EXPERT OPINION

The role of mitochondrial dysfunctions due to oxidative and nitrosative stress in the chronic pain or chronic fatigue syndromes and fibromyalgia patients: peripheral and central mechanisms as therapeutic targets?

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Introduction: Chronic fatigue syndrome (CFS) and fibromyalgia (FM) are characterized by persistent pain and fatigue. It is hypothesized that reactive oxygen species (ROS), caused by oxidative and nitrosative stress, by inhibiting mitochondrial function can be involved in muscle pain and central sensitization as typically seen in these patients.
Areas covered: The current evidence regarding oxidative and nitrosative stress and mitochondrial dysfunction in CFS and FM is presented in relation to chronic widespread pain. Mitochondrial dysfunction has been shown in leukocytes of CFS patients and in muscle cells of FM patients, which could explain the muscle pain. Additionally, if mitochondrial dysfunction is also present in central neural cells, this could result in lowered ATP pools in neural cells, leading to generalized hypersensitivity and chronic widespread pain.
Expert opinion: Increased ROS in CFS and FM, resulting in impaired mitochondrial function and reduced ATP in muscle and neural cells, might lead to chronic widespread pain in these patients. Therefore, targeting increased ROS by antioxidants and targeting the mitochondrial biogenesis could offer a solution for the chronic pain in these patients. The role of exercise therapy in restoring mitochondrial dysfunction remains to be explored, and provides important avenues for future research in this area.

Keywords: ATP, central sensitization, chronic pain, mitochondria, muscle, nitric oxide, NMDA receptor, peroxynitrite, spinal cord, superoxide

Expert Opin. Ther. Targets [Early Online]

1. Introduction

1.1 Defining chronic fatigue syndrome and fibromyalgia

Chronic fatigue syndrome (CFS) is a debilitating and complex disorder, characterized by extreme fatigue [1]. The population prevalence of CFS is between 0.2 and 2.6% (with > 75% female patients [2]) and little is known about the etiology of the illness, making prevention and treatment challenging. In addition to the chronic fatigue, widespread and persistent *pain* is common in individuals with CFS [3-6].

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Article highlights.

- There is evidence for increased oxidative and nitrosative stress in CFS and FM.
- Mitochondria are both the source and the target of increased oxidative and nitrosative stress.
- Mitochondrial dysfunctions are shown in muscle cells of patients with FM.
- Mitochondrial dysfunctions in muscle cells may cause muscle pain, due to increased muscle damage and decreased aerobic capacity.
- If mitochondrial dysfunctions are also present in the cells of the CNS, this may lead to increased sensitivity and thus central sensitization.
- Possible therapeutic targets encompass increasing the antioxidant level or optimizing mitochondrial function.

This box summarizes key points contained in the article.

The syndrome is largely overlapping with fibromyalgia (FM), a chronic pain syndrome that has been defined by widespread pain for > 3 months and the presence of additional symptoms like disturbed sleep, emotional distress, and pronounced fatigue [7,8].

Patients with FM and the majority of patients with CFS suffer from myalgias and chronic widespread pain. Evidence is growing for *central sensitization* as the underlying mechanism explaining chronic pain in CFS and FM [9]. But the mechanism behind the onset of central sensitization seems to be multifactorial. It is hypothesized that reactive oxygen species (ROS), caused by oxidative and nitrosative stress, by inhibiting mitochondrial function can be involved in muscle (peripheral) pain and central sensitization.

1.2 Oxidative and nitrosative stress

Both syndromes are often preceded by a physical trauma, such as from an accident or from surgery, psychological stress, viral, parasitic or bacterial infections, episodes of strenuous physical activity, etc. [10,11]. Following inflammatory stimuli, the production of oxygen radicals (ROS, oxidative and nitrosative stress), H_2O_2 (peroxides), $2O_2^-$ (superoxide) and $ONOO^-$ (peroxynitrite) is increased.

