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MARKERS OF MITOCHONDRIAL ENERGY METABOLISM AND THEIR POTENTIAL RELATIONSHIPS WITH FATIGUE IN HUMAN ADULTS: A SCOPING REVIEW

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Abstract

This scoping review aimed to synthesize the best available evidence of the associations between molecular and genetic markers of mitochondrial metabolism and fatigue in human adults. The research question guiding this review was, “Are there potential relationships between mitochondrial metabolism markers and fatigue?” The literature search used three terms (mitochondria; fatigue; energy metabolism), which yielded 263 manuscripts and 22 theses/dissertations. The studies included in the review had to meet three criteria: (1) Include adult participants (≥18 years of age); (2) Show a relationship between mitochondrial energy metabolism and fatigue; (3) Be published in English, Spanish, or Portuguese. Of the 17 articles included for a full-text review, some had a cross-sectional design (6/17, 35%), and more than half (12/17, 70%) were published between 2015 and 2020. The predominant population studied were patients diagnosed with chronic fatigue syndrome (9/17, 53%). Most studies (15/17, 88%) assessed fatigue with validated instruments. Mitochondrial markers associated with fatigue are a) mitochondrial transport pathways and respiratory chain, b) mutations in mitochondrial DNA, and c) energy disorders in cells of the immune system, such as natural killer cells. Mitochondrial metabolic activities, such as the production and transport of ATP, are significant components that may help understand the etiology of fatigue. Future directions should include longitudinal study designs, characterization of fatigue phenotypes, and the identification of markers involved in production and transport pathways. The clinical relevance in this field can lead to interventions targeting mitochondrial markers to reduce or prevent fatigue.

Keywords: Energy metabolism. Fatigue. Mitochondria. Oxidative phosphorylation. Review.

1. Introduction

Fatigue is a complex and multidimensional symptom defined as a subjective and persistent sensation of tiredness and physical, emotional, and/or cognitive exhaustion disproportionate to recent activities, not improving with rest and sleep, and interfering with several areas of life (Saligan et al. 2015; Feng et al. 2020). Many studies have explored the interindividual variability, persistence, and severity of fatigue in different clinical populations, such as people with cancer (Peoples et al. 2017; Dias et al. 2020; Al Maqbali et al. 2021; Wu et al. 2022), muscle failure (Allard et al. 2018; Norbury et al. 2022), and cardiac pathologies (Schaefer et

al. 2013; Joseph et al. 2021; Cock et al. 2022). The assessment and management of fatigue are still inconsistent, as no specific biomarkers have been validated to assist in a definitive diagnosis that informs appropriate interventions and effective treatments (Saligan et al. 2016; Allard et al. 2018; Lee et al. 2021).

Different biological mechanisms of fatigue have been proposed, including the role of inflammatory cytokines, neurotransmitter dysfunction (Wang et al. 2008; Gheita et al. 2020), and the hypothalamic-pituitary-adrenal axis (Surapaneni et al. 2012; Doerr et al. 2021). More recent studies have focused on in mitochondrial metabolism changes (Tomas et al. 2017; Pierce et al. 2018; Rusin et al. 2021; Hamilton and Jensen 2021; Korzeniewski 2022), as they have become a target of interest for researchers seeking evidence of the biological mechanisms of fatigue (Booth et al. 2012; Hsiao et al. 2019; Rusin et al. 2021; Korzeniewski 2022).

A previous review (Allard et al. 2018) associated mitochondrial energy metabolism disorders with extreme fatigue. People with muscle weakness, fatigue, and clustering of neuropsychological symptoms have lower energy from the impairment in adenosine triphosphate (ATP) production due to changes in oxidative phosphorylation (Booth et al. 2012). Besides severe fatigue, chronic fatigue syndrome (CFS) patients and those with primary mitochondrial diseases also share exercise intolerance and myalgia, often caused by mitochondrial DNA mutations (Allard et al. 2018). These investigations have led experts to propose the contributions of genetic and molecular factors related to mitochondrial metabolism in the pathophysiology of fatigue (Lutgendorf et al. 2011; Hsiao et al. 2019; Hsiao et al. 2021).

Besides the background aforementioned, which provided an initial understanding of the relationships between some biomarkers related to mitochondrial dysfunction and fatigue, the present review collected recent information mainly from the past 10 years when new techniques were developed to measure markers related to mitochondrial metabolism. Therefore, the main goal of this scoping review was to identify and synthesize the most current scientific evidence on the potential relationships between mitochondrial energy metabolism markers and fatigue.

2. Material and Methods

It is a scoping review prepared according to the methodology by the Joanna Briggs Institute (JBI), Reviewer's Manual and theoretical recommendations by Arksey and O'Malley (2005) and based on the Preferred Reporting Items for Systematic reviews and Meta-Analyses extension for Scoping Reviews (PRISMA-ScR) guideline (Page et al. 2021).

