Altered immune response to exercise in patients with chronic fatigue syndrome/myalgic encephalomyelitis: A systematic literature review

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ABSTRACT

An increasing number of studies have examined how the immune system of patients with Chronic Fatigue Syndrome (CFS), or myalgic encephalomyelitis, responds to exercise. The objective of the present study was to systematically review the scientific literature addressing exercise-induced immunological changes in CFS patients compared to healthy control subjects. A systematic literature search was conducted in the PubMed and Web of science databases using different keyword combinations. We included 23 case control studies that examined whether CFS patients, compared to healthy sedentary controls, have a different immune response to exercise. The included articles were evaluated on their methodological quality. Compared to the normal response of the immune system to exercise as seen in healthy subjects, patients with CFS have a more pronounced response in the complement system (i.e. C4a split product levels), oxidative stress system (i.e. enhanced oxidative stress combined with a delayed and reduced anti-oxidant response), and an alteration in the immune cells' gene expression profile (increases in post-exercise interleukin-10 and toll-like receptor 4 gene expression), but not in circulating pro- or anti-inflammatory cytokines. Many of

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these immune changes relate to post-exertional malaise in CFS, a major characteristic of the illness. The literature review provides level B evidence for an altered immune response to exercise in patients with CFS.

Keywords: fatigue, pain, genetics, oxidative stress, complement system, cytokine, inflammation, exercise, physical activity

INTRODUCTION

Chronic fatigue syndrome (CFS), or myalgic encephalomyeltitis, is a condition defined by the 1994 Center for Disease Control and Prevention definition (11, 15). The major symptom is fatigue, of new or definite onset (not been lifelong), lasting for 6 months or longer (11, 15). CFS is diagnosed by exclusion of any other medical condition which might explain the symptoms (e.g. untreated hypothyroidism, sleep apnea, narcolepsy, major depressive disorder, bipolar affective disorders, schizophrenia, anorexia nervosa, bulimia nervosa, severe obesity, etc.) (11, 15). In addition, four or more of the following symptoms must be present for 6 months or longer: impaired memory or concentration; sore throat; tender lymph nodes (cervical or axillary); muscle pain; pain in multiple joints without joint swelling or redness; headaches of a new kind or greater severity; unrefreshing sleep and post-exertional malaise lasting more than 24 hours (11). Symptoms are often exacerbated during and after physical activities (54).

The presence of symptoms like a sore throat, tender lymph nodes, and low-grade fever, as well as flu-like symptoms including widespread muscle pain and severe fatigue, has inspired researchers to search for immune abnormalities in patients with CFS. Several immune abnormalities have been reported in CFS patients: decreased natural killer cell activity (44), altered functional B cell subset populations (3), alterations in cytokine production (52), alterations in inflammatory markers (25), increased nitrosative and oxidative stress pathways (25-27), upregulation of various aspects of the 2'-5' oligoadenylate synthetase/RNase L pathway (28, 32, 43, 50, 51), among others. However, many of the observed abnormalities were not confirmed by others, resulting in inconsistent findings across studies (12, 31). This probably relates to the heterogeneous nature of the illness, the use of different diagnostic criteria for diagnosing CFS, the different laboratory methods used for measuring immune function (i.e. different assays, specimens or stimuli), and the fluctuating nature of CFS.

Besides resting immune function, the immune system of patients with CFS might respond differently to exercise as compared to what we see in healthy, sedentary controls. The 2011 International Consensus Document regarding Myalgic Encephalomyelitis acknowledges the importance of malaise following exercise for the diagnosis of CFS (8). Indeed, in CFS patients too vigorous exercise (1, 18, 20) or a sudden increase in activity (2) frequently triggers a severe increase in symptoms. The severe exacerbation of symptoms following exercise, as seen in CFS patients, is one of the core features of the illness (38, 47).

In addition, the (normal) effects of exercise on the body's immune system are well established, and an ever-growing volume of scientific publications speaks to the rapid growth in understanding of exercise immunology (56). An increasing number of studies have examined the response of the immune system to exercise in CFS patients (e.g. (18, 19, 22, 23, 35, 37, 47)), yet the abnormal immune responses to exercise in CFS patients has not been conclusively defined and it remains unclear whether exercise-induced immune abnormalities can be regarded as biomarker for CFS.

Hence, the objective of the present study was to systematically review the scientific literature addressing exercise-induced immunological changes in CFS patients compared to healthy control subjects. Specifically the review examined whether CFS patients, compared to healthy sedentary controls, have a different immune response to exercise. Thus, the review intends to answer the following question: does the immune system of patients with CFS respond differently to exercise as compared to healthy sedentary controls? It is hypothesized that CFS patients show a more pronounced immune response to exercise as compared to healthy sedentary controls.

METHODS

This systematic review is reported following the PRISMA-guidelines (Preferred Reporting Items for Systematic reviews and Meta-Analyses), which is an updated statement addressing the conceptual and methodological issues of the original QUOROM Statement (29).

Search strategy

The aim of this systematic review was to answer the research question that was formulated using the PICO model; "Do CFS patients (P) have a different immune response (O) to exercise (I) compared to healthy people (C)?". To answer the research question a systematic search of the existing literature up to August 2013 was conducted on the electronic databases PubMed and Web of Science, and is reported following the PRISMA guidelines (21). The search strategy was based on a combination of search terms and Mesh terms, which were derived from the "PICO" research question. Therefore all search terms from "P" (combined with OR) were combined with the possible search terms from "I" (combined with OR) and "O", using the boolean term 'AND'. The used search and Mesh terms, and the construct of the search strategy are presented in Table 1. No filters were used during the search strategy.