There is evidence for increased *oxidative stress* in CFS and FM, with higher oxidized low-density lipoproteins (LDL) and elevated protein carbonyl levels found in blood samples [12-19]. There is also evidence for damage caused by oxidative stress in CFS and FM. The serum IgM antibodies to fatty acids, such as phosphatidyl-inositol (Pi) and malondialdehyde (MDA), which are by-products of lipid peroxidation, are significantly greater in CFS patients compared to healthy controls. This shows that CFS is characterized by an IgM-related immune response directed against disrupted lipid membrane components and by-products of lipid peroxidation, which are normally not detected by the immune system, but due to oxidative damage, have become immunogenic. Thus, the oxidative damage in CFS may have functional consequences: disorders in Pi may have biological effects by interfering with intracellular signaling processes, and the damage to membrane fatty acids may render the peroxidized membrane bilayer more rigid, thus inducing changes in different membrane (including receptor) functions [20].

Inflammation is also accompanied by *nitrosative stress* due to overactivated iNOS (inducible nitric oxide synthase) resulting in excessive nitrogen monoxide (NO) and peroxynitrite ($ONOO^-$) formation by activated neutrophils and monocytes. Both nitrogen monoxide and peroxynitrite levels are reported to be increased in blood peripheral lymphocytes of CFS patients [20]. Peroxynitrite reacts with and inactivates several of the enzymes in mitochondria leading to energy metabolism dysfunction [21,22]. A central tenet of the peroxynitrite hypothesis is that once levels are elevated, there are several potential positive feedback mechanisms which may act to amplify and sustain these elevated levels. The most important thing here is that the mitochondria are both the source and the target of peroxynitrite, sustaining a vicious circle [23].

There is evidence in other different myopathies and peripheral neuropathies [24] that mitochondria contribute to the pathogenesis and/or pathophysiology. In CFS and FM, the role of mitochondria in the chronic widespread pain of these patients remains obscure and underreported. Nevertheless, we hypothesize that mitochondrial function in muscle, blood and spinal cord is impaired in CFS and FM due to increased oxidative and nitrosative stress and that these impairments may lead, among others, to peripheral sources of nociception and central sensitization, explaining the chronic widespread pain and other systemic complaints in these patients. The present report will provide an overview of the existing evidence regarding mitochondrial dysfunction in these patients and explain how these abnormalities may lead to the chronic pain seen in these patients. Furthermore, possible therapeutic targets will be discussed.

2. Current evidence

2.1 Mitochondrial dysfunctions in white blood cells and skeletal muscle of CFS patients?

The available CFS data concerning mitochondrial dysfunction were obtained mainly in two targeted tissues: white blood cells and muscle cells. In white blood cells data are more sound than in the muscle tissue. In white blood cells several researchers reported decreased mitochondrial function resulting in less ATP [25-27]. However evaluating these results more in depth, some authors discussed that the main cause is decreased citrate synthase content, which is an expression of the number of mitochondria [26]. Others find a decreased activity of the different enzymes (complexes) expressed relatively to the citrate synthase content. This means that the enzymes are less active additionally to the decreased number of mitochondria [25,27].

A limited number of studies addressing muscular mitochondrial function in CFS patients are available, but data are conflicting. Some did not found any difference in mitochondrial enzyme activity in CFS patients compared to a matched control group, but only a decreased mitochondrial content [28]. Other studies have shown abnormal mitochondrial degeneration [27,29,30] and severe deletions of oxidative genes in mitochondrial DNA (mtDNA) [27]. These changes result in reduced ATP synthesis and increased lactate concentrations [29]. The most important difference between these studies are different inclusion criteria resulting in a larger heterogeneity of the studied population and the used methodology, for instance, by measuring plasma creatine kinase levels, which is an indirect method [25].

In conclusion, real progress in establishing the etiopathogenesis of CFS as a ROS-dependent process with clear impact on mitochondrial function will only be possible with definitive evidence that an excess of free radicals in CFS muscles (measured in muscle biopsies) is directly influencing the mitochondrial function. At the moment this is fundamentally lacking in the current literature.

2.2 Mitochondrial dysfunction in white blood cells and skeletal muscle of FM patients?

In FM patients the literature is more sound concerning mitochondrial dysfunction. Several authors report mitochondrial dysfunction in white blood cells as well as in muscle biopsies [31,32]. In these patients abnormalities in white blood and muscle cell mitochondria were detected with electron microscopy, namely mitochondria with irregular cristae, single fiber defects of cytochrome-c-oxidase, and deletions of the mitochondrial genome [33].