The research question and search strategy were guided by the PCC (Population, Concept, and Context) tool (Peters et al. 2020). This study considered: P - Human adults; C - Markers of mitochondrial metabolism; C - Fatigue. A combination of descriptors and keywords was used with Boolean operators (Table 1) to search the literature to answer the research question: "Are there potential relationships between mitochondrial metabolism markers and fatigue?".

A comprehensive search was performed in the PubMed, SCOPUS, EMBASE, and Web of Science databases, the screening period was between January 2019 and January 2022, and the search terms were adapted to the specificity of each database.

The gray literature was also consulted in the following databases: Brazil - Thesis and Dissertations Portal for the Coordination of Improvement of Higher Education Personnel (CAPES); Brazil - The Digital Library of Theses and Dissertations of the University of São Paulo; Portugal - Scientific Repository of Open Access of Portugal (RCAAP); Europe E-theses Portal (DART); Canada - Theses Canada And World - Cyberthesis; Australia and New Zealand - The National Library of Australia's Trobe (Trove). These databases were included because they somehow represent research performed on different continents. According to the inclusion criteria of the study, the databases of the Asian continent were not investigated due to language limitations.

Next, all identified citations were organized and uploaded into Rayyan (Ouzzani et al. 2016), a review software used to aid the initial selection of articles by facilitating title and abstract screening of the selected studies. It also allows removing duplicates and provides practicality for reviewers in selecting the articles of interest.

Table 1. Search strategies according to databases.

Databases	Search Strategies
PubMed	(mitochondria[mesh] OR mitochondria[tiab] OR mitochondrial[tiab]) AND (fatigue[mesh] OR fatigue*[tiab]) AND (bioenergy[tiab] OR bioenergetic*[tiab] OR energy metabolism[mesh] OR "energy metabolism" [tiab] OR "energy expenditure*" [tiab]) NOT animals[mesh]
EMBASE	('mitochondrion'/exp/mj OR mitochondria:ti,ab OR mitochondrial:ti,ab) AND ('fatigue'/exp/mj OR fatigue*:ti,ab) AND ('bioenergy'/exp/mj OR bioenergy:ti,ab OR bioenergetic*:ti,ab OR 'energy metabolism'/exp/mj OR 'energy metabolism':ti,ab OR 'energy expenditure'/exp/mj OR 'energy expenditure*':ti,ab) AND [humans]/lim
Scopus	(TITLE-ABS (mitochondria OR mitochondrial) AND TITLE-ABS (fatigue*) AND TITLE-ABS (bioenergy OR bioenergetic* OR "energy metabolism" OR "energy expenditure*") AND (LIMIT-TO (EXACTKEYWORD, "Human") OR LIMIT-TO (EXACTKEYWORD , "Humans"
Web of Science	TOPIC: (mitochondria OR mitochondrial) AND TITLE: (fatigue*) AND TOPIC: (bioenergy OR bioenergetic* OR energy metabolism OR energy expenditure*) TITLE: (mitochondria OR mitochondrial) AND TOPIC: (fatigue*) AND TOPIC: (bioenergy OR bioenergetic* OR energy metabolism OR energy expenditure*)

The inclusion and exclusion criteria were extensively discussed and agreed upon among the reviewers beforehand to minimize potential biases during the full-text review and selection. Therefore, the inclusion criteria were clinical studies (1) including adult participants (≥ 18 years of age); (2) showing a relationship between markers of mitochondrial metabolism and fatigue; (3) written in English, Spanish, or Portuguese. Studies with animal models, case reports, editorials, letters, literature reviews, and conference abstracts were not included.

Four independent researchers (AVAL, PVB, BTB, and JTT) examined the titles and abstracts for inclusion, blinded by all reviewers. After the screening, the team discussed the results, resolved conflicts, and sought consensus on the articles selected for full reading.

All reviewers read the full texts of the selected articles, again in blind mode, and screened them according to the inclusion and exclusion criteria. The final sample was reached after a consensus. Moreover, a manual search was conducted, which involved analyzing the references of each article that met the inclusion criteria. Lastly, as recommended by the JBI guide, a specialist in the field of symptom science (LNS) was consulted to ensure that the main articles on the topic were in the search results.

For data collection, including extracting information directly from articles and other study reports, a database was created according to the author, year, study design, research question/objective, studies focused on mitochondrial metabolism and fatigue, the genetic and molecular factors related to mitochondrial bioenergetics, and the use of tissue and/or blood samples of human adults.

3. Results

Search results

The initial search in the databases and the grey literature yielded 263 articles and 22 manuscripts (theses and dissertations), respectively, and 144 articles did not receive consensus for inclusion and were excluded from the full-text review. Sixty-three articles were selected for full-text reading. Forty-six articles were excluded for not meeting the inclusion criteria, mainly for not relating fatigue and mitochondrial function. No dissertation or thesis (grey literature) was included in the final sample because they did not provide enough scientific evidence regarding the research question, and most just proposed future research directions. Finally, this review included 17 articles (Figure 1).

