Exercise in fibromyalgia and related inflammatory disorders: Known effects and unknown chances.

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ABSTRACT

Fibromyalgia (FM) is characterised by chronic widespread pain and allodynia (pain from stimuli which are not normally painful with pain that may occur other than in the area stimulated) of more than 3 months duration. The current hypothesis of the aetiology of FM includes inflammatory and neuroendocrine disorders. The biophysiology of this syndrome, however, remains still widely elusive, and there are no formally approved therapies. Non-pharmacological interventions in FM patients include habitual exercise programs which improve physical function and quality of life of patients and may even reduce pain. However, the mechanisms through which exercise benefits FM symptoms needs to be elucidated. In this article we firstly review the main topics and characteristics of the FM syndrome, while focusing our attention on the inflammatory hypothesis of FM, as well as on the beneficial effects of habitual exercise as a co-therapy for FM patients. In this context, the latest developments in research on anti-inflammatory effects of exercise are also reviewed and discussed. To find out what is known about the connection between benefits of exercise for FM and anti-inflammatory effects of exercise, we carried out a PubMed search using the term "fibromyalgia" and "exercise" together with "inflammation", and no more than ten published articles were found (six of them reviews), which are also discussed.

In the second part of the article we present a pilot investigation on a group of 14 female FM patients with a diagnosis of FM by a rheumatologist. They took part in a pool-aquatic program in warm water over a period of fourth months (three weekly 60-min sessions). Circulating inflammatory (IL-1β, IL-2, IFNγ, TNFα, IL-8, IL-6, IL-4, IL-10 and CRP) and neuroendocrine (NA and cortisol)

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markers were determined. FM patients showed higher circulating levels of IL-8, IFNγ and CRP, as well as cortisol and NA than age-matched healthy control women. After the exercise program, a significant decrease in IL-8, IFNγ, and CRP were found, in parallel with a decrease in circulating concentrations of cortisol and increased levels of NA.

The results confirm an elevated "inflammatory status" in the FM syndrome and strengthen the hypothesis that the benefits of exercise in FM patients are mediated, at least in part, by its anti-inflammatory effects. A better regulation of the cytokine-HPA axis feedback may be also involved.

Key words: fibromyalgia, inflammation, exercise, cytokines, IL-8, IFNγ, CRP, NA, cortisol, women

INTRODUCTION

Classifying fibromyalgia syndrome: the inflammatory hypothesis

Fibromyalgia (FM) is a form of non-articular rheumatism characterised by long term (>3 months) and widespread musculoskeletal pain, morning stiffness, and pressure hyperalgesia at characteristics sites, called soft tissue tender points. FM usually appears at the age of 30-40 years (after the age of 55 years is rare). It is often associated with symptoms like reduced muscle strength, fatigue, sleep disturbance, irritable bowel syndrome and psychological disturbances (e.g. depression). The currently used classification criteria for FM (American College of Rheumatology, 1990) define FM as the presence of chronic widespread pain and allodynia (in both sides of the body and both over and under the navel) to pressure in more than 11 of 18 specified sites or tender points (83). This definition, based on subjective symptoms and lacking objective criteria, has many times raised doubts about the existence of FM, a situation which can cause additional problems for patients before or after diagnosis. The fact is that until today the biophysiology of FM remains elusive and the treatment remains empirical. Recently, a hypothesis of the aetiology of FM has been put forward by van West and Maes (77) as follows:

– It is an inflammatory disorder accompanied by changes in the neuroendocrine-immune system.
– It is related to dysfunctions in the serotonergic system.
– It is a psychiatric disorder related to major depression.
– It is related to muscle abnormalities or energy depletion in the muscles.
– It is related to the inflammatory response system (IRS) and cytokines.
– It results from mechanical abnormalities of the cervical or lumbar spine.
– It results from aberrant central pain mechanisms with peripheral modulation.