The degenerative muscle changes in membranes, mitochondria, and capillary vessels may be related to defects in membrane ion channeling, oxygen and metabolite transport, and ATP production via oxidative phosphorylation. The decrease in activity of the oxidative enzymes, 3-hydroxy-CoA dehydrogenase, citrate synthase, and cytochrome oxidase, support the proposal of defects in oxidative metabolism and ATP synthesis [34].

2.3 Muscular mitochondrial dysfunctions leading to widespread peripheral muscle pain

Muscular mitochondrial dysfunctions are known to cause (exercise-induced) *peripheral muscle pain*, weakness, fatigability, exercise intolerance, acidosis, etc. [24]. If mitochondria become dysfunctional when they are stressed by one or more stimuli, cells can undergo apoptosis. Mitochondria contain cytochrome c and apoptogens, which enter the cytoplasm and activate caspase, which in turn causes cellular damage and induce nuclear fragmentation.

In FM patients data supporting this view are scarce. In order to show apoptosis and abnormal cell death in FM, Sprott *et al.* investigated DNA fragmentation in the muscle tissue and ultra-structural analysis by electron microscopic features of the muscle in FM patients. DNA fragmentation has been detected to be significantly higher in patients with FM compared to the nuclei in healthy controls [35]. In some genetic connective tissue disorders like Bethlem and Ullrich disease, which are associated with severe hypermobility and have a complaint pattern (pain and fatigue) which is largely overlapping with FM [36], mitochondrial dysfunction and increased apoptosis in skeletal muscle have been reported [37,38].

Furthermore, it has been proposed that ROS damage can increase permeability of transition pores in the mitochondrial inner membrane, leading to a simultaneous collapse of mitochondrial membrane potential and the elimination of dysfunctional mitochondria by mitophagy, as evidenced by the findings of mitophagy in blood mononuclear cells of FM patients. "Mitophagy" is described as the selective removal of mitochondria by autophagy during development and under pathologic conditions. And while normal autophagy is functional, extensive mitophagy comprises cell functionality and may contribute to the pathophysiology of FM [18].

Finally, it seems that mitochondrial dysfunction is related to the circulating cytokine levels in patients with FM and thus contributes to the pathophysiology of FM. Inversely, reducing oxidative stress in patients with FM improved mitochondrial function, reduced cytokines levels, and reduced pain scores and tender points [39]. It is indeed shown that lipid peroxidation in blood cells (due to mitochondrial ROS) is significantly correlated to pain scores, fatigue, stiffness, etc., in patients with FM [40].

But besides increased inflammation and mitophagy in FM, other mechanisms leading to peripheral pain due to possible mitochondrial dysfunction in CFS or FM are not yet clear. To date, it is not known whether mitochondrial dysfunction could, for example, lead to muscle damage and reduced aerobic capacity in CFS or FM, leading to muscle pain and peripheral sensitization (by, e.g., acidosis, hypoxia and by-products of muscle damage) and the typical pain exacerbation after exercise.

2.4 Mitochondrial dysfunctions in central nervous system leading to central sensitization and chronic pain

In FM and CFS, a body of evidence is available for central sensitization as the underlying mechanism of chronic pain [41-45]. This heightened general pain sensitivity may be sustained and amplified by continuous upgrading of peripheral nociceptive input [33]. The proposed basis for this enhancement of secondary hyperalgesia is the activation of the *N*-methyl-D-aspartate (NMDA) receptors. The NMDA receptors in the dorsal horn of the spinal cord and periaqueductal gray have been demonstrated to play a role in the development of central sensitization [46].