Study characteristics

Most published articles were by authors from the United States (47%) (Hsiao et al. 2014; Wawrzyniak et al. 2016; Tomas et al. 2017; Pierce et al. 2018; Hsiao et al. 2019; Feng et al. 2020; Hsiao et al. 2021; Hamilton and Jensen 2021). The remaining articles were from Australia (Reuter and Evans 2011; Nguyen et al. 2018; Missailidis et al. 2020), the UK (Myhill et al. 2009; Booth et al. 2012), China (Lau et al. 2015), the Netherlands (Vermeulen et al. 2010), Germany (Herpich et al. 2021), and Denmark (Fernandez-Guerra et al.

2021). Of the 17 articles included for full-text review, more than half (12/17, 70%) were published recently (2015-2021), some were cross-sectional or longitudinal (6/17, 35% each) or case-control studies (23%), and one was an open-label study (5%).

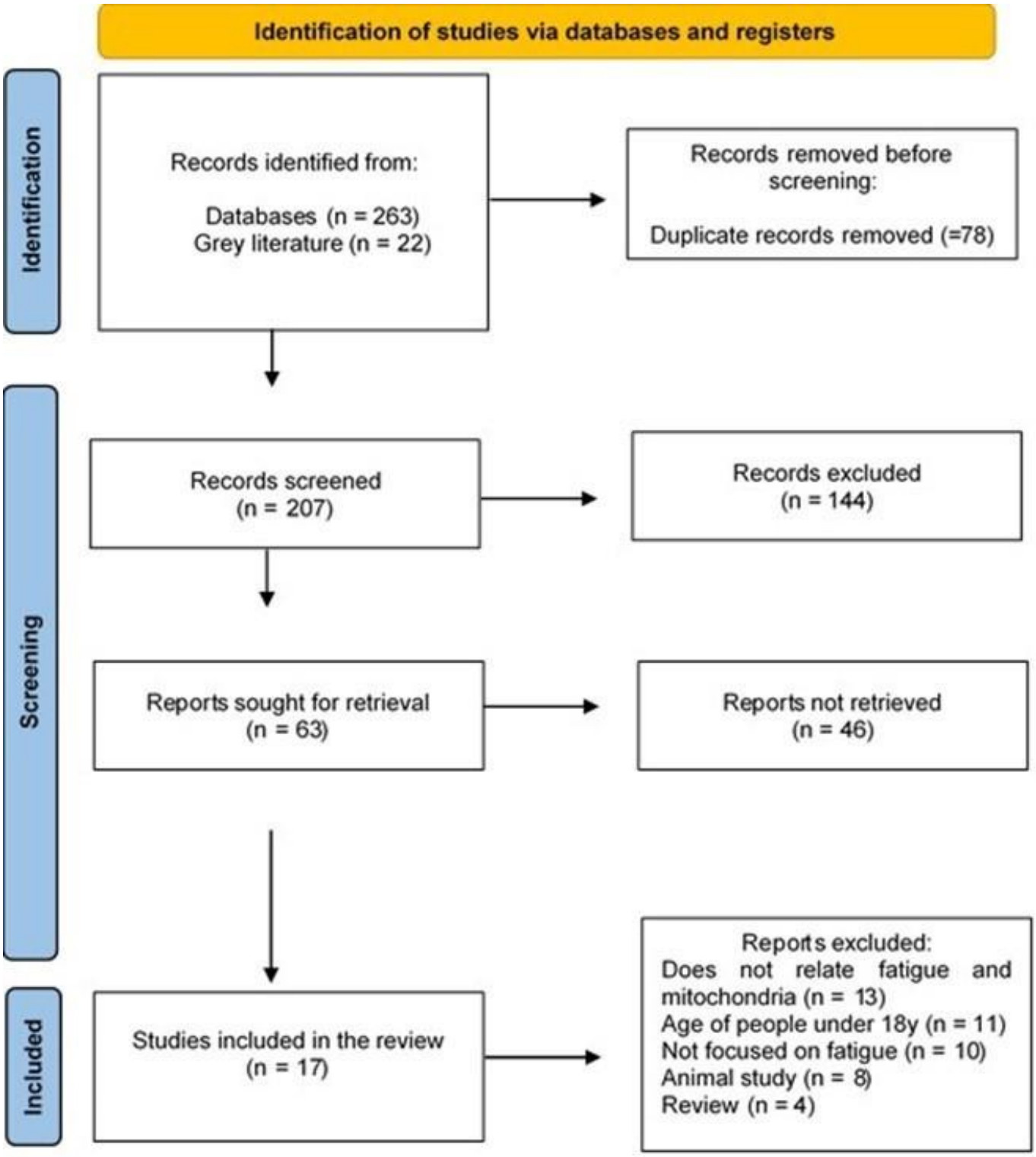

Figure 1. Flowchart for selecting publications in the databases.