Taking into account the variety of the biophysiologic aspect underlying in the FM aetiology, Müller and co-workers (47) propose that FM cannot be considered as a single clinical entity, but rather a syndrome with multiple causes, therefore the

term fibromyalgia syndrome must always be applied. They have established different subgroups of FM syndrome: in principle, FM is categorized as primary or secondary FM. While studies of primary FM do not reveal any definitive organic factor triggering the syndrome, in secondary FM underlying diseases, such as inflammatory rheumatic processes, are frequently diagnosed. In turn, primary FM is divided into four groups (47):

1. FM with extreme sensitivity to pain but no associated psychiatric problems (21): FM might be caused by mild inflammatory diseases, since elevated cytokine concentrations have been found in serum (47, 48) and supernatants of blood monocytes (79) in FM patients.
2. FM and co-morbid, pain-related depression: FM and depression coexist. The alleviation of pain with antidepressants is taken as an indication that depression constitutes the triggering factor in the second and third groups.
3. Depression with concomitant FM syndrome.
4. FM due to somatisation: patients have an ongoing history of psychological strain, and possibly an abnormal affective modulation of the disorder leads to development of pain symptoms (45).

There are some other classifications to define subgroups of FM patients. However, they are very similar to the previous one, and they all state that chronic inflammation is one of the causes of the pain symptoms in FM, even in the absence of other inflammatory diseases. Pain sensation has an important physiological role in inflammation by alerting the organism to injuries/abnormalities of the damaged tissue. Based on the hypothesis that the origin of all pain is inflammation and the inflammatory response (52), special attention has been focused on the inflammatory hypothesis of FM (53). Since 1988 it has been known that increased levels of the inflammatory transmitter Substance P (SP) are found in the spinal fluid of FM patients (69; 74). After excitation of pain receptors in the periphery, nerve impulses arriving in the spinal cord stimulate the release of SP, which is closely associated with pain. In recent years, inflammatory cytokines have also been suggested to be involved in the FM syndrome. This hypothesis was based on the assumption that IL-6 and IL-8, release of which is stimulated by SP, may have an important role in FM symptoms, since IL-8 promotes sympathetic pain and IL-6 is associated with hypersensitivity to pain. Besides generation of pain and hyperalgesia in inflammatory conditions, pro-inflammatory cytokines, such as IL-1β, TNFα, IL-8, or inflammation associated cytokines like IL-6 may also induce other characteristic symptoms of FM syndrome, such as stress, fatigue, sleep disorders and depression symptoms; and in turn the anti-inflammatory cytokine IL-10 (which is induced by IL-6 and decreases TNFα production) may block pain (53, 77-79). Taken together, the profile of pro- and anti- inflammatory cytokines in FM patients has recently attracted considerable attention. Several studies have focused on circulating pro-inflammatory cytokines as possible "inflammatory markers" in FM patients. With some exceptions, normal circulating concentrations of IFNγ (79), TNFα (4, 79), IL-1β (27, 79), IL-2 (79) and IL-6 (27, 28, 35, 72, 80) have been reported in FM patients. However, most studies found increased serum levels of IL-8 (6, 27, 28, 79, 80). On the other hand, results for IL-10, an anti-inflammatory cytokine which is an antagonist of TNFα and IL-1β, appear to

be controversial, since both increases (6) or no significant changes (6, 72, 79) have been reported in serum IL-10 concentration in FM patients compared with healthy controls. Normal production of IL-1β, TNFα and IL-10 by peripheral mononuclear cells (PBMC) has been reported in FM patients (4, 79), but increased levels of IL-6 have been found in the supernatants of cultured PBMC from FM patients (79). Furthermore, higher circulating levels of IL-1 receptor antagonist (IL-1ra) (79) and IL-6 receptor (IL-6R) have been reported in FM patients than in healthy volunteers, but IL-6R was only found in FM patients with associated depression (35). In addition, both the serum concentration and production of IL-6, IL-8 and IL-1ra increased with the symptoms time-frame, and since IL-8 promotes sympathetic pain and IL-6 induces hyperalgesia, fatigue and depression, the authors suggested that these cytokines play a modulating role in FM syndrome (78, 79).

The sources of inflammation triggering the FM syndrome remain to be elucidated. It has been proposed that FM is due to neurogenic inflammatory response to allergens, infectious agents, chemicals or emotional stress (53). Bearing in mind that mechanical abnormalities of the cervical or lumbar spine are included among the possible aetiology of FM, an open question may be that neck or lumbar disorders can be a source of inflammation, and thus may be one cause of FM rather than a consequence. As reviewed by Omoigui (53) back and neck pain results from injury to the muscle, disk nerve or ligaments, with subsequent inflammation. Herniation of disk tissue produces a profound inflammatory reaction releasing TNFα and subsequently other inflammatory mediators, such as nitric oxide, prostaglandin E2 and also IL-6. However, circulating concentrations of these inflammatory mediators are not clearly increased in FM patients, and further studies on this are needed.