Study selection

To be included in this systematic review, papers had to fulfill predefined inclusion criteria regarding type of report, topic and population studied. Only full text clinical reports / (original) research reports (= *type of repor*t) which examined exercise induced immunological changes (= *topic*) in adult (≥18 years) CFS patients and compared these with the results of healthy controls (= *population studied*) were eligible. No language, publication date, or publication status restrictions were imposed, and all clinical study designs were eligible. Non-clini-

Table 1. The search terms used for the literature review.

Patient		AND	Intervention		AND	Outcome	
Keywords Pubmed	**Keywords Web of Science**		**Keywords Pubmed**	**Keywords Web of Science**		**Keywords Pubmed**	**Keywords Web of Science**
Chronic fatigue syndrome [Mesh]	Chronic fatigue syndrome		Exercise [Mesh]	Exercise		Immune system phenomena [Mesh]	Immune system phenomena
	Myalgic encephalomyelitis			Physical exercise		Immune system	Immune system
	Postviral fatigue syndrome			Aerobic exercise		Immune function	Immune function
	Chronic fatigue disorder		Motor activity [Mesh]	Motor activities		Immunology	Immunology
				Physical activity		Immunity	Immunity
				Locomotor activity		Cytokines [Mesh]	Cytokines
			Exercise therapy [Mesh]	Exercise therapy		Lymphokines [Mesh]	Lymphokines
						Lymphocytes [Mesh]	Lymphocyte
							Lymphoid cells
						Leukocytes [Mesh]	Leukocyte
							White blood cell
						Complement System Proteins [Mesh]	Complement system proteins
							Complement protein
							Complement
						Natural Killer T-cells [Mesh]	Natural killer t-cell
							NKT Cells
						T-Lymphocytes, Cytotoxic [Mesh]	Cytotoxic t-lymphocyte
							Cell-mediated lympholytic cells
						Cytotoxicity, Immunologic [Mesh]	Immunologic cytotoxicity
						Gene expression [Mesh]	Gene expression
						Gene expression profiling [Mesh]	Gene expression profiling
						Apoptosis [Mesh]	Apoptosis
							Programmed cell death, type I
							Intrinsic pathway apoptosis
							Extrinsic pathway apoptosis
						Necrosis [Mesh]	Necrosis
						Autophagy [Mesh]	Autophagy
						Biological Markers [Mesh]	Biological marker
							Biomarkers
							Clinical marker
							Immunologic marker
							Viral marker
							Serum marker
							Biochemical marker
							Laboratory marker
						Laboratory tests	Laboratory tests

cal reports such as reviews, abstracts, posters, letters to the editor, and editorials were excluded. First, the title and abstract of all citations retrieved using the search strategy were screened using the inclusion criteria. If it was not clear from the abstract whether the study was eligible for study inclusion, the full-text was consulted. The full text versions of all papers that met the inclusion criteria were retrieved for quality assessment and data extraction.

Qualification of searchers

The literature was searched and screened independently by AN and JVO. The risk of bias was assessed by 3 researchers (AN, JVO and JN), who were blinded from each other's assessment. AN holds a bachelor degree in rehabilitation sciences, and was trained by the final author. JVO and JN hold a PhD degree and have published several systematic literature reviews.

Risk of bias assessment

Each study that fulfilled the inclusion criteria was assessed for methodological quality using the evaluation 'Evidence Base Richtlijn Ontwikkeling' (EBRO) criteria for case-control studies as recommended by The Dutch Cochrane Centre (http://dcc.cochrane.org/sites/dcc.cochrane.org/files/uploads/patient-controleonderzoek.pdf). The evaluation criteria for case-control studies assess 6 items. First it is assessed whether an adequate definition of the case group is given (C1), and secondly if this is also the case for the control group (C2). Therefore we assessed whether the total sample size and demographical characteristics were described for each group. For the case group of CFS patients the fulfilled CFS diagnostic criteria had to be mentioned. In case only sedentary or moderately active subjects were included in the control group, a description of the authors' understanding of sedentary or moderately active was expected. The 3rd item controls for exclusion of selection bias (*C3*), which implied that studied groups needed to be representative of the general population. This item was scored negative when the included age range was not representative for the general adult population or when only male CFS patients were recruited. A negative assessment was given in case the patient sample was recruited solely from one sort of setting such as a specific hospital department or only through a patient support group, as combining a variety of recruitment procedures is recommended to prevent recruitment bias (33). The 4th item entails a clear description of the exposure and an adequate method for assessment (*C4*), The 5th item was related to blinding of the involved assessors (*C5*). In case an assessor could influence the results, non-blinding or failure to mention blinding was penalized. This included that saliva and blood processing / analyses was performed by personnel blinded to the subject's health status. As a final point identification or accounting for confounders was assessed (*C6*). Possible confounders related to exercise performance and immunology are body mass index (BMI), physical activity levels, and menstrual phase. Furthermore, pooling of gender data has been identified as an important source of bias in studies of exercise physiology in CFS patients (42).

