment of Cancer Therapy– Fatigue subscale (FACT-F). This is a validated 13-item questionnaire exploring fatigue symptoms in various populations, including cancer patients and healthy participants. This questionnaire has shown good test-retest reliability ($r = 0.90$), and internal consistency ($\alpha = 0.93$ and 0.95) 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). The FACT-F can also be used to measure minimally important changes that may be clinically relevant. A greater than or equal to 3-point decrease in the FACT-F score is considered to be the threshold denoting a minimally-important change that may be clinically relevant (Cella, Eton, Lai, Peterman, & Merkel, 2002).

HRQOL was measured 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 $\alpha = 0.87$ to 0.89) (Esper et al., 1997; Cella, Nichol, Eton, Nelson, & Mulani, 2009).

Mitochondrial measures. Peripheral blood collected at both study time points was used to obtain protein markers of mitochondrial function.

Cell lysate collection. Peripheral whole blood samples collected using ethylenediamineteraacidic (EDTA) tubes were centrifuged immediately after collection at 3,000 rpm in 4˚C for 10 minutes. After the plasma was extracted from the tube, the remaining cell sample was divided into 500 µl aliquots and stored in -80ºC freezers until batch analysis. The cells from the whole blood samples were lysed with a cell extraction buffer (10mM Tris, pH 7.4, 100mM NaCl, 1mM EDTA, 1% Trition X-100, 10% Glycerol, and 0.1% SDS). A protease inhibitor was added to the buffer to prevent the degradation of enzymes. Protein content 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.

Serum preparation. Peripheral whole blood collected using serum-separating tubes (SST) was centrifuged immediately after collection at 3,000 rpm and 4˚C for 10 minutes, then the serum was divided into 250 µl aliquots that were stored in -80ºC freezers until batch analysis.

Two areas of mitochondrial function were investigated in this study: (a) energy metabolism and (b) oxidative stress.

*Energy metabolism: Mitochondrial electron transport chain***.** The respiratory chain is comprised of five enzyme complexes, respiratory complexes I-V (Azzam, Jay-Gerin, & Pain, 2012). Dysfunction in any complex could interrupt the transfer of electrons, thereby disrupting efficient and effective energy metabolism. The Human Profiling ELISA kits (Abcam®, Cambridge, MA) were used for the quantitative detection of the mitochondrial respiratory enzyme complexes (complexes I-V; 1 kit for each complex) from cell lysates.

Oxidative stress: Antioxidants. Manganese superoxide dismutase (MnSOD) provides the first line of defense against ROS in the mitochondria, countering oxidative stress (Lustgarten et al., 2011). Serum MnSOD levels were assessed by the MnSOD ELISA kit (Abcam®, Cambridge, MA).

Statistical Analysis

Descriptive statistics were used to illustrate the demographic characteristics of participants. If variables were normally distributed, means and standard deviations were reported versus medians and ranges data that was not normally distributed. Medians and ranges were calculated for fatigue questionnaires, and means and standard deviations were calculated for HRQOL questionnaires. Mitochondrial enzymes were described with medians and ranges. Nonparametric Wilcoxon matched-pairs rank-sum tests were used to analyze changes in levels of fatigue between baseline and completion of EBRT. A paired *t*-test was used to analyze changes in levels of HRQOL between baseline and completion of EBRT. Mitochondrial data were log-transformed and paired *t*-tests were used to analyze changes in levels of biomarkers of mitochondrial function over time. Estimated correlations of mitochondrial biomarkers, fatigue (as assessed by rPFS and FACT-F scores), and HRQOL were calculated using Pearson's product-moment correlation coefficients if both variables were normally distributed and

Spearman's rank correlation coefficients if one or more of the variables was not normally distributed. Participants in this study were grouped into clinically significant (high) and non-clinically significant (low) fatigue groups based on changes in FACT-F scores from baseline to completion of EBRT. High fatigue groups had a decrease of 3 or more points in FACT-F scores, while those who had less than a 3-point decrease in FACT-F scores were categorized in the low fatigue group.

Power analysis. The primary purpose of this dissertation study is to explore the relationship between mitochondrial function and fatigue intensification during EBRT in men diagnosed with NM-PC who completed an NINR protocol (# 10-NR-0128) at the NIH, Bethesda, MD. Minor diagnostic criteria for primary respiratory chain (RC) disorders in adults include a 30%–40% activity (effect size 0.30 - 0.40) of any RC complex, as observed in fibroblasts or lymphoblasts (Bernier, 2002). An n = 25 has an 80% power to show a difference in levels of mitochondrial markers before and after EBRT at an effect size of 0.58 with an alpha of 0.05. A secondary aim of this study is to examine relationships over time with changes in levels of fatigue, mitochondrial function, and HR-QOL. A study by Hsiao et al. (2013) found significant correlations between fatigue scores and differential expression of mitochondrial genes during EBRT with an n = 15. Therefore, the sample size n = 25 will be sufficient to address a secondary aim of this study. However, due to sample degradation, n = 22 participants were included in this study.

Results

Sample demographics. Twenty-two men (mean age = 65.86 $\pm$ 6.87 years) were included in the study. All participants were able to carry on all pre-disease 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 clinical t-stage (Cheng et al., 2012) of T1c (22.72%), T2a (27.27%), or T2c (22.72%), indicating that most participants had disease that had not spread outside the prostate gland. Participants received EBRT five days a week; 17 participants received a total dose of 75.50 Grays for 42 days, while 5 participants who had prior prostatectomies, received 68.40 Grays of EBRT for 38 days.