Frequently, FM and depression present symptomatic similarities. If FM and depression coexist, the question is whether depression must be regarded as an associated affection or whether it is the actual cause of the chronic pain disorders (47). Several studies indicate that major depression is accompanied by the activation of inflammatory response, with an increased production of pro-inflammatory cytokines (35, 77). In fact, many FM patients are treated with antidepressant agents, which may suppress the production of pro-inflammatory cytokines, such as IFNγ, and stimulate the production of anti-inflammatory cytokine IL-10 (34, 35). However, it is known that FM and depression do not always coexist (classified as Group I by Müller et al, 2007; 47); even different profiles of pro- inflammatory and anti-inflammatory cytokines between patients with or without diagnosis of depression have been reported. For example, Müller and co-workers (47) reported higher circulating levels of pro-inflammatory (IL-1α and TNFα) and anti-inflammarory (IL-10) cytokines in FM patients without signs of depression. However, Bazzichi and co-workers (6) found increased levels of IL-10 and IL-1 in patients with depression, but they did not find differences in the circulating level of IL-8 between FM patients with or without depression, both of them strongly higher compared with the levels found in control healthy people. This finding strongly suggested that IL-8 may be an “inflammatory marker” for FM syndrome regardless of associated depression.

Pro-inflammatory cytokines and IL-6 can also alter the sympathetic nervous system (SNS) and the hypothalamic-pituitary-adrenal (HPA) axis activity during

inflammation and/or stress conditions, perhaps with special importance in FM syndrome (73, 77) which has been proposed to be included in the spectrum of "stress-related illnesses" (13). It is shown in several studies that FM are part of a spectrum of conditions that include an inflammatory disorder accompanied by changes in the neuroendocrine-immune system (4, 47, 77). Neuroendocrine abnormalities in FM include altered HPA axis activity with integrated mildly elevated cortisol secretion, altered circadian cortisol release, an exaggerated adrenocorticotrophic hormone (ACTH) response to corticotrophin-releasing hormone (CRH) and a paradoxical fall in cortisol levels after exercise (13, 14, 26, 73, 75). Increased basal levels of noradrenaline (NA) have also been observed among female FM patients compared with age-matched female controls reflecting an abnormal regulation of the sympathetic nervous system in FM patients (73). Taking into account that NA and glucocorticoids modulate the production of inflammatory cytokines by immune cells and, in turn, inflammatory cytokines modulate the release of cortisol and NA, designing therapeutic strategies (such as habitual exercise programs and others) focused on the regulation of FM syndrome-associated disorders in the sympathetic and HPA axis activity, as well as in the pro/anti-inflammatory cytokine balance, could be useful in FM patients.

Currently, there is no therapy formally approved by the European Agency for the Evaluation of Medicinal Products or by the US Food and Drugs Administration for treatment of the pain of FM or the syndrome as a whole (42). Nowadays, treatment of the FM syndrome includes both pharmacologic and non-pharmacologic therapies. Pharmacologic therapies are based on antidepressants, analgesics, muscle relaxants and antiepileptics. Non-pharmacologic therapies include exercise, massage, cognitive behavioural therapy etc (42). Today non-pharmacological therapies, such as exercise, are recommended in the management of FM symptoms together with pharmacological treatment. In fact, it has been shown that aerobic exercise training improves physical function, psychological distress and other quality of life parameters in FM patients. However, non-pharmacological therapies cannot replace pharmacological ones yet.

Exercise co-therapy in patients with FM syndrome:

Compared with healthy people, FM patients have fatigue and stiffness and display reduced physical performance capacity. They have difficulties in performing everyday activities such as walking, working with the arms and prolonged sitting and standing. Non-pharmacological treatment in FM patients (such as exercise) has focused on improving health, function and independence (36, 37). Exercise training interventions for these patients aims to improve fitness (thereby reducing fatigue), and enhance muscle strength (thereby enabling the patient to more easily manage their daily life activity). They also experience that the pain threshold can be exceeded, which leads to a change in pain perception and in the pain threshold (64).