According to the authors, adults of both sexes experience fatigue similarly (13/17, 76%) in different clinical conditions. The relationships of markers of mitochondrial energy metabolism have been studied mainly in people with chronic fatigue syndrome (CFS) (9/17), followed by prostate cancer patients (4/17). Also, muscle and mental fatigue (2/17), migraine (1/17), and heart failure (1/17) have been considered (Table 2).

Markers of mitochondrial energy metabolism were investigated, especially with peripheral blood (15/17, 88%), to evaluate: a) Gene expression related to mitochondrial function; b) Mitochondrial

dysfunction in natural killer cells (NK); c) Neutrophil counts; d) L-carnitine and acylcarnitine levels. The vastus lateralis muscle biopsy was also used as biological material to study these markers. One study did not state the biological markers explored, and another obtained data from a medical records review.

Fatigue was measured primarily with specific psychometric instruments (15/17, 88%), including the Patient-Reported Outcomes Measurement Information System-Fatigue (PROMIS-F), Fatigue Severity Scale (FSS), Functional Assessment of Cancer Therapy-Fatigue (FACT-F), Piper Fatigue Scale (PFS), Canadian Consensus Criteria (CCC), Brief Fatigue Inventory (BFI), Bell CFS Ability Scale, and the Vigor subscale.

4. Discussion

The main biological aspects and pathophysiology of fatigue are still unknown (Saligan et al. 2016; Feng et al. 2020). However, symptom science experts support that genetic and molecular factors, such as changes in gene expression, structure proteins, and metabolic pathways, may relate to mitochondrial biogenesis and bioenergetics in fatigue (Tomas et al. 2017; Hsiao et al. 2019; Hamilton and Jensen 2021). Significant results from this review confirmed that mitochondrial deficits, as discussed below, contribute to fatigue, establishing a direct relationship with the lack of energy (ATP) and its low production, directly affecting mitochondrial bioenergetics.

Mitochondria are intimately involved in cellular homeostasis, producing most energy used in our entire metabolism through the respiratory chain (Tomas et al. 2017). Thus, mitochondrial dysfunction or changes in the composition of enzymes that participate in ATP production resulting from DNAmt mutations may cause apoptosis, contributing to the emergence of mitochondrial diseases and adjacent syndromes such as chronic fatigue syndrome (CFS) (Tomas et al. 2017; Pierce et al. 2018; Fernandez-Guerra et al. 2021; Hamilton and Jensen 2021).

The ATP molecule is present in cells that form a complex with magnesium; when hydrolyzed to diphosphate (ADP), it represents the main source of energy for muscle, skeletal, and cardiac tissues, among others (Myhill et al. 2009). The mitochondrial membrane consists of five complexes (I, II, III, IV, and V) responsible for maintaining the optimal functioning of mitochondrial bioenergetics (Tomas et al. 2017). Complex V synthesizes ATP, which is used as an energy source for several activities in cell metabolism (Hsiao et al. 2019; Fernandez-Guerra et al. 2021). A recent study found that ATP synthesis by complex V was inefficient in lymphoblasts of people diagnosed with CFS (Missailidis et al. 2020), with a significantly low rate compromising respiratory synthesis, which could be directly related to the fatigue symptom reported by patients. Even considering this abnormality in complex V functioning, the other complexes (I-IV) maintained a normal flow of electrons and levels of ATP homeostasis. Therefore, the defect in oxidative phosphorylation is isolated in ATP synthesis by complex V (Missailidis et al. 2020).

Missailidis (2020) highlights questions about the reasons for changes in complex V functioning. Among them are mutations that might affect the subunits of this complex or the structure of proteins included in it (Missailidis et al. 2020; Feng et al. 2020). However, research has already been exploring the mutations of this complex, which so far has been discarded as a possible cause of dysfunction in this structure (Missailidis et al. 2020; Feng et al. 2020; Fernandez-Guerra et al. 2021).

Another reason is the absence of protons to produce ATP, termed a “leak in protons”, rendering complex V inefficient and with increased consumption in other processes. However, this study showed that the electron transport capacity in lymphoblasts is more than sufficient to allow complex V to operate with regular efficiency (Missailidis et al. 2020).

Lastly, it could be related to the deregulatory inhibition of complex V, considering that mitochondrial ATP synthase activity can be regulated by a variety of proteins, small molecules, and signaling pathways, some of which work through the AIF1 inhibitory subunit of complex V (Campanella et al. 2009; Garcia-Bernudez and Cuezya 2016). As this is a recent discovery in the field, further studies are needed with other methods to understand and evaluate the possible causes of complex V inefficiency in mitochondria and how it contributes to fatigue symptoms (Missailidis et al. 2020).