Table 1. Demographic and Clinical Characteristics of Sample

Variable	Mean (*SD*)	*n* (%)	Median (Range)
Age	65.86 (6.87)		
Race			
White		16 (72.73)	
A.A./Black		4 (18.18)	
Hispanic		2 (9.09)	
Ethnicity			
Hispanic/Latino		3 (13.64)	
Not Hispanic/Latino		17 (77.27)	
Disease Characteristics			
Gleason	7.59 (.91)		
Clinical T-Stage			
T1c		5 (22.72)	
T2a		6 (27.27)	
T2b		2 (9.09)	
T2c		5 (22.72)	
T3a		1 (4.55)	
T3b		1 (4.55)	
Pre-EBRT Levels			
PSA (0-3.99 ng/mL)		3.69 (5.24)	
Testosterone (181-758 ng/dL)		220.37 (166.02)	
WBC (4.23-9.07 K/µL)		6.13 (1.67)	
RBC (4.63-6.08 M/µL)		4.61 (0.44)	
Hemoglobin (13.70-17.50 g/dL)			13.95 (12.80-15.60)
Albumin (3.70-4.70 g/dL)			4.10 (2.70-4.40)
Body Mass Index (18.50-24.90)			29.85 (22.90-40.70)

SD = standard deviation, A.A. = African American, T-stage = tumor stage (TNM classification for tumor staging), PSA = prostate specific antigen, WBC = white blood cell, RBC = red blood cell

Fatigue. The median fatigue score on the FACT-F at baseline was 47.50 (range 28.00-52.00) and at treatment completion was 43.00 (range = 20.00-52.00); lower scores on the FACT-F indicate higher fatigue. The median score on the rPFS at baseline was 0.93 (range = 0-4.41) and at treatment completion was 3.68 (range = 0-6.73); higher scores on the rPFS indicate more fatigue.

There was a significant change in fatigue scores detected on both instruments from baseline to the completion of EBRT for the total sample (FACT-F $p = 0.02$; rPFS $p = 0.004$). There was no significant difference in the change in FACT-F scores from baseline to completion of EBRT between subjects who received 38 versus those who received 42 days of treatment ($p = 0.24$). Although the change in rPFS scores over time was significant, the actual change in the level of fatigue was not considered to be clinically relevant, 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 was both statistically and clinically significant.

Participants were grouped into those with a clinically significant increase in fatigue from baseline to completion of EBRT ($n = 12$) and those who did not have a clinically significant increase ($n = 10$). Within the high fatigue group there was a significant decrease in FACT-F scores from baseline (median = 48.00 [range = 28.00-52.00]) to completion of EBRT (median = 35.50 [range = 20.00-46.00], $p = 0.002$), while there was no significant change in median FACT-F score from baseline (44.00 [range = 30.00-52.00]) to completion of EBRT (47.50 [range = 41.00-52.00], $p = 0.12$) in the low fatigue group. Table 2a describes the fatigue and HRQOL characteristics of the all participants and Table 2b describes the fatigue characteristics for those with high versus low fatigue scores.

Table 2.

2a. Fatigue and HRQOL Scores for all Study Participants

Changes in Fatigue and HRQOL Scores during EBRT

		n	Baseline	Last day of Treatment	Range	*p*-value
Fatigue median (range)	rPFS	22	0.93 (0-4.41)	3.68 (0-6.73)	0-10	0.004
	FACT-F	22	47.50 (28-52)	43.00 (20-52)	0-52	0.02
HRQOL Mean (*SD*)	FACT-P	19	132.79 (12.85)	123.74 (18.24)	0-156	0.003

2b. Fatigue Scores for those with High versus Low Fatigue

Changes in FACT-F Scores of High and Low Fatigue Groups during EBRT

		n	Baseline Median (Range)	Last day of Treatment Median (Range)	Range	*p*-value
FACT-F	High	12	48 (28-52)	35.5 (20-46)	0-52	0.002
	Low	10	44 (30-52)	47.50 (41-52)	0-52	0.12

HRQOL= health-related quality of life, EBRT = external beam radiation therapy, SD = standard deviation, FACT-F= Functional Assessment of Cancer Therapy-fatigue subscale, FACT-P = Functional Assessment of Cancer Therapy-Prostate questionnaire

HRQOL. The mean FACT-P score at baseline was 132.79 (*SD* = 12.85) and at completion of EBRT was 123.74 (*SD* = 18.24); lower scores on the FACT-P indicate lower HRQOL. This was a significant decline in HRQOL from baseline to completion of treatment ($p = 0.003$). At the completion of EBRT, the HRQOL of the participants was below the standardized HRQOL score of 126.30 for the general population on the FACT-P (Wei et al., 2002). There was a significant decline in HRQOL from baseline (mean = 132.78, *SD* = 10.05) to completion of treatment (mean = 118.44, *SD* = 17.11, $p = 0.01$) in those with high fatigue. In contrast, there was no significant decline in HRQOL from baseline (mean = 132.80, *SD* = 15.50) to completion of EBRT (mean = 128.50, *SD* = 18.75, $p = 0.12$) in the low fatigue group.