The first studies examining the effectiveness of exercise in the management of FM symptoms date from the late 1980s, and the studies suggested that exercise programs can reduce the pain (41). In the last years a large number of land-based exercise programs for this patient group have been published. Jones and colleagues (31) reviewed that the mean length for this training programs was 12 weeks, although they ranged from 4-24 weeks. The number of exercise sessions

ranged from 1-5 times per week, most commonly 2-3 times weekly, lasting from 15-180 minutes per session with the average being 60 minutes. Most of these exercise intervention programs comprised the three major modes of exercise: aerobic, strength and flexibility training, either alone or in combination (31). Aerobic training includes walking, calisthenics, cycling, dance or tai-chi. It has been shown that low or moderate intensity activities (1, 49, 71, 76) are more effective to improve FM symptoms than higher intensity activities (31). Thus, low- to moderate aerobic exercise increases overall well-being (by 7 points on a scale of 0 to 10), improves tender-point pain threshold (an increase of 0.23 kg/cm^2) and reduces pain rating (1.3 on a scale of 0 to 10). On the contrary, its effects on fatigue, depression and stiffness are uncertain (9). On the other hand, strength training programs include static or dynamic exercises. It has been reported that strengthening programs increase overall well-being (by 41 points on a scale of 0 to 100), reduces pain rating (by 49 points from 0 to 100) and improves tender-point status (9). It seems that both aerobic and strength training programs improve FM symptoms, but on the contrary, the benefits of flexibility training programs, which include static stretching of the major muscle groups, are still unclear (9). However, a recent study suggests that flexibility training combined with aerobic exercise can improve the symptom severity in FM patients (68).

Apart from land-based training programs, water-based exercises have been presented in recent studies as an effective therapeutic option in FM patients. In fact, pool exercise is another exercise modality in the therapy for sedentary patients suffering from pain. The temperature (commonly between 30 and 34 ºC), the viscosity and the buoyancy of water seems to reduce stiffness and pain, provides resistance in aerobic and stretching exercises and facilitates the performance of movements (36, 37). Pool exercise programs usually consist of aerobic, strength and flexibility exercises (18, 25, 30, 46), but some studies have also comprised proprioceptive exercise (29), deep water running (15), and balneotherapy (the treatment of disease by bathing that may involve hot or cold water and massage via moving water) (85). The duration of these programs ranged from 6-24 weeks, with 1-3 sessions per week lasting 30-60 minutes. It seems that the traditionally known utility of warm water alone is enough to improve some but not all symptoms of FM. In a recent study comparing the effects of pool exercise with balneotherapy, it was concluded that both groups improved in terms of pain, fatigue and stiffness, but only the exercise group shows improved distress, furthermore pool-based exercise had a longer-lasting effect on FM symptoms (3). In support of this, Mannerkorpi and colleagues (38) showed improvements in physical function, pain severity, social functioning, psychological distress, and quality of life in a group of patients who received a combination treatment of pool-based exercise and education for 6 months compared to a control group with no treatment. Furthermore, Jentoft and co-workers (30) compared the effects of pool-based and land-based exercise, and the authors concluded that pool-based exercise can also be effective in increasing physical capacity and may have some additional effects on the symptoms. In conclusion, pool exercise appears to improve physical capacity (measured by 6-minute walk test) and strength, symptom severity and distress in FM patients. These improvements are still present 6 months later. Moreover, water-based exercise could decrease injuries since movements in the water are more easily performed and the exercise load can be adjusted to each patient's limitations (36).

To summarize, there is strong evidence supporting the positive effects of habitual exercise training programs on FM symptoms, and on physical fitness and quality of life. In addition, most of the trials studying the beneficial effects of exercise training programs on FM syndrome found that these benefits (improved physical functioning, less pain and fatigue, self-efficacy for controlling symptoms, and well-being in general) are still present several months after finishing the program (64). It is also important to highlight that Pedersen and Saltin (64) emphasize that there are no contraindications for any form of physical training in FM patients.

Nevertheless, the mechanisms through which exercise exerts these effects remain unknown. However, it is known that exercise modulates the inflammatory response through neuroendocrine mechanisms. Since FM syndrome seems to be associated to a deregulation of the inflammatory response, and an unbalanced HPA axis and SNS activity, it could be plausible that exercise therapies could work at this level in FM patients.