When a study fulfilled a criterion a positive score was given, when a criterion was applicable but the study did not comply with the criterion a negative was given. When no adequate information was present regarding an applicable criterion, that

item was scored as negative. If a criterion was not applicable to the design and purpose of the study it was not scored. Fulfillment of the criteria was assessed by 3 independent, blinded researchers (JVO, JN and AN) using a score sheet. After rating the selected articles, the results of all three researchers were compared, and the amount of agreement (in percentages) between the 3 researchers was calculated. In case of disagreement, the final risk of bias score was the score which was given by 2 out of the 3 researchers. Finally, a total score for methodological quality was computed by adding up the scores for each of the related criteria which were applicable. The maximum total score that could be achieved was 6, and was also presented as a percentage to facilitate comparison between studies. In table 2 the risk of bias scores of the studies are presented. Levels of evidence were defined based on study design and quality, according to the EBRO-guidelines.

Table 2. Risk of bias and level of evidence of the exercise immunology studies in CFS patients.

References	Criteria						Total quality score	Total quality %	Level of evidence
	1	2	3	4	5	6			
Cannon et al. 1999 (7)	+	+	+	+	-	+	5	83,3	B
Light et al. 2012 (22)	+	-	+	+	+	+	5	83,3	B
Jammes et al. 2009 (17)	+	+	+	+	-	-	4	66,7	B
Jammes et al. 2012 (16)	+	+	+	+	-	-	4	66,7	B
LaManca et al. 1999 (19)	+	+	-	+	-	+	4	66,7	B
Light et al. 2009 (23)	+	-	-	+	+	+	4	66,7	B
White et al. 2012 (59)	+	-	+	+	-	+	4	66,7	B
Nijs et al. 2010 (37)	+	+	+	+	-	-	4	66,7	B
Robinson et al. 2010 (41)	+	+	-	+	-	+	4	66,7	B
Jammes et al. 2005 (18)	+	+	-	+	-	+	4	66,7	B
Smylie et al. 2013 (45)	+	+	-	+	-	+	4	66,7	B
Suarez et al. 2010 (49)	+	+	-	+	-	+	4	66,7	B
Thambirajah et al. 2008 (53)	+	+	-	+	-	-	3	50,0	B
Peterson et al. 1994 (39)	+	+	-	+	-	-	3	50,0	B
White et al. 2010 (58)	+	+	-	+	-	-	3	50,0	B
Broderick et al. 2013 (4)	+	-	-	+	-	+	3	50,0	B
Cannon et al. 1997 (6)	+	+	-	-	-	+	3	50,0	B
Whistler et al. 2005 (57)	+	+	-	-	-	-	2	33,3	B
Sorensen et al. 2003 (47)	+	+	-	-	-	-	2	33,3	B
Lloyd et al. 1994 (24)	-	-	-	+	-	+	2	33,3	B
Sorensen et al. 2009 (46)	-	+	-	-	-	-	1	16,7	B
Gupta et al. 1999 (13)	-	-	-	-	+	-	1	16,7	B
Steineau et al. 2004 (48)	-	-	-	-	-	-	0	0,0	B

Criterion 1: adequate definition of the case group
Criterion 2: adequate definition of the control group
Criterion 3: exclusion of selection bias
Criterion 4: exposure is clearly defined and method for assessment of exposure is adequate
Criterion 5: blinded assessor
Criterion 6: identification or accounting for confounders
0: criterion not fulfilled; 1: criterion fulfilled; NA: criterion not applicable

RESULTS

Study selection

The search of the databases provided a total of 584 citations. After removing duplicates 254 studies remained. Of these, 231 studies were discarded after reviewing the title and abstract or when necessary the full text paper. More specifically, studies were excluded because they had a study type (n=80), population (n=139), or topic (n=217) offline with the predefined selection criteria (figure 1). Twenty-three studies met all inclusion criteria.

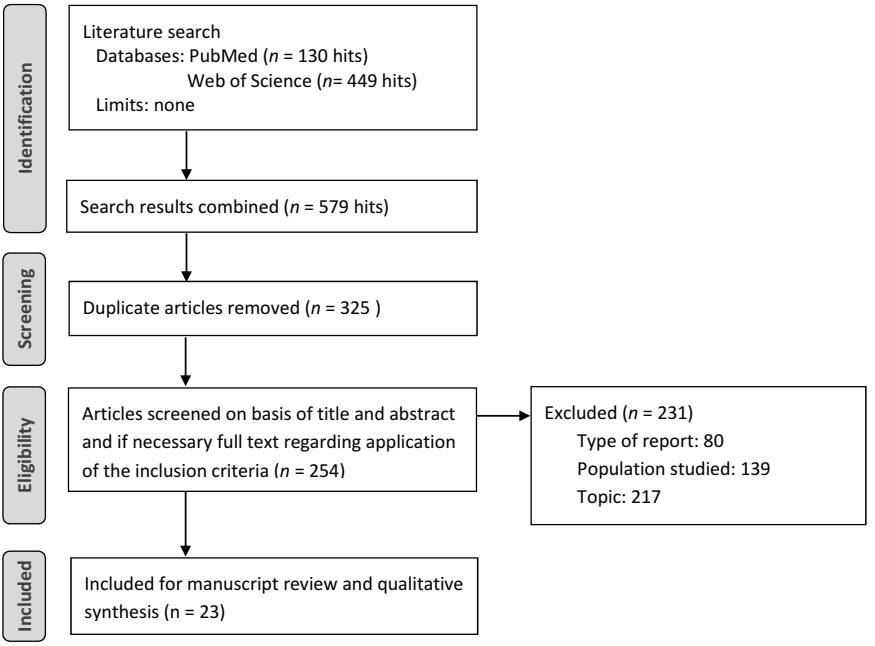

Figure 1. PRISMA flow diagram of the literature search and study selection.