Mitochondrial measures. Five mitochondrial enzymes (complex I-V) and one mitochondrial antioxidant (SOD2) were measured to assess mitochondrial function. There were no significant changes in the mean optical densities of complex I-IV and SOD2 enzymes from

baseline to the end of EBRT for the participants as a whole. However, when participants were categorized into high and low fatigue groups, patterns in the directions of the optical densities of the mitochondrial enzymes were observed. Table 3 lists each of the mitochondrial enzyme levels for baseline and at completion of EBRT for the high and low fatigue groups.

Table 3. Mitochondrial Enzymes between High and Low Fatigue Participants

Level of Fatigue		**Mean (*SE*)**	**Observed Pattern**	***p***
High Fatigue (N=12)	Complex I Baseline	-0.05 (0.17)	Increase	0.68
	Complex I Completion	0.01 (0.14)		
	Complex II Baseline	-1.35 (0.18)	Increase	0.93
	Complex II Completion	-1.04 (0.07)		
	Complex III Baseline	0.14 (0.20)	Increase	0.54
	Complex III Completion	0.30 (0.19)		
	Complex IV Baseline	-0.32 (0.08)	Increase	0.51
	Complex IV Completion	-0.24 (0.12)		
	SOD2 Baseline	3.12 (0.04)	Decrease	0.79
	SOD2 Completion	3.11 (0.03)		
Low Fatigue (N=10)	Complex I Baseline	0.14 (0.13)	Decrease	0.20
	Complex I Completion	-0.05 (0.13)		
	Complex II Baseline	-1.34 (0.19)	Decrease	0.97
	Complex II Completion	-1.35 (0.06)		
	Complex III Baseline	0.66 (0.12)	Decrease	0.16
	Complex III Completion	0.34 (0.24)		
	Complex IV Baseline	-0.15 (0.14)	Decrease	0.61
	Complex IV Completion	-0.22 (0.13)		
	SOD2 Baseline	3.04 (0.04)	Increase	0.91
	SOD2 Completion	3.05 (0.05)		

Complex I. For the participant group as a whole, the median mitochondrial enzyme optical density at baseline was 1.21 (range = 0.10-6.37) and at treatment completion was 0.92 (range = 0.19-15.2). There was no significant change in mean enzyme optical density from baseline to completion of EBRT (t (21) = 0.57, p = 0.58).

Complex II. For the participant group as a whole, the median mitochondrial enzyme optical density at baseline was 0.07 (range = 0-0.14) and at treatment completion was 0.07 (range = 0.03-0.14). There was no significant change in mean enzyme optical density from baseline to completion of EBRT (t (12) = -1.38, p = 0.19). There was no significant difference in the mean complex II optical density between the high and low fatigue groups at baseline, but there was a significant difference at completion of EBRT (p = 0.01), with the high fatigue group having higher optical densities.

Complex III. For the participant group as a whole, the median mitochondrial enzyme optical density at baseline was 2.79 (range = 0.14-17.53) and at treatment completion was 2.19 (range = 0.05-34.67). There was no significant change in mean enzyme optical density from baseline to completion of EBRT (t (21) = 0.33, p = 0.75). At baseline, there was a significant difference in the mean Complex III optical density between the groups (t (17.44) = -2.22, p = 0.04), with the low fatigue group being higher than the high fatigue group, but this was not observed at the last day of EBRT.

Complex IV. For the participant group as a whole, the median mitochondrial enzyme optical density at baseline was 0.42 (range = 0.21-4.14) and at treatment completion was 0.36 (range = 0.22-6.90). There was no significant change in mean enzyme optical density from baseline to completion of EBRT (t (21) = -0.14, p = 0.89).

Complex V. A colorimetric change was observed for complex V enzyme; however, the change was not measurable. A multitude of different serial dilutions were attempted; however, even completely undiluted cell lysate produced no signal above background after repeated attempts.

SOD2. For the participant group as a whole, the median mitochondrial enzyme optical density at baseline was 1195.19 (range = 696.66-2003.01) and at treatment completion was 1193.72 (range = 685.73-2007.62). There was no significant change in mean enzyme optical density from baseline to completion of EBRT (t (21) = 0.15, p = 0.88).

Correlations. Significant correlations for the participant group as a whole were observed between mitochondrial complex II optical density and FACT-F scores at baseline (r = -0.57, p = 0.04), and at completion of EBRT (r = -0.64, p = 0.02). A significant correlation for the whole sample was also observed between the difference in FACT-P scores from baseline to treatment completion with difference in levels of complex III (r = -0.64, p = 0.003) and complex IV (r = -0.59, p = 0.01) from baseline to completion of EBRT.

For those in the high fatigue group, significant correlations were observed at baseline between complex II and FACT-F (r = -0.85, p = 0.01) and HRQOL (r = -0.87, p = 0.01) as well as between complex III and rPFS (r = -0.69, p = 0.01). Significant correlations were also observed between the difference in HRQOL from baseline to treatment completion and mitochondrial enzymes (complex III: r = -0.68, p = 0.05), complex IV: r = -0.79, p = 0.01) from baseline to treatment completion in the high fatigue group. There were no significant correlations observed for mitochondrial enzymes and fatigue scores or HRQOL in the low fatigue group.