Inflammation and Exercise: anti-inflammatory hypothesis of exercise

The cellular innate immune responses (carried out mainly by macrophages and neutrophils) together with cytokines (pro- and anti-inflammatory ones) and other inflammatory mediators (vasoactive amines and peptides, lipid mediators, proteolytic enzymes) are crucial in the inflammatory mechanisms. It is well known that both intense and moderate exercise activate the innate and/or inflammatory response in healthy sedentary men and women as well as in athletes in terms of phagocyte function (20, 56-58, 60) as well as in terms of "inflammatory markers" such as the release of inflammatory cytokines (22, 44, 51, 61, 70). Nevertheless, the physiological relevance of this activation could be different when exercise is performed by healthy people or by people with inflammatory diseases. It is known that while a controlled inflammatory response is beneficial for providing protection against infection, a deregulated inflammation (triggered for example by tissue stress and malfunction) can be detrimental, for example causing inflammatory diseases (43). In this way, it is still not really known which is the optimal level of exercise that improves, but does not impair or over-stimulate, a healthy immune function. Well-regulated stimulation can help to prevent infections, but exercise in inadequate doses might also exacerbate symptoms in patients with inflammatory or autoimmune diseases (17) specially in women who are more susceptible to these diseases (22, 23, 82) - as could be the case in FM patients. In addition, some of the exercise-induced changes in the innate/inflammatory response are mediated by "stress hormones". This is true for catecholamines but, under given circumstances, also for low doses of glucocorticoids (55, 59), both of which also may be contribute to the greater inflammatory status in women (23). Thus, in our opinion it is necessary to be sensible regarding relative intensity, regularity and duration when recommending exercise to patients, particularly women, with inflammatory or stress-related illnesses.

During a local inflammatory response TNFα, IL-1 and IL-6 are the main cytokines released (43, 70), followed by anti-inflammatory cytokines, such as IL-1ra and IL-10. A good balance of the pro-/anti-inflammatory cytokines is increasingly recognized to be important in the maintenance of health and in the development of immune-based diseases, including infections, autoimmune/inflammatory

and allergic diseases (16, 17). The first cytokines in the cytokine cascade during an inflammatory response are TNFα, IL-1β, IL-6, IL-1ra and sTNF-R. This response is accompanied by a systemic response known as the acute-phase response, which includes the production of a large number of hepatocyte-derived acute phase proteins, such as C-reactive protein (CRP) (66). Since 1983 (11), studies in healthy people (mainly athletes) have lead many researchers to support the concept that the same cytokine cascade as started by TNFα and IL-1 during sepsis might also take its course in exercise-induced cytokine changes, because these conditions showed some analogy. Subsequent studies demonstrated that anti-inflammatory cytokines, such as IL-1ra and IL-10, are also secreted into the circulation after endurance exercise (70). Pedersen and co-workers reported that strenuous exercise is accompanied by an increase in circulating pro-inflammatory and inflammation responsive cytokines (TNFα, IL-1, IL-6, IL-1ra, TNF-R, IL-10, IL-8 and macrophage inflammatory protein-1) being to some extent similar to sepsis and trauma responses (62, 63). However, today it seems to be accepted that the cytokine response to exercise differs from that elicited by sepsis. Based on many studies (mainly in athletes) which showed that circulating concentrations of TNFα and IL-1β are either unchanged following exercise, or exhibit relatively small increments (70), Petersen and Pedersen (66) proposed that IL-6 is the first and most marked cytokine present in the circulation during exercise, followed by an increase in IL-1ra and IL-10 (66). They also presented evidence that IL-6 is produced by working muscle itself in the absence of injury or signs of inflammation. They hold that IL-6 acts as an anti-inflammatory cytokine, since IL-6 exerts inhibitory effects on TNFα and IL-1 production (for example by LPS-stimulated monocytes). In addition, IL-6 also stimulates the production of IL-1ra and IL-10, and it is one of the primary inducers of CRP, which can also have anti-inflammatory properties, all of this supporting the anti-inflammatory effects of exercise (66). All this does, however, not exclude, that in case of strenuous, exhaustive exercise which causes sizable tissue injury, the inflammation induced cytokine cascade analogous to sepsis might also be operable. Indeed, this is highly likely, since prolonged or biphasic kinetics of IL-6 have been shown in such conditions.