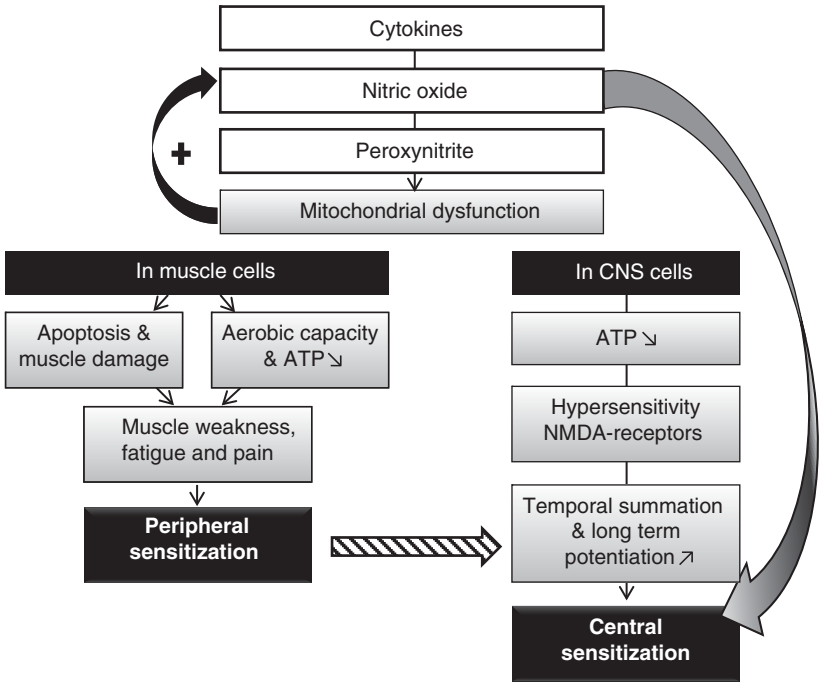

Figure 1. Relation of oxidative and nitrosative stress together with mitochondrial dysfunction to central sensitization.

It is well known that when neurons containing NMDA (*N*-methyl-D-aspartic acid) receptors have lowered ATP pools, their NMDA receptors become hypersensitive to stimulation due to lowering of the plasma membrane potential, leading to an immediate increase in NMDA activity and stimulate NMDA-dependent temporal summation of pain long-term potentiation, thus producing longer term increases in synaptic activity. Due to the interaction with peroxynitrite ATP production, this possibly explains the contribution to central sensitization [47].

Earlier studies implicated increased sensitivity of central NMDA receptors as playing a primary role in FM, as evidenced by a significant reduction in symptoms among a large subset of patients in response to low doses of ketamine, a non-competitive NMDA receptor antagonist [48]. Furthermore, ketamine attenuates pain, pain thresholds, referred pain and temporal summation when administered intravenously to patients with FM [49,50].

Another emerging concept in pain research is that oxidative stress play a critical role in *chronic pain due to central sensitization*. Elevated spinal ROS levels by increased production of mitochondrial superoxide lead to central sensitization and consequently pain without peripheral nerve injury or tissue inflammation [51]. Superoxide can cause peripheral and central sensitization and alter nociception [52], resulting in hyperalgesia mediated by both local and spinal oxidant mechanisms. Also NO has, for example, the capability of inducing peripheral and central sensitization by reducing receptor thresholds [53] and is able to reduce the inhibitory activity of the central nervous system leading to central sensitization of dorsal horn neurons [54]. Evidence for the involvement of ROS in pain has been provided in a number of studies mainly by demonstrating the antinociceptive effect of some antioxidants [55,56], superoxide dismutase (SOD; neutralizes superoxide) mimetics [57] and inhibitors of electron transport chain complexes [58]. Furthermore, increased levels of ROS or their by-products are shown in specific tissues in animal models of persistent pain [59] and SOD inhibitors further enhanced secondary capsaicin-induced hyperalgesia [57]. Pain modulation can be accomplished by altering superoxide in the spinal cord [57]. Joseph and Levine demonstrated a role of the mitochondrial electron transport chain in neuropathic and some forms of inflammatory pain. The contribution of the mitochondrial electron transport chain in neuropathic pain is ATP-dependent [58].

However, this hypothesis with an interaction of oxidative and nitrosative stress, mitochondrial function and chronic pain due to central sensitization has not yet been studied in CFS.

Figure 1 presents a possible mitochondrial-related mechanism underlying central sensitization in CFS and FM.

3. Conclusion

To conclude, it seems plausible that oxidative and nitrosative stress together with mitochondrial dysfunction play an important role in both syndromes. Nevertheless, the exact mechanisms and the clinical relevance require stronger evidence.

Table 1. Therapeutic targets for oxidative stress and mitochondrial dysfunction in CFS and FM.

Objective	Possible tools
Antioxidant level ↗	Coenzyme 10 Carnosine
Mitochondrial biogenesis ↗	Resveratrol
Mitochondrial content/enzymes ↗	Exercise (cave NO!)