Discussion

To our knowledge, this is the first report of measures of mitochondrial enzyme function in relationship to fatigue prior to and at the completion of EBRT in men with prostate cancer. There were significant increases in fatigue and a significant decrease in HRQOL from baseline to the completion of EBRT. In the participant sample as a whole, there was no significant change in mitochondrial function from baseline to completion of treatment. When patients were

characterized into groups based upon change in level of fatigue from baseline to EBRT completion, we observed preliminary evidence to support the possibility of distinct patterns between the groups in mitochondrial enzymes. Low fatigue participants tended to have higher relative levels of mitochondrial enzymes at baseline compared to the high fatigue participants, and the enzyme levels for the low fatigue group tended to decrease during therapy. The opposite was observed in the high fatigue group, such that lower relative mitochondrial enzyme levels were noted at baseline compared to the low fatigue group, and the enzyme levels tended to increase during EBRT. Opposite patterns were observed with the relative SOD2 enzyme levels, such that a decreasing pattern in relative SOD2 levels was noted in the high fatigue group, and an increasing pattern was observed in the low fatigue group. These preliminary findings suggest that alterations in energy metabolism may contribute to fatigue intensification during radiation therapy.

The observed patterns in mitochondrial enzymes between participants with high and low fatigue levels may signal that there is a difference in energy demands between the two groups. High fatigue subjects may require more energy to maintain metabolic needs during therapy, thus driving the increase in mitochondrial ETC enzymes. On the other hand, those in the low fatigue group had higher baseline mitochondrial enzyme levels, which would in theory, enhance cellular energy and thereby support their metabolic needs during therapy.

Declines in mitochondrial enzymes and an increase in mitochondrial antioxidant levels have been reported in cells exposed to radiation therapy (Kam & Banati, 2013; Turrens, 2003; Yoshida et al., 2012). Reactive oxygen species are a byproduct of energy metabolism, and antioxidants such as SOD2 are protective agents against oxidative stress resulting from increased ROS production (Chan, 2006; Turrens, 2003). These preliminary data for the low fatigue group

revealed a pattern that mirrored the pattern observed in previous studies for radiation exposure -- a pattern towards decreased mitochondrial ETC enzymes and increased SOD2 levels. However, our preliminary data in the high fatigue group was the opposite from what would be expected with radiation exposure -- mitochondrial ETC enzymes increased in this group and SOD2 levels decreased. This discrepancy provokes a question as to whether the high fatigue subjects attained a less ideal EBRT outcome in terms of cancer cell death. We compared the mean PSA of high ($0.26 \pm 0.0.74$) and low fatigue (0.25 ± 0.39) participants post-EBRT and found no significant difference ($p = 0.20$). These findings suggest the possibility that there is an increase in ETC enzyme energy metabolism in EBRT-related fatigue, resulting in an increase in ROS production and a decrease in antioxidant availability. However, further research is necessary to determine the clinical relevance, if any, of the relationship of high fatigue during EBRT to treatment outcomes.

Previously published research with persons with chronic fatigue has documented reduced levels of succinate reductase (complex I), cytochrome-c oxidase (complex IV), and CoQ10 (electron shuttle from complex I and II to complex III), as well as disrupted oxidative stress as evidenced by decreased levels of antioxidants (McArdle et al., 1996; Kurup & Kurup, 2003a, 2003b). Our findings revealed opposite patterns; relative levels of complexes I and IV enzymes were increasing, while relative SOD2 levels were decreasing among high fatigue subjects during EBRT. This discrepancy suggests that unlike chronic fatigue, treatment-related fatigue may entail a rapid increase in energy demand, requiring an escalation of mitochondrial energy metabolism. As the fatigue persists or becomes chronic, the compensation mechanism may begin to fail, exhausting the mitochondria as evidenced by reduction in mitochondrial enzyme levels, and in turn, resulting in increased ROS and reduced levels of antioxidants. This line of thinking

is consistent with the mitochondrial ETC decline implicated in aging and various neurodegenerative diseases (Enns, 2003; Wallace, 1999).

The study also brings attention to the possible associations of complexes II and III with fatigue intensification and complexes II, III and IV with HRQOL during EBRT. Complex II dysfunction is often associated with a wide range of clinical manifestations including neuroendocrine disorders, metabolic issues, and skeletal myopathies, which may explain the observed relationship between complex II with fatigue and with HRQOL (Ackrell, 2002; Rutter, Winge, & Schiffman, 2010). Complex III plays a pivotal role in health and disease as it is one of the primary sites of ROS generation (Koopman et al., 2010; Rigoulet, Yoboue, & Devin, 2011). ROS production has been associated with many negative health implications (Alfadda & Sallam, 2012) and could explain the observed association between increased complex III (increased ROS) and decreased fatigue and HRQOL. Complex IV is the final enzyme in the ETC responsible for the reduction of O_2 to H_2O (Mimaki, Wang, McKenzie, Thorburn, & Ryan, 2012). Complex III and complex IV deficiencies are often associated with global, multi-systemic clinical presentations such as those seen in liver failure, renal tubulopathy, myopathy, and cardiomyopathy (Bénit, Lebon, & Rustin, 2009; Antonicka et al., 2003). The broad nature of complex IV dysfunction may explain the observed association between complex IV and global HRQOL. The findings suggest that as mitochondrial enzyme levels increase, HRQOL declines.

This exploratory study identified novel relationships between mitochondrial ETC enzyme dysfunction, fatigue, and HRQOL prior to and at the completion of EBRT; however, the results are limited by the small sample size and the exploratory nature of the group comparisons. The differentiation between high and low fatigue scores on the FACT-F does not control for baseline fatigue levels; however, there were no differences noted between groups in the baseline fatigue

scores. Further investigations are warranted to identify specific mitochondrial activities and structures related to the ETC complexes that may directly influence fatigue and HRQOL using larger samples and more diverse populations. Additionally, as mitochondrial disorders have a large genetic component, the epigenetic impact of cancer therapies on mitochondrial DNA and its potential consequences for behavior need further investigation.