In a systematic review based on a search of literature on the effect of exercise on CRP and other inflammatory markers, such as cytokines, Kasapis and Thompson (32) conclude that exercise produces a short-term inflammatory response, whereas both cross-sectional comparisons and longitudinal exercise training studies demonstrated a long-term anti-inflammatory effect, also suggesting that anti-inflammatory responses may contribute to the beneficial effects of habitual physical activity. The authors emphasise that this anti-inflammatory response of habitual exercise is mainly highlighted by changes in the circulating concentration of CRP, supporting the idea that habitual exercise training reduces CRP levels by altering the inflammatory process (32).

Although it has been generally accepted that some of the beneficial effects of exercise involve the stimulation of the innate and/or inflammatory response preventing the organism against infection, today many researchers support the concept that the beneficial effects of exercise are mainly mediated by its anti-inflammatory properties. This fact becomes important in order to use exercise as a "therapeutic help" in people with inflammatory disorders. However, most of the studies have been performed in athletes following high endurance or intense train-

ing. So, the main question now is whether these responses to exercise will be similar in sedentary people who start performing regular exercise; and, above all in patients suffering from inflammatory diseases (such as FM patients) who perform endurance training as adjuvant therapy.

In 1989, it was already suggested that a subgroup of FM patients could have a low-grade inflammatory response (12). Chronic low-grade systemic inflammation has been introduced as a term for conditions with a typically two- to threefold increase in the systemic concentration of TNFα, IL-1β, IL-6, IL-1ra, sTNF-R, and CRP (66). Increased circulating concentrations of IL-8, IL-1ra, IL-10 and CRP (as well as IL-6 produced by monocytes) have been reported in patients diagnosed with FM, supporting the hypothesis that in the FM syndrome a low-grade systemic inflammation is underlying. Several reports also support that regular exercise offers protection against chronic diseases associated with chronic low-grade systemic inflammation as reflected by increased levels of inflammatory cytokines. Bearing this in mind, and taking into account that exercise improves the physical conditions and quality of life, and may also contribute to reduce the pain in FM patients, it is plausible to think that the beneficial effects of habitual exercise training in FM patients are also mediated through their anti-inflammatory effects. However, it is surprising that studies conducted in this way are rare. Very few articles on fibromyalgia, exercise and inflammation are identified via a PubMed search: for example, a PubMed search using the term fibromyalgia and exercise (and/or physical activity) together with inflammation came up with no more than 10 published articles, six of them reviews and only two in some way related to the anti-inflammatory effects of exercise. When the term cytokine (or particularly IL-1, IL-2, IL-4, IL-6, IL-8, IL-10, TNF or IFN) or CRP were introduced together with fibromyalgia and exercise, no related articles were found. This situation and the knowledge that 1) FM is an inflammatory disorder accompanied by changes in the neuroendocrine immune system; 2) exercise (above all pool-aquatic habitual exercise) show benefits in FM patients; and 3) there is a substantial lack of studies related to the anti-inflammatory effects of exercise as one of the mechanisms underlying the benefits of exercise on this syndrome, prompted us to carry out a pilot investigation that could serve as a first step to deal with new research in the use of exercise as "therapeutic help" in the FM syndrome.

POOL-AQUATIC HABITUAL EXERCISE AS "ANTI-INFLAMMATORY THERAPY" FOR FM PATIENTS

METHODS

Study design and FM patients:
14 women (age range 30-60 years) diagnosed with FM by a rheumatologist (they fulfilled the ACR criteria for FM) were enrolled in the study. FM patients belonged to the Fibromyalgia Association of D. Benito (Badajoz, Spain). They were requested to fill out a questionnaire about lifestyle (diet, habits etc), medication and other previous or current concomitant illnesses. All procedures were carried out with written consent of the subjects. FM patients had already undergone

all conventional forms of biomedical treatment. Exclusion criteria consisted of tumor illness (diagnosis from history), infection, cardiopulmonary, vascular or other internal medical conditions, or use of oral or local corticosteroids or anticytokine therapy that could influence the level of cytokines. All of the participants were advised not to do any other regular physical activity outside the exercise program. FM patients were then classified according the classification criteria from Müller and colleagues (47). Some of them (n=10) were classified as primary fibromyalgia patients (with no definitive organic factor triggering the syndrome) and the rest (n=4) as secondary fibromyalgia patients (with other diagnosed underlying disease, such as inflammatory rheumatic processes). Primary fibromyalgia patients belonged to the Group I of the classification (fibromyalgia with sensitivity to pain but no diagnosed of depression or other relevant psychiatric disorder).