Some reviewed articles cited the dysfunctions in these mitochondrial membrane complexes as the possible etiology of fatigue (Vermeulen et al. 2010; Reuter and Evans 2011; Hsiao et al. 2019; Missailidis et al. 2020; Feng et al. 2020; Herpich et al. 2021). Another significant function of the mitochondrial membrane,

Table 2. Summary of the studies investigating the relationship between mitochondrial energy metabolism and fatigue (N = 17).

Authors/ year/ country	Goal	Study design	Sample characteristics	Fatigue measurement	Biomarker assessed	Sample source	Main outcomes
Hsiao et al. (2014), U.S.	To explore the relationships between changes in gene expression related to mitochondrial biogenesis/bioenergy and fatigue in men with prostate cancer receiving external beam radiation therapy (EBRT).	Longitudinal; Tree Time points: Baseline, Days 19-21 (midpoint of EBRT) and Days 38-42 (completion of EBRT).	N=50 men with prostate cancer (25 = scheduled for EBRT and 25 = active surveillance as matched controls, age-, gender-, and race-matched).	Patient-Reported Outcomes Measurement Information System-Fatigue (PROMIS-F)	168 genes involved in mitochondrial function.	Blood	Four genes (BCL2L1, BCS1L, SLC25A37, and FIS1) showed changes in expression that were significantly associated with changes in fatigue during EBRT.
Vermeulen et al. (2010), Netherlands.	To investigate the possibility that a decreased mitochondrial ATP synthesis causes muscle and mental fatigue and plays a role in the pathophysiology of chronic fatigue syndrome (CFS).	Longitudinal; Time points: Baseline and 24 hours later.	N=15 female patients with CFS. Controls: 15 healthy sedentary women.	Fukuda Diagnostic criteria for CFS	Peripheral blood mononuclear cells (PBMC).	Blood	No deficiencies in mitochondrial ATP productions. Hypothesized two explanations for insufficient energy production in CFS: lower transport capacity of oxygen or mitochondrial insufficiency.
Tomas et al. (2017), U.S.	To specifically examine the major parameters of mitochondrial function, including the two main energy-producing pathways in the cell.	Cross-sectional.	N=52 patients with Chronic Fatigue Syndrome. Controls: 35 healthy individuals.	Fukuda Diagnostic criteria for CFS	Peripheral blood mononuclear cells (PBMC).	Blood	Statistically significant differences in the bioenergetic profile of PBMC from CFS patients compared to the control group.
Pierce et al. (2018), U.S.	To understand, through symptoms sciences, using ubiquinol and D-ribose (therapeutic interventions) to achieve mitochondrial bioenergetics to reduce the complex symptoms of patients with cardiac problems.	Longitudinal; Time points: Baseline and at 12 weeks.	N=276 patients with heart failure with preserved left ventricular ejection fraction (HFpEF).	Did not use a fatigue scale. Used the Vigor subscale from PROMS.	Not informed.	Blood	The data of a pilot study with patients with HFpEF showed that two supplements (ubiquinol and D-ribose) added to their usual HF care enhanced their myocardial energetics and diastolic function, resulting in decreased symptom burden. Both ubiquinol (the active form of coenzyme Q10) and D-ribose play a vital role in mitochondrial ATP production.