↗: Increase; NO: Nitric oxide.

Especially, the relation of oxidative and nitrosative stress together with mitochondrial dysfunction to central sensitization has not been examined yet. Given the consistent and overwhelming evidence supporting a cardinal role for central sensitization in both FM and CFS, this is an important shortcoming of our current understanding of these syndromes. Further research should focus on these lacunas, because the present rationale offers interesting therapeutic targets in these populations: targeting the antioxidant status and optimizing mitochondrial function.

4. Expert opinion

As mentioned above, the presence of mitochondrial dysfunction in CFS as well as FM patients is a relevant plausible hypothesis, based on the current evidence, which potentially explains pain and fatigue in these patients. Mitochondrial dysfunction in CFS and FM patients in muscle, peripheral as well as central nervous tissue, can be induced by a large amount of ROS. These oxygen radicals are produced by the mitochondria themselves. The increased amount of free radicals in this tissue is a consequence of a negative balance between ROS-production of the mitochondria, on the one hand, and the antioxidant content in the tissue, on the other hand. The relative rates of ROS production and decomposition determine their steady state concentration and ultimately, their potential to cause tissue injury.

It is possible that the production of ROS is increased due to an increased frequency of action potentials registered in the neurons of the pain system (peripheral as well as central) due to a traumatic injury or infection which often precedes the syndromes. Due to the injury or infection, different cells will become necrotic and several humoral factors, such as bradykinine and potassium, will leak into the environment, possibly targeting nociceptors of the pain neurons. In this case, there is a constant imbalance at the level of the membrane potential which has to be restored by Na/K-ATPase pumps. These pumps are ATP-dependent, which stresses the mitochondrial function, resulting in an increased production of oxygen radicals. This hypothesis is supported by Mattson and Liu who reported that mitochondria in axons and presynaptic terminals provide sources of ATP to drive the ion pumps that are concentrated in these structures, for rapid restoration of ion gradients following depolarization and neurotransmitter release [60].

Mitochondria may also play important roles in the regulation of synaptic function because of their ability to regulate calcium levels and ROS production. ROS generated in response to synaptic activity are known to contribute to the regulation of long-term structural and functional changes in neurons. The high-energy demands of synapses, together with their high levels of ROS production, place them at risk during conditions of increased stress, which occur in aging, neurodegenerative disorders such as Alzheimer's and Parkinson's diseases, and after acute traumatic and ischemic insults.

Besides an increased ROS production, it is also possible that the levels of several antioxidants in the tissue are lowered [61]. The elimination of strong oxidants is a non-enzymatic process and is done by vitamin C, vitamin E, ubiquinone, carnosine, etc. The elimination of less reactive species is done by specific enzymes, present in the cytosol, peroxisomes or mitochondria. The mitochondrion has its own set of enzymes and other antioxidants. The mitochondrial matrix contains Mn-superoxide dismutase (SOD-2) and the intermembrane space contains the cytosolic isoform of superoxide dismutase (Cu, Zn-SOD or SOD-1) [62,63].

Besides increased ROS production and lowered levels of antioxidants, inflammatory substances, such as TNF-α and interleukins, can also have a direct impact on the mitochondrial function in the inflamed environment. In liver tissue, Weidinger *et al.* reported that inflammation, due to hypoxia, induced mitochondrial dysfunction [64]. In CFS and FM patients, increased biomarkers of inflammation have been reported [65], and it can therefore be hypothesized that increased levels of inflammation may lead to mitochondrial dysfunction.

Based on the aforementioned evidence and knowledge, different therapeutic targets can be defined, as presented in **Table 1**.

To counter the cause of the mitochondrial dysfunction, therapy can focus on keeping up the levels of antioxidants and on mitochondrial antioxidant function.

In FM patients as well as in CFS patients, several authors reported decreased levels of Coenzyme Q10 and several minerals as zinc, copper, magnesium and manganese, which would be related to pain complaints [66-69]. Cordero reported that supplementation of Coenzyme Q10 had positive effects on mitochondrial function by decreasing oxidative stress and thereby having a positive impact on headache symptoms, tender points, VAS score and the Fibromyalgia Impact Questionnaire in FM patients [16,39,70]. In juvenile FM patients, supplementation also reduced self-reported fatigue [13]. No studies evaluating the effect of Coenzyme Q10 supplementation on pain in CFS are available to the best of our knowledge. Further clinical trials focusing on the antioxidant status in these patients and focusing on supplementation with antioxidants with interest for oxidative stress, mitochondrial function measured in blood cells and muscle tissue and

evaluating association with several complaints, mainly pain and fatigue, are required.