13 healthy women (who had no pain disorders or infectious illness at the time of blood sampling) served as the matched-control group (age range 28-55 years). Control women had to be physically inactive having undertaken no exercise program during the previous 12 months. The same exclusion criteria as for FM volunteers were applied for the control group.

Peripheral venous blood samples were drawn by antecubital vein puncture. The sampling was carried out before (basal status) and two days after finishing the exercise program (to avoid the effect of acute exercise) with the participants fasting and at rest for at least 1 h before.

The investigation was approved by the Ethical Committee of the University of Extremadura (Spain) according to the guidelines of the European Community Council Directives and the Declaration of Helsinki.

Habitual exercise training program:

FM patients took part in an aquatic fitness program over a period of four months (October- January). All of them completed the program with a minimum of attendance of 90% of the sessions. The training program consisted of three weekly 60-minute sessions. These characteristics are the most frequent in other similar programs (31). The exercise sessions were carried out in an indoor swimming pool (depth 1.1 m, mean water temperature 32 ± 1º C), and were performed as follows: a) stretching exercises out of the water (5 min); b) aerobic warm-up in the water: slow walking and easy movement in different directions (5 min); c) passive stretching of the main muscle groups in the water: elbow, trunk, and knee extensors and flexors (5 min); d) aerobic aquatic choreography: fast walking and more difficult movements (25 min); e) strength exercises involving the main muscle groups of the upper limbs (with and without overload): chest anterior and posterior muscles, elbow extensors and flexors, forearm supinator and pronator muscles (15 min); f) cool down: breathing and passive stretching exercises (10 min). The program was designed in training standards of the American College of Sports Medicine (ACSM) recommendations (1998). Parts a, b, c, and f were performed at low exercise intensity (40-50% of maximal heart rate). Part d was performed at low-moderate intensity (50-60% of maximal heart rate) at the beginning of the program, and the exercise intensity increased at the end of the aquatic training program (65-75% of maximal heart rate). This intensity is recommended for these kind of studies (39).

Noradrenaline (NA) determination: 40μl of a solution containing 900 mg of EGTA and 700 mg of glutathione in 100 ml of 0.1 M NaOH was added to 2 ml of each blood sample before separation of the plasma. Plasma NA concentration was determined by ELISA (Labor Diagnostika Nord GmbH & Co. KG; LDN), with a sensitivity (limit of detection) of 5 pg/ml and a tested normal range in healthy people of 120-680 pg/ml.
Cortisol determination: serum cortisol concentration was determined by electrochemiluminescence immunoassay using an automatic analyzer (Roche Elecsys). The tested normal range in healthy people with this method is 6-23μg/dl.
C-reactive protein (CRP) determination: serum concentration of CRP was determined by ELISA (CycLex Co. Ltd.), with a sensitivity (limit of detection) of 29 pg/ml and a tested normal range in healthy people of less than 0.5 μg/ml (60% of healthy people) and less than 1μg/ml (80% of healthy people).
Cytokine determination: serum concentrations of IL-1β, IL-2, IFN-γ, TNF-α, IL-8, (pro-inflammatory cytokines), IL-6, and IL-10 and IL-4 (anti-inflammatory cytokines) were determined by ELISA (Diaclone). Sensitivity and normal values in healthy people for each cytokine are indicated in Table 1.
Physical fitness of FM patients
Aerobic fitness was assessed by the 6-min walk test (10), which is a reliable and valid measure in FM patients (33). Subjects were instructed to walk as far and fast as they could during 6 min. Walk distance was recorded by a researcher.
Health-related quality of life (HRQoL):
Perceived HRQoL was assessed by self-administration of the SF-36 Health Survey questionnaire (81) and Fibromyalgia Impact Questionnaire (8). The SF-36 questionnaire has been translated to Spanish (2), and validated by Ayuso-Mateos and colleagues (5). The SF-36 survey questionnaire assessed eight domains of HRQoL: physical function, role-physical, bodily pain, general health, vitality, social function, role-emotional, and mental health. These domains were quantified from 0 to 100, where 0 corresponds to "very poor health" and 100 to "very good health". The FIQ, developed and validated by Burckhardt and colleagues (8), is a specific health questionnaire which evaluates current health status in patients with FM, and it is today one of the most commonly used tools for clinical researchers in patients with FM. It measures physical function, work, well being, and it contains visual analogue scales (VAS) for pain, sleep, fatigue, stiffness, anxiety and depression. A total score may be obtained after normalization of some items (between 0-10) and summing with all VAS. Total score ranges between 0-80 (without job items), where a higher score indicates a negative impact. The used Spanish version of FIQ is a reliable, valid and responsive to changes questionnaire for measuring health status and assessing physical function in female patients with FM (67).
Statistical analysis:
Values are given as mean ± SEM. The variables were normally distributed (as determined by a normality test). To evaluate the effects of the exercise program, a statistical comparison was conducted between the pre- and post-test results in FM patients using the paired t-test for the normally distributed variables. Comparisons of the results between FM patients and healthy controls were carried out by using the unpaired t-test. The significance level was set at $p<0.05$. All tests were completed using SPSS (version 15.0).