Nguyen et al. (2018), Australia	To determine the metabolic function of resting Natural Killer cells in patients with chronic fatigue syndrome (CFS).	Cross-sectional.	N=6 patients with CFS. Controls: 6 healthy, non-fatigued controls, age- and sex-matched.	Fatigue Severity Scale (FSS).	Natural Killer Cells.	Blood	NK cells showed difficulties in regulating glycolysis for the effective function of immunological responses. This happens because their glycolytic reserve significantly decreases, which reduces their energy due to hypomethylation in specific genes of CFS patients.
Wawrzyniak et al. (2016), U.S.	To determine the metabolic function of resting NK cells in patients with ME/CFS.	Cross-sectional.	N=20 patients with idiopathic chronic fatigue. Controls: 28 non-fatigued age-matched individuals.	FACIT-F.	Vastus lateralis muscle biopsies.	Vastus lateralis muscle biopsies	Mitochondrial dysfunction in NK cells represents a plausible mechanism underlying CFS because the energy production (ATP) in cells is compromised, which is evident in muscle tissue, especially in older people.
Lau et al. (2015), China.	To examine the association between fatigue and migraine using a national population-based database in Taiwan.	Retrospective case-control.	N=6902 patients newly diagnosed with migraine. Controls: 27,608 migraine-free individuals randomly selected as the comparison cohort.	Not informed.	Not informed.	Medical records	Impaired oxidative phosphorylation results in mitochondrial dysfunction, so it may be included in the pathophysiology of disorders such as CFS in the elderly.
Booth et al. (2012), U.K.	To test the hypothesis that fatigue and accompanying CFS symptoms are due to defects in the cellular energy supply, and to understand the pathophysiology of the defects so that effective medical intervention can be implemented.	Cross-sectional.	N=138 adults aged 18 to 65 years diagnosed with CFS. Controls: 53 healthy+A2:O12y individuals.	Bell CFS Ability scale.	Neutrophils.	Blood	Free DNA measurements in patients with CFS have abnormally high levels in damaged and necrotic cells and a strong correlation to mitochondrial dysfunction, which directly affects their bioenergetics.
Reuter and Evans (2011), Australia.	To comparatively examine complete profiles of endogenous carnitine in patients with chronic fatigue syndrome and healthy controls.	Cross-sectional.	N=44 CFS patients. Controls: 49 healthy age- and gender-matched healthy controls.	Fatigue severity scale.	L-carnitine and acylcarnitine.	Blood	CFS patients exhibited significantly changed concentrations of C8:1, C12DC, C14, C16:1, C18, C18:1, C18:2, and C18:1- OH acylcarnitines. Particularly, oleyl-L-carnitine (C18:1) and linoleyl-L-carnitine (C18:2) were, on average, 30–40% lower in patients than in controls (P < 00001). There were also significant correlations

							between acylcarnitine concentrations and clinical symptomology.
Myhill et al. (2009), U.K.	To perform interventions based on disease biochemistry (fatigue), specifically on mitochondrial function of ATP production, energy currency for all body functions, and ADP recycling to replenish ATP supply as needed, aiming at a better quality of life.	Cross-sectional.	N=71 patients with CFS. Controls: 53 healthy volunteers.	Bell CFS ability Scale.	Neutrophils.	Blood	The immediate cause of CFS/ME symptoms is mitochondrial dysfunction. The ATP profile results indicate mitochondrial dysfunction of the neutrophils in the patients, and the degree of dysfunction is strongly correlated to the severity of their illness.
Hsiao et al. (2019), U.S.	To examine the relationship between the expression of mitochondrial gene BCS1L, integrated mitochondrial OXPHOS, ETC enzymatic activity, and CRF in prostate cancer patients receiving XRT, and to compare them to patients undergoing active surveillance (AS).	Longitudinal; Three times during radiotherapy.	N=52 patients with prostate cancer.	Piper Fatigue Scale.	Peripheral blood mononuclear cells (PBMC), Gene BCS1L.	Blood	Differences in fatigue scores at the midpoint and endpoint between XRT and AS groups. Significant differences in changes of OXPHOS, ETC activity, and BCS1L gene expression over time in patients receiving XRT; Lower OXPHOS decreased the activity of complex III and downregulated BCS1L gene expression in peripheral mononuclear cells at midpoint and endpoint in patients receiving XRT. Also, worsened fatigue symptoms are associated with decreased complex III oxidation and BCS1L downregulation.
Missailidis et al. (2020), Australia.	To measure mitochondrial function and cellular stress sensing in actively metabolizing patient blood cells.	Longitudinal and case-control.	N=73 Women of European descent. 51 = CFS, 22 = Healthy women.	Canadian Consensus Criteria.	Peripheral blood mononuclear cells (PBMC).	Blood	ME/CFS lymphoblasts show an isolated inefficiency of complex V in ATP synthesis in the final stage of mitochondrial oxidative phosphorylation. Another possibility is the already high TORC1 activity, considering that TORC1 is an inhibitor of the upstream pathways that activate AMPK. In any case, if this "chronic cell fatigue" is present in other cell types, it may contribute to the unexplained fatigue experienced by patients with ME/CFS, as suggested by the fact that all mitochondrial