An interesting antioxidant is carnosine (β-alanyl-L-histidine), a dipeptide of the amino acids β-alanine and histidine. It is highly concentrated in muscle and central nervous system.

Carnosine has been proven to scavenge reactive oxygen species (ROS) as well as α–β unsaturated aldehydes formed from peroxidation of cell membrane fatty acids during oxidative stress.

In animal models carnosine has been shown to retard cancer growth and protect against alcohol-induced oxidative stress as well as ethanol-induced chronic liver damage. Carnosine is also neuroprotective against permanent cerebral ischemia in mice [71].

Carnosine has been shown to counteract peroxynitrite-dependent protein alterations such as tyrosine nitration and thereby cell damage [72]. Boldyrev *et al.* [71] reported in two recent clinical trials that carnosine levels were depressed in Parkinson's disease and after brain stroke, and that supplementation resulted in a decrease in blood plasma protein carbonyls and lipid hydroperoxides (ROS indicators) and this was accompanied with increased cerebral blood flow, locomotor and cognitive function. It should be interesting to evaluate the content of carnosine in muscle tissue and blood in CFS and FM patients and if carnosine is decreased supplementation studies should be done.

A third element in the possible solutions of decreasing ROS and increasing antioxidant capacity in the cell are medication-specific targeting the mitochondrial biogenesis. As an example we name resveratrol or esveratrol (3,5,4′-trihydroxy-*trans*-stilbene) which is a stilbenoid, a type of natural phenol, and a phytoalexin produced naturally by several plants, especially the roots of the Japanese Knotweed, from which it is extracted commercially.

The effects of resveratrol are currently a topic of numerous animal and human studies. In mouse and rat experiments, anticancer, anti-inflammatory, blood sugar-lowering and other beneficial cardiovascular effects of resveratrol have been reported [73].

It could be speculated that these types of medication can increase the content of mitochondria and thereby the content of antioxidants, which would help to decrease the amount of ROS, but further research is required in patients with FM and CFS.

Finally, due to the fact that these patients have decreased mitochondrial content due to the pathology but also due to physical deconditioning, it is desirable to increase this by increasing physical activity, for example, by physical exercise. In healthy individuals, aerobic, strength, combined exercise training and interval training induces morphological, metabolic and gene-regulating adaptations in skeletal muscle including increases in muscle respiratory capacity, components of the mitochondrial respiratory chain, ATP synthase, and enzymes of both the Kreb's cycle and β-oxidation pathways [74]. The effect of exercise training on antioxidant function of mitochondria remains however element of debate. Physical activity normally causes an increase in NO production [75,76]. If this effect would be reinforced by the already elevated amounts of NO this could explain the typical pain exacerbation, malaise and the delayed recovery after physical activity as typically seen in FM and CFS patients. The results of Jammes *et al.* indeed confirm a lengthened and accentuated oxidative stress after exercise in patients with CFS, sufficient to explain muscle pain and postexertional malaise [77]. Additionally, a subgroup of CFS patients presents a significantly lower ATP synthesis rate during recovery, indicating impaired mitochondrial oxidative phosphorylation [78].

Furthermore, pathological overproduction of NO will decrease oxygen consumption [79] and increase anaerobic glycolysis (lactate production) by modulating mitochondrial respiration [80] and iron metabolism [81]. These effects in addition to the oxidative damage of cell membranes [82], structural proteins as actin and DNA [83], may compromise exercise capacity and worsen physical activity responses. So, although the increase of NO may be one among more beneficial effects of exercise in healthy people, in CFS and FM patients more research is required to study the effect of exercise on NO production, to elucidate the benefits or the dangers of exercise [84]. Additionally, further investigation into the possible positive effects of exercise training on mitochondrial function has to be elaborated and certainly the most optimal form with respect to the complaints.

Declaration of interest

The authors state no conflict of interest and have received no payment in preparation of this manuscript.

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