Risk of bias and level of evidence

The agreement between the three raters was 70.3 % (97/138), 73.2 % (101/138) and 75.4 % (104/138). All three raters agreed on the scores of 83 out of 138 items. An adequate definition of the case group was given in 19 out of 23 studies, and for the control group this was the case in 16 studies (table 2). As different criteria exist for the diagnosis of CFS, and for the definition of sedentary or moderate activity levels, authors should at least refer to the criteria which were used. There was a risk of selection bias in 17 studies because the included subjects were not representative for the population which was studied. The authors failed to mention from where the subjects were recruited or they recruited solely from one sort of setting. The studies by Gupta et al. 1999 (13), Light et al. 2009 (23), Sorensen

et al. 2009 (46), and Steinau et al. 2004 (48) describe that (a part of) the included subjects were selected from a previous study, but fail to mention why exactly these subjects were selected. Most studies (17 out of 23) provided a clear description of the physical exercise which was performed by the subjects and the methods for assessing outcome measures. Failure of blinding formed an important source of bias in 20 studies. In most cases, it was not mentioned whether blood samples were coded and stored anonymously, and whether blood processing / analyses were performed by personnel blinded to the subject's health status. We assume that this was the case in many studies, but that authors simply failed to mention whether they prevented this form of assessment bias. Eleven studies did not prevent or account for important confounders related to exercise immunology. The most frequent cause of penalization was pooling of gender data. The EBRO-classification assigns a B score for individual studies which use a case-control study design. Because all the included studies were comparative studies without randomization of the study subjects, an evidence level B was applicable for all studies.

Study characteristics

All included studies applied a case-control design, comparing CFS patients with healthy controls. In each of the included studies, immune variables were measured at rest (pre-exercise) and following one bout of exercise, with post-exercise measurements performed immediately and/or up to days following exercise. All studies examined the acute effects of exercise on the immune system (i.e. the effects of one exercise bout on the immune system). None of the studied applied a true experimental design, or studied the effects of exercise *therapy* on the immune system in CFS patients.

ARTICLE RESULTS and DISCUSSION

The main study findings are summarized below and discussed together in view of the risk of bias scores. Given the focus of the review, only the study findings addressing exercise immunology in CFS patients versus controls are presented. Other findings, like baseline (resting) differences in immune function or differences in exercise physiology unrelated to the body's immune system, are not presented. Study findings are organized in 4 major categories, namely cytokines, complement system, oxidative stress and leukocyte gene expression.

Cytokines

The cytokine response to exercise of CFS patients in comparison with healthy controls has been examined thoroughly (6, 7, 13, 17, 19, 24, 37, 39, 41, 45, 47, 58). Lloyd et al. did not find alterations in the blood level of interferon-γ, interferon-α, interleukin-1β, or tumour necrosis factor-α at baseline, during, and up to 24 hours following 30 minutes of submaximal isometric (hand-grip) exercise (24). They compared twelve male CFS patients with 13 male matched healthy controls (24). Similar findings were reported in the Peterson et al. study, who failed to detect alterations in serum cytokine levels of interleukin-1β, interleukin-6, or tumour necrosis factor-α in any of the participants (CFS-patients or healthy) com-

paring levels at rest with values immediately and 40 minutes following walking on a horizontal treadmill at a speed of 1 mph for a maximum of 30 minutes or until exhaustion (39). This is surprising given the well-established cytokine response to strenuous exercise in healthy people (56, 60), questioning the validity of the study. The risk of bias assessment of these studies revealed rather low scores of 33% and 50% for the Lloyd et al. (24) and Peterson et al. (39) studies respectively. Still, the lack of alterations in blood interleukin-1β in response to exercise in CFS patients was later confirmed in a larger study (n=22 per group) performing quantitative *in vitro* detection of human interleukin-1β using two different assays (37). Others were similarly unable to find alterations of interleukin-1β in response to exercise in CFS patients (7, 39, 47). The Peterson et al. study, however, did detect serum transforming growth factor-β differences at rest between CFS patients and healthy controls, with higher values in the CFS group, but this cytokine did not respond to exercise either (39).

Others used a moderate whole-body exercise task (working both arms and legs) for 25 minutes to examine whether the flare in symptoms, up to 48 hours following exercise, was related to changes in peripheral blood cytokines and CD40 ligand (cluster of determination 40 ligand) of CFS patients (58). CD40 ligand is a pro-inflammatory marker, linked to platelet activation. Increased levels have been linked to cardiovascular diseases, while low levels may be related to increased risk for opportunistic infections (which are often seen in CFS (36)). CD40 ligand was lower in the CFS patients versus healthy controls, a difference that remained following exercise, and the level of CD40 ligand decreased similarly in both groups following exercise (58). Besides from changes in red blood cell count, which are of less relevance to this literature review, no major changes in cytokine response to exercise were observed between CFS and healthy controls (58). However, when dividing the CFS group into high and low symptom flares following exercise, it was found that CFS patients with high symptom flares had a pattern of pro- (interleukin-6, interleukin-1β, interleukin-12) as well as anti-inflammatory cytokines (interleukin-10, interleukin-13) at 8 hours post-exercise compared to the low symptom flare group. Still, the findings from this study should be interpret with caution, as not all participants in the control group were sedentary, the control group differed in gender distribution and body mass index from the CFS group, and the cytokine changes were not controlled for these possible confounders. Pooling of gender data has been identified as an important source of bias in studies addressing exercise physiology in CFS patients (42).