							abnormalities observed were correlated to the severity of patient symptoms measured by Weighted Waiting Time.
Feng et al. (2020), U.S.	To explore the influence of androgen deprivation therapy (ADT) and chemical castration on fatigue progression and mitochondrial function during RT for localized prostate cancer.	Case-control.	N=64 Men over 18, diagnosed with non-metastatic prostate cancer.	FACIT-F.	Peripheral blood mononuclear cells (PBMC).	Blood	The combination of ADT and RT caused aggravated fatigue associated with anemia and mitochondrial dysfunction. This combined effect of ADT and radiation therapy appeared to be specific for fatigue, as depressive symptoms were not affected. The study also suggests that self-reported subjective fatigue appeared to be associated with decreased ATP coupling efficiency, indicative of less efficient mitochondrial ATP production.
Herpich et al. (2021), German	To evaluate mitochondrial respiration of peripheral blood mononuclear cells (PBMCs) in older patients with and without fatigue.	Case-control.	N= 23 geriatric patients with fatigue and 22 without fatigue.	Brief Fatigue Inventory (BFI).	Peripheral blood mononuclear cells (PBMC).	Blood	The study shows, for the first time, that fatigue in older patients is associated with functional impairments and reduced mitochondrial bioenergetics in PBMCs. Whether this is the result of lower mitochondrial mass per cell and the reduced PBMC mitochondrial respiration in older patients with fatigue is also accompanied by a reduced mitochondrial activity in other organs and tissues remains to be elucidated.
Hsiao et al. (2021), U.S.	To discover potential biomarkers associated with chronic CRF in men with prostate cancer receiving radiation therapy.	Longitudinal repeated-measures.	N = 20 men with prostate cancer undergoing radiation therapy.	Patient-Reported Outcomes Measurement Information System for Fatigue (PROMIS-F) Short Form.	Peripheral blood mononuclear cells (PBMC).	Blood	Peripheral blood mononuclear cell messenger RNA for BCS1L may be a potential biomarker and therapeutic target for radiation therapy-induced chronic CRF in this clinical population.
Fernandez-Guerra et al.	To perform deep profiling of the mitochondrial function and evaluate its	Case-control.	N = Six female patients between 30 and 50 years old.	Fukuda Criteria, Revised Canadian	Peripheral blood mononuclear cells (PBMC).	Blood	PBMCs from ME/CFS patients showed significantly lower mitochondrial coupling efficiency. They exhibited

(2021), Denmark	association with symptom burden in ME/CFS.			Criteria, International Consensus Criteria (ICC), and American Institute of Medicine (IOM) criteria.			proteome changes, including altered mitochondrial metabolism, centered on pyruvate dehydrogenase and coenzyme A metabolism, decreasing the capacity to provide adequate intracellular ATP levels. These results indicate that PBMCs from ME/CFS patients have a decreased ability to fulfill their cellular energy demands.
Hamilton & Jensen (2021), US	To evaluate the effects of ATP 360, a nutraceutical energy formula, in people experiencing long-term fatigue affecting daily living. To explore the use of ex vivo mitochondrial stress testing to evaluate cellular energy improvements with nutraceutical support.	Open-label.	N = 11 consumed the nutraceutical energy formula for 8 weeks.	Piper Fatigue Scale .	Peripheral blood mononuclear cells (PBMC).	Blood	Drug consumption was associated with reduced symptoms of moderate to severe fatigue, with alleviation of some of the symptoms within 1 week. Inflammation and mitochondrial dysfunction contribute to the development and persistence of symptoms in chronic illness. NEF offers an option to improve the health of chronically ill patients by alleviating some of the most debilitating symptoms of fatigue.

which can directly affect energy production if modified, is partially blocking the translocator (TL) that converts ADP (adenosine triphosphate) into ATP. TL plays an essential physiological role, causing ADP recycling and potentially being used as an energy source in the cytosol, making mitochondrial metabolism work correctly. The functional changes of this mechanism compromise the other processes that depend on energy, such as oxidative phosphorylation and the Krebs cycle (Booth et al. 2012). The reviewed articles highlight the need for investigating their findings better, and it is worth noting that, so far, there is still no further study on TL functioning in fatigued individuals.

Technologies were used to assess mitochondrial metabolism functioning, such as the Mito Stress technique (Feng et al. 2018). This test is based on mitochondrial cell stress and allows visualizing oxygen consumption levels during drug application (Feng et al. 2018), comparing healthy and fatigued individuals. However, this research did not focus on studying the complexes separately, so it does not indicate the specific processes that would be compromised.

However, a more recent study by the same researchers, with glucose metabolism, specifically the GLUT4 gene (glucose transporter), identified decreased levels of expression of this gene in mitochondrial dysfunction, which may contribute to lower ATP production and, consequently, fatigue. Furthermore, this finding represented a potential marker of mitochondrial health (Feng et al. 2020).

Other techniques, such as the ATP Profile, are used to diagnose CFS patients more precisely. This technique consists of measuring ATP supply in neutrophils extracted from venous blood where ATP is measured with luciferin-luciferase and luminescence is provided by a magnesium (mg) tag on energy molecules (ATP) (Myhill et al. 2009; Booth et al. 2012). This study showed a marked correlation between the Mitochondria Energy Score (MES) and the degree of CFS (Booth et al. 2012).

The information in the literature shows that besides changes in ATP production, there possible problems in the transport of electrons used by mitochondrial metabolism for energy production. For studying such transport pathways, electron transport measurement techniques were used with spectrophotometry (Hsiao et al. 2019; Fernandez-Guerra et al. 2021).