Conclusions

This study explored the association between mitochondrial function, fatigue intensity, and health-related quality of life in men with prostate cancer receiving external beam radiation therapy. The most important preliminary finding from this study is the possibility that mitochondrial respiratory ETC complex enzymes might be related to fatigue intensification during EBRT. However, confirmatory research with a larger sample is needed. These future studies will be critical to determine if these preliminary findings are replicable, and if so, whether there are potential therapeutic targets in individuals at highest risk for fatigue intensification during EBRT, in order to optimize fatigue management and improve HRQOL.

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Chapter Four

To understand the mechanisms related to etiology of cancer-related fatigue (CRF), this dissertation research initially focused on understanding how fatigue intensifies immediately after completion of a localized radiation therapy for a non-metastatic cancer. As a certified oncology nurse, my interest in CRF primarily stems from my clinical encounters with oncology patients who battled through this debilitating symptom, sometimes even years after completion of their cancer treatments. Secondly, CRF is an important, discrete, and approachable problem. Unlike fatigue associated with other diseases such as chronic fatigue syndrome, fibromyalgia, or traumatic brain injury, the trajectory of CRF and the potential biological mechanisms can be monitored from the time of diagnosis, and its triggers, such as stage of disease as well as dose and type of treatment, can be identified and examined.

The biological pathways responsible for CRF have not yet been established. CRF is commonly believed to be a multidimensional symptom that involves multiple psychosocial and physiologic mechanisms (Wang, 2008), with most studies focused on markers of inflammation such as cytokine levels. However, another plausible mechanism for fatigue is disturbances in mitochondrial function. With my specific interest in the role of mitochondrial function in CRF, my first step in this exploration was to conduct a literature review to examine markers of mitochondrial function that have been shown to be associated with fatigue in order to identify areas needing further research. The findings from this review provided an empirical foundation

for my program of research. Based on the findings of this review, an ideal starting point for my investigation involved the measurement of critical mitochondrial enzymes such as complexes I-V and mitochondrial-specific oxidative stress marker before and after radiotherapy.

Using the knowledge gained from the literature review, I developed a proposal to investigate – a potential association between altered energy metabolism as evidenced by mitochondrial measures and levels of fatigue – in the oncology population. To my knowledge, the research that followed is the first to describe the levels of and relationship between mitochondrial enzyme activity and fatigue prior to and at the completion of external beam radiation therapy (EBRT) in men with prostate cancer. The primary aim of the research was to describe levels of biomarkers of mitochondrial function, fatigue, and health-related quality of life (HRQOL) before and at the completion of EBRT. A secondary analysis was conducted of a descriptive, longitudinal study, *Relationship between Mitochondrial Dysfunction and Fatigue in Cancer Patients Following External Beam Radiation Therapy* that was approved by the National Institutes of Health (NIH) Institutional Review Board (#10-NR-0128), using a subsample of twenty-two men with nonmetastatic prostate cancer. There were significant increases in the levels of fatigue and declines in HRQOL from baseline to the completion of EBRT. However, there was no significant change in the selected biomarkers of mitochondrial function from baseline to completion of treatment. This result was unexpected, given the a priori expectation of declines in mitochondrial enzymes and an increase in the mitochondrial antioxidant. My hypothesis was based upon the findings of my literature review which showed that in various fatigued populations, several mitochondrial enzymes involved in oxidative phosphorylation were significantly decreased with a significant increase in oxidative stress (McArdle et al., 1996; Kurup & Kurup, 2003a, 2003b; Maes et al., 2009a, 2009b; Segal et al., 2012). Additionally,

declines in mitochondrial enzymes and an increase in mitochondrial antioxidant levels have been reported in cells exposed to radiation therapy (Yoshida, Goto, Kawakatsu, Urata, & Li, 2012). Given the exploratory nature of the study, the dissertation research team decided to further investigate the patient sample to understand the relationship of fatigue and mitochondrial function in a well-characterized fatigue phenotype. We decided to categorize the participants into those who developed fatigue intensification and those who did not develop fatigue intensification during EBRT. The fatigue categories were based upon a cut-score of a greater than or equal to 3-point decrease in the Functional Assessment of Cancer Therapy-Fatigue subscale (FACT-F) score, which has been shown using this validated measure to represent the minimally important difference in this fatigue score that requires clinical intervention (Cella, Eton, Lai, Peterman, & Merkel, 2002).

When patients were characterized into groups based upon change in level of fatigue from baseline to EBRT completion, there was preliminary evidence to support the possibility of patterns of mitochondrial enzyme levels between the two fatigue groups; however, these differences were not statistically significant. Those with non-clinically significant (low) fatigue had higher relative levels of mitochondrial electron transport chain (ETC) enzymes at baseline and lower levels of the antioxidant superoxide dismutase 2 (SOD2) at baseline compared to the clinically significant (high) fatigue participants. Further, the enzyme levels for the low fatigue group decreased during EBRT. The opposite was observed in the high fatigue group, such that lower relative mitochondrial ETC enzyme levels and higher antioxidant levels were noted at baseline in comparison to the low fatigue group, and the enzyme levels increased during EBRT. Opposite patterns were observed with the relative SOD2 enzyme levels, such that a decreasing pattern in relative SOD2 levels was noted in the high fatigue group, and an increasing pattern

was observed in the low fatigue group from baseline to completion of EBRT. These preliminary findings suggest that alterations in energy metabolism may contribute to fatigue intensification during radiation therapy. However, replication with a larger sample is necessary. Although our preliminary evidence was not statistically significant, the results of this dissertation study need to be replicated in light of the potential clinical implications of mitochondrial activity and CRF.