In a small (n=6 male subjects per group), but otherwise methodologically sound study, the response of interleukin-6, its soluble receptors (sIL-6R and sgp130) and F2-isoprostanes to submaximal exercise were studied (41). Interleukin-6 increased from rest to the end of the exercise and returned to resting values 24 hours post-exercise, but there were no group X time interactions, indicating that the increase in interleukin-6 in response to exercise was similar in both groups (41). Likewise, there were no group X time interactions for sIL-6R, sgp130 and F2-isoprostanes. Another study examined the interleukin-6 response to exercise in CFS, this time using 30 minutes of fatiguing non-dominant limb exercise (13). In the CFS group, and not in the control group, spontaneous interleukin-6 produc-

tion by monocytes increased following exercise. Phytohemagglutinin-induced (for lymphocytes) and lipopolysaccharide-induced (for monocytes) production of interleukin-6 did not differ following exercise across groups. Given the small sample size (5 CFS patients vs. 4 healthy controls) and the low risk of bias score (17 %), the weight of these findings is limited.

Two similar studies by Cannon, et al. (6, 7) used an exercise protocol involving 15 minutes of stepping on and off a platform, and compared peripheral blood mononuclear cells' secretion of interleukin-1β, interleukin-1 receptor antagonist, soluble interleukin-1 receptor type II (6), secretion of interleukin-6 in unstimulated cultures and interleukin-6 secretion in lipopolysaccharide-stimulated cultures (7). However, they were unable to find exercise-induced changes in any of the immune parameters studied, including α2-macroglobulin (6, 7). The exercise challenge might have been too low to enable immune alterations; the lack of exercise-induced symptom-increases in the CFS group (7) supports this notion.

In response to 20 minutes of steady-state stationary cycling at 70% of the subject's predicted maximum workload, patients with CFS (n=32), compared to healthy controls (n=29), did not show statistically significant differences in pro-inflammatory (interleukin-1β, interleukin-6, tumour necrosis factor-α, interferon-α) or anti-inflammatory cytokines (interleukin-10) detected in peripheral blood mononuclear cells (47). However, there was a trend of an increase in pro-inflammatory cytokines in the CFS group at 6 hours post-exercise, whereas at the same time, the values for pro-inflammatory cytokines decreased in the control group (47).

The latter study findings are in line with those reported by La Manca et al. 1999 (19). A rigorous study – the risk of bias assessment yielded a score of 67 % - investigated the differences in cytokines, leukocytes, granulocytes, monocytes and lymphocyte subsets in response to a graded treadmill exercise until exhaustion in 20 CFS patients and 14 sedentary controls (19). Blood samples were collected up to 24 hours post-exercise. Although changes in peripheral lymphocytes' cytokines were found from pre- to post-exercise, there were no time X group interactions for any of the cytokines (interleukin-2, -4, -10, -12, interferon-γ, and tumour necrosis factor-α) examined (19). The same applies to the leukocytes, granulocytes, monocytes and lymphocyte subsets (including T-helper cells, suppressor T, cytotoxic T, and natural killer cells) studied: most of them responded acutely to exercise, returning to baseline values relatively quickly (after 1 to 24 hours), but again no time X group interactions were found (19). Interestingly, in another rigorous but small (n=9 CFS versus 9 controls) study, venous levels of interleukin-6 and tumour necrosis factor-α increased in response to maximal cycling exercise in the healthy controls, but remained unchanged in the CFS group (17), suggesting a depressed cytokine response to exercise in the CFS group. The oxidative stress findings of that study are presented below.

Finally, Smylie et al. studied the cytokine response to a peak graded exercise stress test in patients with CFS (n=22), Gulf War Illness (n=30) and healthy controls (n=30) (45). They correctly separated sexes for running the data analysis, but focused on searching an immune signature / biomarker among the 16 cytokines

examined at baseline, at peak effort and 4 hours post-exercise. Therefore they did not report time X group interactions, making the report less appropriate for the present review.

To summarize the findings addressing the cytokine response of CFS patients to exercise, from the available literature data there is moderate evidence that CFS patients have a normal circulating cytokine (e.g. interleukin-1β, interleukin-6, interleukin-10, tumour necrosis factor-α) response to exercise. Exercise does not result in abnormally higher levels of pro- or anti-inflammatory cytokines in patients with CFS.

Complement system

Nijs et al. (described above) compared the immune response of CFS patients (n=22) and sedentary, healthy controls (n=22) to two types of exercise: a submaximal bicycle exercise and a self-paced, physiologically limited bicycle exercise (37). The study was primarily interested in examining the changes in blood elastase level and complement C4a split product levels in people with CFS versus healthy sedentary control subjects. Elastase is a proteolytic enzyme produced by monocytes and neutrophils during the inflammatory response. In a previous study of people with CFS it was found that baseline elastase level was predictive of the respiratory exchange ratio and the oxygen uptake at the anaerobic threshold (35). In this exercise immunology study, neither type of exercise altered blood levels of elastase activity, interleukin-1, or complement C4a split product levels in people with CFS or healthy sedentary control subjects. However, the change in complement C4a level was strongly related to the increase in pain and fatigue 24 hours following the self-paced, physiologically limited exercise (37). Post-exercise elastase activity level and the change in elastase activity level were inversely related to the fatigue increase one hour following the self-paced, physiologically limited exercise (37). These findings suggest that subtle alterations in blood elastase activity level and complement C4a split product levels account in part of post-exertional malaise in people with CFS.