Mitochondrial dysfunctions could also be related to differentiations in the expression of some genes and their proteins. A study on BCS1L gene expression in complex III could re-establish a relationship between the impaired activity of these mitochondrial membrane components, production, and lack of energy (ATP) with fatigue (Hsiao et al. 2019; Hsiao et al. 2021). Techniques such as real-time polymerase chain reaction (PCR) were performed to analyze this gene, using TaqMan Chemistry from Applied Biosystems.

Under normal conditions, the BCS1L gene is responsible for expressing a specific protein (Rieske protein) that works on complex III. It has a crucial role in oxidative phosphorylation, in which oxygen and simple sugars are used to produce ATP (Hsiao et al. 2019).

Differential gene expressions such as BCL2L1, BCS1L, SLC25A37, and FIS1 found in current studies, assume that these mitochondrial genes can be vital biomarkers for understanding the etiology of fatigue (Tomas et al. 2017; Hsiao et al. 2019; Hsiao et al. 2021).

There is also a current study by Missailidis (2020) with a key regulator of one of the central mitochondrial functions, which regulates cell growth and activates ATP production pathways. This gene, called TORC1 (complex I), positively regulates the expression of nuclear-encoded mitochondrial proteins, among which are the subunits of the OXPHOS complexes (a potential response to low pH and lactate clearance). Parameters of mitochondrial function in immortalized lymphocytes were compared to explain the roles of aberrant mitochondrial function and TORC1 signaling in ME/CFS.

The current findings correlating mitochondrial dysfunctions and fatigue, based on different molecular and genetic techniques, show a possible change in MES by production or transport, which is directly involved with the origin and permanence of the fatigue symptom (Myhill et al. 2009; Booth et al. 2012; Tomas et al. 2017; Hsiao et al. 2019; Feng et al. 2020; Missailidis et al. 2020; Herpich et al. 2021).

The symptom of fatigue was evaluated in several clinical conditions, such as CFS, heart failure, muscle failure, and cancer, using validated scales, most of them considered the gold standard in the study of this symptom. Despite these dissimilar clinical conditions, these studies shared common findings, such as changes in mitochondrial complex activities (mainly in complexes III and V) and problems in ATP production and metabolic pathways, hypothesized in different ways. The instruments used were the Bell CFS Ability scale (Bell 1994), Piper Fatigue Scale (rPFS) (Piper and Cella 2010), Fatigue Severity Scale (FSS) (Krupp et al. 1989), FACIT-F (Yellen et al. 1997), and Patient-Reported Outcomes Measurement Information System-Fatigue (PROMIS-F) (Hsiao et al. 2014). These instruments used in symptom science research can assist in assessing possible interventions and monitoring patient evolution, for example, in longitudinal studies (Pierce et al. 2018; Hsiao et al. 2019; Missailidis et al. 2020; Hsiao et al. 2021).

Besides using scales to identify the symptom, genetic and molecular techniques based on mitochondrial markers have been used to measure and evaluate fatigue (Hsiao et al. 2019; Feng et al. 2020; Missailidis et al. 2020). There has been a recent emergence of new laboratory technologies, which allow further symptom science research and favor understanding previously unknown information about the possible etiology of fatigue (Hsiao et al. 2019; Feng et al. 2020). Thus, combining validated scales for symptom identification and laboratory techniques with specific markers reduces the risk of bias and makes results more reliable (Feng et al. 2018).

Most recent studies published in the last five years come from the United States, and North-American researchers are mainly responsible for publishing laboratory protocols used in most studies included in this review (Feng et al. 2018; Hsiao et al. 2019; Feng et al. 2020). Nonetheless, it is worth noting that symptom sciences are in full development, showing a promising scenario for new research worldwide (Dorsey et al. 2019).

Limitations of this study

The present scoping review included the current main databases for searching for scientific evidence. Therefore, one of the limitations of this study is the non-inclusion of other, less-known databases in the

health field. It is also worth noting that the authors of published articles may not have used the best descriptors for the field of study, making it impossible to find such articles through search strategies.

5. Conclusions

The findings of this scoping review identified mitochondrial markers associated with fatigue and particularly related to mitochondrial transport pathways, the mitochondrial complex (III and V), the respiratory chain, mitochondrial DNA changes, and energy disorders in immune cells. Mitochondrial metabolism and its main activities, such as ATP production and transport and functions of complexes III and V are essential pathways to understanding the etiology of fatigue.

Mitochondrial energy metabolism abnormalities establish a consistent link with fatigue. However, whether abnormalities occur because of fatigue or cause the disease remains unknown. Therefore, fundamental molecular explanations for the underlying pathophysiology of fatigue and reliable biomarkers must remain potential targets for further investigation. This could lead to a faster and more accurate diagnosis and rational and effective treatments in the long term.

Further research should include longitudinal study designs, characterize fatigue phenotypes, and identify markers involved in the production and transport of mitochondrial pathways. The clinical relevance in this field can lead to interventions targeting mitochondrial markers to reduce or prevent fatigue.

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