The plans for the continuation of this program of research encompass a three-part trajectory: a clinically based inquiry, an animal model, and cell-based investigation. The future clinical research arm of my program of research will include replication of the dissertation research with a larger sample size of the same clinical population of men with non-metastatic prostate cancer receiving localized radiation therapy. Replication in other cancer populations (e.g., persons with lung, brain, and breast cancer) receiving radiation therapy will also be needed to test for the presence of a common physiologic pathway for the etiology of fatigue across irradiated populations. One such pathway might involve the association between mitochondrial dysfunction, inflammation, and neurodegenerative pathways.

Investigation of the upstream pathway of genes encoding the mitochondrial complexes that correlated with fatigue in the dissertation findings would also be an important project to pursue, especially genes encoding complex II, which was significantly correlated with fatigue scores at baseline and at completion of EBRT. This upstream investigation would require the use of genomic and epigenetic platforms. Cancer and cancer therapy have been observed to initiate genetic and epigenetic modifications, such as deoxyribonucleic acid (DNA) methylation changes (Dobosy, Roberts, Fu, & Jarrard, 2007; Li, Carroll, & Dahiya, 2005). Wang et al. (unpublished data) from the NINR lab recently observed loss of DNA methylation in the promoter region of genes encoding a mitochondrial function-related gene for which expression was significantly

associated with fatigue during EBRT in men with prostate cancer. These epigenetic modifications may be reversible through lifestyle remodeling, an important implication for the role of nursing interventions for those with cancer (Alegría-Torres, Baccarelli, & Bollati, 2011).

Further, investigating the functional activity of the mitochondria to see if active mitochondrial respiration dysfunction is associated with fatigue during cancer therapy is worth pursuing. Aligning with mitochondrial experts at the National Institute of Nursing Research, Virginia Commonwealth University, and the University of Florida who can provide mentorship and opportunities in developing skills required to observe actively respiring mitochondria will be a crucial next step.

The goal of my program of clinical research is to identify a biomarker of mitochondrial function that relates to the development of fatigue during radiation therapy. Ultimately, identification of the biologic mechanisms underlying CRF can lead to the development of potential therapeutic targets. Future animal and cell-based studies will be incorporated into my research trajectory to enable enhanced mechanistic investigations of the etiology of CRF. In the near future I will replicate the dissertation procedures in a murine model to determine sample-specific central and peripheral markers that may establish the relationship of mitochondrial function and fatigue, using genomic and proteomic approaches. This animal investigation may assist in plotting important central and peripheral neuromuscular circuits that influence the development or intensification of fatigue behaviors in the animals. If therapeutic targets can be identified in the murine model of radiation-related fatigue, then therapeutic manipulations can be explored to potentially mitigate the fatigue response.

In clinical practice, vitamin supplements such as riboflavin B2, niacin B3, vitamin E and other mitochondrial cofactors including levo-carnitine, lipoic acid, and acetyl-l-carnitine are used

as supplemental treatment for mitochondrial disorders in order to enhance either ETC enzyme activity or antioxidant defenses (Cohen, 2000). The efficacy of these vitamins and mitochondrial cofactors as treatments for mitochondrial disorders remain controversial and more research is needed, especially concerning the benefit of these supplements in an oncology population (Cohen, 2000). The mouse model will be essential to determine the effects of these therapies on mitochondrial function and fatigue prior to translation to human trials.

The cell-based model will be equally important to enhance exploration into the mechanisms involved in CRF. The cell-based models will be utilized to provide a deeper understanding of the effects of radiation on the body. It has been widely reported that inflammation plays a role in CRF (Bower & Lamkin, 2013). Monocytes and other cell lines can be irradiated, cultured, and manipulated in various ways to replicate inflammatory responses in humans during radiation treatment. Additionally, the mitochondrial activity within these irradiated monocytes can be investigated to determine the effect of radiation on the mitochondria in this inflammatory cell.

This planned program of research is designed to enhance understanding of the biomarkers of mitochondrial dysfunction that might contribute to fatigue. This program utilizes a three-model approach to maximize the knowledge that can be gained and the skills that can be acquired to answer this important research question. Understanding the mechanisms behind CRF will ultimately enable identification of therapeutic targets and treatment strategies that can improve health-related quality of life of oncology patients.

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Appendix

The following measures will be used for the proposed study and are standardized forms that are validated. All measures have been completed by participants and data confidentially stored.