These findings are in line with those by Sorensen et al. (47), who reported statistically significant correlations between the increase in C4a and total symptom score, as well as with individual symptoms like headache, joint problems and cognitive difficulty in CFS patients. Moreover, they showed that people with CFS respond to an exercise challenge with increased expression of the lectin pathway (C4 and mannan-binding lectin serine protease 2) in peripheral blood mononuclear cells, resulting in significant increase of C4a split product, but not C3a or C5a (46, 47). The fact that Nijs et al. (37) did not find changes in C4a are not in contradiction with the earlier reports. Firstly, in the study by Sorensen et al. the increase in complement C4a split product became apparent only at 6 hours after exercise (47). In the study by Nijs et al. (37) peripheral blood levels of C4a were measured only at 1 hour after exercise, a time point at which Sorensen and colleagues were unable to find changes in circulating C4a levels either (47).

In the Sorensen et al. study, the eosinophilic cationic protein, a protein released during eosinophil degranulation and consequently related to inflammation, responded differently to exercise in CFS patients compared to healthy controls (47). In CFS patients, eosinophilic cationic protein levels decreased from baseline to post-exercise, followed by an increase 6 hours later and that remained at 24 hours post-exercise. Healthy controls had a similar acute decrease of eosinophilic cationic protein levels post-exercise, but the values did not increase to the same extent in the next hours as the CFS group (significant group-by-time interaction) (47).

Taken together, moderate evidence suggests that CFS patients, compared to healthy controls, respond to strenuous exercise with a slow (not earlier than 6 hours post-exercise) but stronger increase in blood complement C4a split product levels. Importantly, these alterations appear of clinical importance as two independent studies have confirmed the relationship between altered complement response and post-exertional malaise in patients with CFS. Findings such as altered eosinophilic cationic protein response to exercise in CFS require replication.

Oxidative stress

The oxidative stress response to exercise in patients with CFS has been the subject of five studies (16-18, 41, 53). As mentioned above, Robinson et al. did not find group X time interactions for F2-isoprostanes (41) (a marker of lipid peroxidation), but they only included 6 patients per group. In 2005, Jammes et al. were the first to report a dysfunctional oxidative stress response in CFS patients (18). They studied the oxidative stress response in venous blood of 15 CFS patients and 11 healthy controls to a maximal graded bicycle stress test, and found an earlier and longer increase in thiobarbituric acid reactive substances, a byproduct of lipid peroxidation and reflecting oxidative stress damage. Also an enhanced post-exercise decrease in the antioxidant ascorbic acid was found in those with CFS, although glutathione levels did not respond differently to exercise compared to the healthy controls (18).

In a later study Jammes et al. again found an early and longer increase in oxidative stress response to strenuous exercise in CFS patients compared to healthy controls (17). Thiobarbituric acid reactive substances were accentuated and increased early following exercise in the CFS group compared to the healthy controls (17). They also reported a delayed and reduced increase of ascorbic acid and heat shock proteins 27 and 70 following exercise (17), which implies a reduced anti-oxidant status post-exercise in the CFS group. This was confirmed more recently in a similar study by the same group, this time using a much larger sample (n=43 CFS patients and n=23 healthy, sedentary controls) (16). Another group used treadmill exercise for 18 minutes to study the heat shock protein response to exercise in 6 CFS patients and 7 healthy controls; heat shock proteins 27, 60, 70 and 90 were determined in peripheral blood mononuclear cells of the study partic-

ipants before, immediately after, and 1 and 7 days following the exercise challenge (53). A group X time interaction was only found for heat shock protein 27, as heat shock protein 27 remained unchanged in the healthy people, and declined following exercise in the CFS group (53).

Interestingly, Jammes et al. (16) found that exercise-induced oxidative stress levels were higher in those CFS patients who had regularly taken part in sports activity (> 6 hours per week) for more than 6 years prior to CFS onset, and/or suffered from a severe infection (peritonitis, pneumonia or encephalomyelitis) within 3-4 months preceding CFS onset (16).

One study examined the nitrosative stress response of female CFS patients (n=44) versus healthy women (n=25) to two bicycle exercise bouts (one maximal test to exhaustion and a second personalized submaximal bout), and found that nitric oxide metabolites (nitrates) become much higher post-exercise among the CFS patients (49). This was true for both conditions: the maximal and submaximal exercise bout. These findings require replication.

Summarizing the findings in relation to oxidative stress, cumulating evidence indicates that oxidative stress following exercise occurs earlier and lasts longer in CFS patients, and also that the anti-oxidant response post-exercise is delayed and reduced. However, nearly all studies come from the same laboratory and hence require replication.

Leukocyte gene expression

Immune response to exercise can be studied at the cellular level, at the protein level, but also at the gene level. Six studies have examined immune cell gene responses to exercise in patients with CFS (4, 22, 23, 48, 57, 59). The most important findings are summarizes below.

Whistler, et al. studied blood mononuclear cell gene expression in response to 20 minutes of steady-state bicycle exercise at 70% of the predicted maximum work load in 5 women with CFS and 5 healthy controls (57). They first identified 21 genes as being differentially expressed in response to exercise in healthy subjects (as a normal response to exercise). When comparing the gene response of CFS patients with the normal response, they identified differences in exercise-responsive genes in CFS subjects before and after exercise. A lower expression of the identified genes, as observed in response to exercise in the CFS group, may have a subtle effect on immune functioning (57). More specifically, gene regulation in chromatin structure was the most obvious change following exercise in CFS patients, and they also observed that the complement pathway showed significant differences between CFS and control subjects after exercise (57). This is important as it shows that exercise results in a stronger complement activation in CFS patients, not only at the protein level (i.e. gene product level), but also at the transcript level. In addition, nucleosome assembly, cytoplasmic vesicles, membrane

transport, and G protein-coupled receptor ontologies were found in those with CFS.