1. Sociodemographic Data Sheet
2. revised Piper Fatigue Scale
3. Functional Assessment of Cancer Therapy- Fatigue subscale
4. Functional Assessment of Cancer Therapy-Prostate

Study ID: _____

Sociodemographic Data Sheet

Date: _____

Race: ___ White
___ Black/African American
___ Native Hawaiian/Other Pacific Islander
___ Asian
___ Native American/Alaskan Native
___ Other (specify: _____)
___ Unknown

Ethnicity: ___ Hispanic/Latino
___ Not Hispanic/Latino
___ Unknown

Education: ___ 8th or less
___ 9-12th grade, did not graduate
___ High school grad/GED
___ Vocational/technical school
___ Assoc degree/some college
___ Bachelor's degree
___ Advanced degree
___ Other (specify: _____)
___ Unknown

Marital Status: ___ Married
___ Widowed
___ Single
___ Divorced/Separated
___ Living as married
___ Unknown

Persons in Household: ___ Live alone ___ Live with 1 other person
___ Live with 2-4 others ___ Live with 5 or more others
___ Unknown

Employment Status: ___ Employed outside home/full time ___Employed outside home/part time
___ Homemaker, employed at home ___ Retired
___ Disabled ___ In school
___ Not working ___ Unknown

Annual Household Income: ___ < $8,000 ___ $8000-$14,999
___ $15,000 - $24,999 ___ $25,000 - $34,999
___ $35,000 - $49,999 ___ >$50,000
___ Unknown

Subject ID # _____

Piper Fatigue Scale

Directions: Many individuals can experience a sense of unusual or excessive tiredness whenever they become ill or receive treatment for their illness. This unusual sense of tiredness is not usually relieved by either a good night's sleep or by rest. Some call this symptom fatigue to distinguish it from the usual sense of tiredness.

For each of the following questions, please circle the number which best describes the fatigue you are experiencing now or for today. Please make every effort to answer each question to the best of your ability. If you are not experiencing fatigue now or for today, circle a "0" for your responses. Thank you very much!

1. How long have you been feeling fatigued? (Check one response only.)
   a. _____ minutes
   b. _____ hours
   c. _____ days
   d. _____ weeks
   e. _____ months
   f. _____ other (please describe):

---

2. To what degree is the fatigue you are feeling now causing you distress?

0	1	2	3	4	5	6	7	8	9	10
No distress										**A great deal of distress**

3. To what degree is the fatigue you are feeling now interfering with your ability to complete your work or school activities?

0	1	2	3	4	5	6	7	8	9	10
None										**A great deal**

4. To what degree is the fatigue you are feeling now interfering with your ability to visit or socialize with your friends?

0	1	2	3	4	5	6	7	8	9	10
None										**A great deal**

5. To what degree is the fatigue you are feeling now interfering with your ability to engage in sexual activity?

0	1	2	3	4	5	6	7	8	9	10
None										**A great deal**

Continued on next page.

Subject ID # _____

6. Overall, how much is the fatigue which you are experiencing now interfering with your ability to engage in the kind of activities you enjoy doing?

0 1 2 3 4 5 6 7 8 9 10
None **A great deal**

7. How would you describe the degree of intensity or severity of the fatigue which you are experiencing now?

0 1 2 3 4 5 6 7 8 9 10
Mild **Severe**

8. To what degree would you describe the fatigue which you are experiencing now as being:

0 1 2 3 4 5 6 7 8 9 10
Pleasant **Unpleasant**

9. To what degree would you describe the fatigue which you are experiencing now as being:

0 1 2 3 4 5 6 7 8 9 10
Agreeable **Disagreeable**

10. To what degree would you describe the fatigue which you are experiencing now as being:

0 1 2 3 4 5 6 7 8 9 10
Protective **Destructive**

11. To what degree would you describe the fatigue which you are now experiencing as being:

0 1 2 3 4 5 6 7 8 9 10
Positive **Negative**

12. To what degree would you describe the fatigue which you are now experiencing as being:

0 1 2 3 4 5 6 7 8 9 10
Normal **Abnormal**

13. To what degree are you now feeling:

0 1 2 3 4 5 6 7 8 9 10
Strong **Weak**

Continued on next page.

Subject ID # _____

14. To what degree are you now feeling:

0	1	2	3	4	5	6	7	8	9	10
Awake										**Sleepy**

15. To what degree are you now feeling:

0	1	2	3	4	5	6	7	8	9	10
Lively										**Listless**

16. To what degree are you now feeling:

0	1	2	3	4	5	6	7	8	9	10
Refreshed										**Tired**

17. To what degree are you now feeling:

0	1	2	3	4	5	6	7	8	9	10
Energetic										**Unenergetic**

18. To what degree are you now feeling:

0	1	2	3	4	5	6	7	8	9	10
Patient										**Impatient**

19. To what degree are you now feeling:

0	1	2	3	4	5	6	7	8	9	10
Relaxed										**Tense**

20. To what degree are you now feeling:

0	1	2	3	4	5	6	7	8	9	10
Exhilarated										**Depressed**

21. To what degree are you now feeling:

0	1	2	3	4	5	6	7	8	9	10
Able to concentrate										**Unable to concentrate**

Continued on next page.

Subject ID # _____

22. To what degree are you now feeling:

0	1	2	3	4	5	6	7	8	9	10
Able to remember										**Unable to remember**

23. To what degree are you now feeling:

0	1	2	3	4	5	6	7	8	9	10
Able to think clearly										**Unable to think clearly**

24. Overall, what do you believe is most directly contributing to or causing your fatigue?_____
_____
_____

25. Overall, the best thing you have found to relieve your fatigue is: _____
_____
_____

26. Is there anything else you would like to add that would describe your fatigue better to us? _____
_____
_____

27. Are you experiencing any other symptoms right now? _____
_____
_____

FACIT Fatigue Scale (Version 4)

Below is a list of statements that other people with your illness have said are important. Please circle or mark one number per line to indicate your response as it applies to the past 7 days.