The differences in gene expression suggest important perturbation in biochemical activity, including ion transport and ion channel activity of lymphocyte and monocyte peripheral blood fractions from CFS subjects in response to exercise (57). It suggests that immune cells of CFS do not respond normally to an exercise challenge. However, the findings should be interpreted with caution, as this small scale study had a high risk of bias assessment score (33%). Hence, these findings should be viewed as pilot data, as is the case with the report by Steinau et al., who studied only one CFS patient versus one matched healthy control (48).

Three additional gene expression exercise immunology studies in CFS patients obtained higher risk of bias assessment scores (table 2). Light et al. studied gene expression in leukocytes (venous blood samples) obtained from 19 CFS patients and 16 healthy controls 48 hours before, and up to 48 hours following, 25 minutes of submaximal whole-body exercise (combined arm and leg cycle ergometer) (23). Although baseline (resting) mRNA levels were similar in both groups, in response to exercise they found group differences for metabolite detecting genes, adrenergic genes, and immune genes (23). More specifically, and in line with the hypothesis of the present review, CFS patients showed larger post-exercise increases in interleukin-10 and Toll-like receptor 4 gene expression. Toll-like receptors are important for the activation of both the innate and acquired (specific) immune system.

The same group later partly confirmed these findings in a larger study (n=48 CFS patients and n=49 healthy controls), using the same protocol as the previous study (22). This time, exercise in CFS patients led to increased expression of certain sensory ion channel, adrenergic and immune genes, which do not occur in healthy controls. Addressing the genes closely related to immune function, the previous finding of greater post-exercise increases in Toll-like receptor 4 gene expression in the CFS group was not confirmed, even though they confirmed the larger increase in interleukin-10 gene expression (22). No changes in interleukin-6 or CD14 mRNA were observed in either study.

Thus, although none of the studies summarized above found meaningful changes following exercise in the circulating anti-inflammatory cytokine interleukin-10 (19, 47, 58), two similar studies from the same group reported increased interleukin-10 mRNA (gene expression) in peripheral leukocytes following exercise in CFS patients (22, 23). No changes in interleukin-6 mRNA were observed in either study.

The discrepancies across studies are most likely due to the different sample sizes, range of ages, differences in disease severity, and possibly partly due to medication differences as the more recent study allowed pain medication and anticonvulsant medication use. Importantly the post-exercise increases in interleukin-10 mRNA in the CFS group correlated with increases in post-exercise pain and fatigue (22), pointing to its clinical relevance in relation to post-exertional malaise in CFS.

The larger post-exercise interleukin-10 gene expression increases in CFS patients in response to exercise might indicate too strong and prolonged anti-inflammatory action following exercise, which increases the risk of opportunistic infections. Opportunistic infections have increased prevalence rates in patients with CFS compared to healthy people (5, 30, 36). However, it remains to be established whether exercise responses at the gene level have implications for immune function as well. For instance, although an abnormally high post-exercise interleukin-10 increase was found at the gene level in CFS patients (22, 23), blood levels of interleukin-10 did not change following exercise in CFS patients (19, 47, 58).

Still, the clinical importance of the differences in gene expression following exercise was further substantiated in a similar study from the same group, applying similar methods for comparing CFS (n=22) with healthy controls (n=23) and patients with relapsing-remitting multiple sclerosis (n=20), another chronic illness characterized by chronic fatigue (and to a lesser extend pain) (59). Toll-like receptor 4 gene expression differed between CFS and multiple sclerosis, with a post-exercise decrease in the latter group (59). Unfortunately, all three studies (22, 23, 59) from this group pooled gender data.

Gender is an issue when interpreting the findings of a study that examined gene expression of peripheral blood mononuclear cells of male subjects (n=7 CFS, n=20 Gulf War Illness, and n=22 healthy, sedentary veterans) in response to a standard, maximal graded cycling stress test (4). Given their focus on Gulf War Illness rather than CFS, the choice for male participants is understandable, but it limits the external validity of the study findings for CFS patients (even though the patients with Gulf War Illness complied with the CDC criteria for the diagnosis of CFS as well). Although differences were found between groups (e.g. subdued cell cycle progression and immune signaling in CFS), no time X group interactions were found, indicating the gene expression of peripheral blood mononuclear cells does not respond differently between male CFS patients, patients with Gulf War Illness and healthy sedentary controls (4).

Summarizing the findings of gene expression profiling of CFS patients in response to exercise, there is moderate evidence that CFS patients showed larger post-exercise interleukin-10 and Toll-like receptor 4 gene expression increases, which accounts in part for post-exertional malaise. Although compelling, these findings have been confirmed solely by the same laboratory and hence require replication by independent researchers.

RESEARCH AGENDA

In addition to the above outlined need for replicating findings in independent laboratories, several other recommendations for further research can be formulated based on the study of the scientific literature in this area (table 3 summarizes these recommendations).

First, it is important to make a distinction between the effects from therapeutic interventions using exercise *therapy* in CFS (e.g. reference (55)) and findings from studies examining the exercise immunology*/physiology* of people with CFS (e.g. references (37, 47)). The latter often use one bout of exercise to examine the acute response to (often very strenuous) exercise. Such exercise physiology studies provide us with valuable information on the biology of post-exertional malaise of CFS, but the exercise response may be very different in longer-term low-intensity exercise programs. These are two distinct issues. Studies examining the effects of exercise therapy on immune function in CFS patients are essentially lacking. Given the compelling findings addressing acute responses of the immune system to exercise in CFS patients as reviewed here, this is an important avenue for future research in this area.