		Not at all	A little bit	Some-what	Quite a bit	Very much
H7	I feel fatigued	0	1	2	3	4
H12	I feel weak all over	0	1	2	3	4
An1	I feel listless ("washed out")	0	1	2	3	4
An2	I feel tired	0	1	2	3	4
An3	I have trouble starting things because I am tired	0	1	2	3	4
An4	I have trouble finishing things because I am tired	0	1	2	3	4
An5	I have energy	0	1	2	3	4
An7	I am able to do my usual activities	0	1	2	3	4
An8	I need to sleep during the day	0	1	2	3	4
An12	I am too tired to eat	0	1	2	3	4
An14	I need help doing my usual activities	0	1	2	3	4
An15	I am frustrated by being too tired to do the things I want to do	0	1	2	3	4
An16	I have to limit my social activity because I am tired	0	1	2	3	4

FACT-P (Version 4)

Below is a list of statements that other people with your illness have said are important. **By circling one (1) number per line, please indicate how true each statement has been for you during the past 7 days.**

	PHYSICAL WELL-BEING	Not at all	A little bit	Some-what	Quite a bit	Very much
GP1	I have a lack of energy	0	1	2	3	4
GP2	I have nausea	0	1	2	3	4
GP3	Because of my physical condition, I have trouble meeting the needs of my family	0	1	2	3	4
GP4	I have pain	0	1	2	3	4
GP5	I am bothered by side effects of treatment	0	1	2	3	4
GP6	I feel ill	0	1	2	3	4
GP7	I am forced to spend time in bed	0	1	2	3	4

	SOCIAL/FAMILY WELL-BEING	Not at all	A little bit	Some-what	Quite a bit	Very much
GS1	I feel close to my friends	0	1	2	3	4
GS2	I get emotional support from my family	0	1	2	3	4
GS3	I get support from my friends	0	1	2	3	4
GS4	My family has accepted my illness	0	1	2	3	4
GS5	I am satisfied with family communication about my illness	0	1	2	3	4
GS6	I feel close to my partner (or the person who is my main support)	0	1	2	3	4
Q1	*Regardless of your current level of sexual activity, please answer the following question. If you prefer not to answer it, please check this box* ☐ *and go to the next section.*					
GS7	I am satisfied with my sex life	0	1	2	3	4

FACT-P (Version 4)

By circling one (1) number per line, please indicate how true each statement has been for you during the past 7 days.

	EMOTIONAL WELL-BEING	Not at all	A little bit	Some-what	Quite a bit	Very much
GE1	I feel sad	0	1	2	3	4
GE2	I am satisfied with how I am coping with my illness	0	1	2	3	4
GE3	I am losing hope in the fight against my illness	0	1	2	3	4
GE4	I feel nervous	0	1	2	3	4
GE5	I worry about dying	0	1	2	3	4
GE6	I worry that my condition will get worse	0	1	2	3	4

	FUNCTIONAL WELL-BEING	Not at all	A little bit	Some-what	Quite a bit	Very much
GF1	I am able to work (include work at home)	0	1	2	3	4
GF2	My work (include work at home) is fulfilling	0	1	2	3	4
GF3	I am able to enjoy life	0	1	2	3	4
GF4	I have accepted my illness	0	1	2	3	4
GF5	I am sleeping well	0	1	2	3	4
GF6	I am enjoying the things I usually do for fun	0	1	2	3	4
GF7	I am content with the quality of my life right now	0	1	2	3	4

FACT-P (Version 4)

By circling one (1) number per line, please indicate how true each statement has been for you during the past 7 days.

	ADDITIONAL CONCERNS	Not at all	A little bit	Some-what	Quite a bit	Very much
C2	I am losing weight	0	1	2	3	4
C6	I have a good appetite	0	1	2	3	4
P1	I have aches and pains that bother me	0	1	2	3	4
P2	I have certain parts of my body where I experience significant pain	0	1	2	3	4
P3	My pain keeps me from doing things I want to do	0	1	2	3	4
P4	I am satisfied with my present comfort level	0	1	2	3	4
P5	I am able to feel like a man	0	1	2	3	4
P6	I have trouble moving my bowels	0	1	2	3	4
P7	I have difficulty urinating	0	1	2	3	4
HL2	I urinate more frequently than usual	0	1	2	3	4
P8	My problems with urinating limit my activities	0	1	2	3	4
HL5	I am able to have and maintain an erection	0	1	2	3	4

Vita

Kristin Ashley Filler was born on December 8, 1986 in Fairfield, California, and is an American citizen. She graduated from Bishop Ireton High School, Alexandria, Virginia in 2005. She received her Bachelor of Science, major in nursing, from Virginia Commonwealth University (VCU), Richmond, Virginia in 2009. She worked as a registered nurse on the inpatient oncology unit at the VCU Health Systems and at the Hospital Corporation of America (HCA) Johnston-Willis Hospital from 2009-2012. In addition, she also worked as a research assistant on two R01 funded nursing studies at the VCU School of Nursing from 2009-2012. During her doctoral studies she has had the honor of receiving support from the American Cancer Society, the Jonas Center for Nursing Excellence/AACN as well as a research traineeship with the National Institute of Nursing Research at the National Institutes of Health from 2012-2014.