All studies examined here, used standardized exercise protocols. Physical activities like walking long distances or cycling are not applicable, or possible, for all CFS patients. In addition, such studies were often conducted in laboratory settings. Hence, such studies have limited ecological validity. Therefore, there is a need to study exercise immunology using physically demanding functional tasks for CFS patients, like stair climbing and ironing, rather than graded bicycle or treadmill tests. Stair climbing has been used for studying CFS patients (14, 34), but not from an exercise immunology perspective. It remains to be established whether the observed exercise immunology abnormalities (e.g. increased oxidative stress response, enhanced complement activation) are specific for (sub)maximal exercise, or can be extrapolated to activities of daily living.

Table 3. Research agenda for exercise immunology in patients with chronic fatigue syndrome.

	Recommendations for future studies addressing exercise immunology
1.	Examining whether exercise responses at the gene level have implications for immune function in CFS patients.
2.	Examining whether tissue cytokines (e.g. inflammatory cytokines in muscle tissue) respond different to exercise in CFS patients.
3.	Replication of the findings of enhanced oxidative stress response to exercise in CFS patients.
4.	Examining the effects of exercise therapy, rather than acute exercise bouts, on immune function in CFS patients.
5.	Examining whether immune responses to exercise account for post-exertional malaise in CFS.
6.	Examining the immune response to daily life physical activities in CFS patients.
	Methodological recommendations
1.	Apply a true experimental design (randomized cross-over study design)
2.	Use blinded assessments / assessors.
3.	Do not pool gender data.
4.	Apply appropriate statistical methods for a priori determination of the required sample size.
5.	Identify and if required account for possible confounders when designing the study, or the very least when (re)analyzing the data.

Previous exercise immunology studies in the field of CFS used case-control rather than experimental designs. This implies that previous observations regarding exercise immunology in CFS patients did not control for potential bias due to emotional stressors or the fluctuating nature of CFS. Therefore, it is recommended that future studies apply a true experimental design (randomized study design) controlling for emotional stressors.

Only 4 (13, 22, 23, 59) of the 23 studies used blinded assessors, and 3 out of 4 studies are from the same group. Traditionally, this represents an important shortcoming, but in the area of exercise immunology one can question its importance. If a standardized exercise protocol is used in all comparison groups, as is the case in all studies included in this review, then little bias is to be expected from a lack of blinding of the assessors. After all, the outcomes are laboratory analyses, and (blood) samples are typically coded and transferred blindly to the laboratory for analysis. A more important shortcoming is the sample size. The majority of the studies had less that 20 subjects in the CFS group, and only one study (37) based the sample size on an a priori sample size estimation. It is advised that future studies in the field use established statistical methods for a priori determination of the required sample size.

The analysis for blood samples or mRNA extraction varied between studies. This may account for some of the discrepancies across studies. From the available literature it is concluded that CFS patients have a normal circulating cytokine response (e.g. interleukin-1β, interleukin-6, interleukin-10, tumour necrosis factor-α) to exercise (7, 17, 19, 24, 37, 39, 41, 47, 58). Still, tissue cytokines and their response to exercise have hardly been studied in CFS patients. For instance, it would be worthwhile examining whether pro- and anti-inflammatory cytokines in working muscles of CFS patients respond different to (local) exercise than in healthy sedentary controls.

To date, few studies have carefully examined whether exercise-induced immune changes in patients with CFS account for the symptom exacerbations as typically seen in these patients (i.e. post-exertional malaise). As outlined in the introduction section, post-exertional malaise is now increasingly recognized as a prominent characteristic of CFS. Future studies are advised to explore this relevant feature.

Finally, pooling of gender data has been identified as an important source of bias in studies addressing exercise physiology in CFS patients (42), and exercise immunology more in particular (45). Despite this important methodological finding, pooling of gender data remains a common shortcoming in exercise immunology studies in CFS patients, and should be addressed in future work. In general, it is recommended that future studies account for potential confounders when designing the studies and when analyzing the data. Few studies have reanalyzed the dataset accounting for possible confounding factors, as reflected by the low number of studies (12 out of 23) fulfilling criterion 6 (identification or accounting for confounders) in the risk of bias assessment score sheet (table 2).

CONCLUSION

The intention of this review was to answer the following question: does the immune system of patients with CFS respond differently to exercise as compared to healthy sedentary controls? Based on the available research data, our hypothesis that CFS patients show a more pronounced immune response to exercise as compared to healthy sedentary controls can be confirmed. Indeed, the literature review provides evidence for an altered immune response to exercise in patients with CFS. More specifically, compared to the normal response of the immune system to exercise as seen in healthy subjects, patients with CFS have a more pronounced response in the complement system (i.e. C4a split product levels), oxidative stress system (i.e. an enhanced oxidative stress combined with a delayed and reduced anti-oxidant response), and the immune cells' gene expression profile (larger post-exercise interleukin-10 and toll-like receptor 4 gene expression increases), but not in circulating pro- or anti-inflammatory cytokines. Many of these immune changes relate to post-exertional malaise in CFS, a major characteristic of the illness. Future research in this area should apply a true experimental design, extend findings to other tissues than blood samples, control for covariates, and examine the immune response of CFS patients following long-term exercise therapy.

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