Table 1. Circulating concentrations of "inflammatory markers" before (Pre-) and following (Post-) 4 months of the aquatic exercise program.

		WHOLE FM GROUP (n=14)		PRIMARY FM GROUP (n=10)		SECONDARY FM GROUP (n=4)	
	HW (n=13)	Pre-	Post-	Pre-	Post-	Pre-	Post-
IFN-γ (<5 pg/ml)	<5	39 ± 24 *	26 ± 16 +	23 ± 14 *	12 ± 8 +	80 ± 80	62 ± 53
TNF-α (<8 pg/ml)	<8	11 ± 11	9 ± 9	<8	<8	43 ± 43	35 ± 34
IL-1β (<7 pg/ml)	<7	189 ± 151	61 ± 47	<7	<7	661 ± 493	213 ± 149
IL-2 (<7 pg/ml)	<7	<7	<7	<7	<7	<7	<7
IL-6 (<2 pg/ml)	<2	<2	<2	<2	<2	<2	<2
IL-4 (<0,7 pg/ml)	<0,7	2±2	1±1	<0,7	<0,7	7 ± 7	4 ± 4
IL-10 (<8 pg/ml)	<8	9 ± 9	<8	<8	<8	28 ± 28	16 ± 15
IL-8 (<29 pg/ml)	<29	157 ± 35 ***	<29 +++	191 ± 46 ***	<29 +++	74 ± 21 *Δ	29 ± 29
CRP (<0,5 µg/ml)	0.2 ± 0.1	6 ± 2 *	3 ± 2 *+	5 ± 2 *	3 ± 1 *+	7 ± 3 *	3 ± 2 *+

Results represent the mean concentration (in pg/ml for cytokine and µg/ml for CRP) ± SEM of a set of independent experiments performed in duplicate (one experiment per volunteer). The HW column shows the mean values found in the age-matched control group (healthy women). The expected range of concentrations in healthy people as indicated by the commercial kits is given in parentheses. * P<0.05; *** p<0.001 compared with HW values (unpaired *t*-test); +p<0.05; +++ p<0.001 compared with Pre- values (paired *t*-test); Δ p<0.05 compared with the values found in the primary FM group (unpaired *t*-test).

RESULTS AND DISCUSSION

The study was carried out on women who had a diagnosis of FM by a rheumatologist (all of them belonging to a FM Association). All volunteers (n=14) were accepted and enrolled in the investigation. Attending to a strict diagnostic by a medical doctor, they were divided into two groups according to the classification proposed by Müller and co-workers (47): primary (n=10) and secondary (n=4) FM. Women classified in the primary FM group, presenting high sensitivity to pain in absence of relevant psychiatric co-diagnoses were considered to be highly suitable for evaluating inflammatory and/or stress markers that may trigger the clinical picture of FM syndrome. The control group consisted of age-matched healthy women (n=13). Pool-aquatic exercise in warm water has been presented as one of the interventions that can induce more benefits in FM patients. The duration (4 months) and intensity of the exercise program was in line with those that have reported improvements in the physical fitness and quality of life (31, 36, 37).

Circulating concentrations of the "inflammatory markers" found in FM volunteers in their basal state (Table 1), suggest the presence of potential inflammation in our group of FM patients. Significantly higher serum concentrations of IL-8 and CRP were clearly found in the whole FM group (and also in the primary and secondary FM groups separately) than in the control group of HW. Most of the few previous studies carried out by other groups have also found increased serum levels of IL-8 in FM patients (6, 28, 79, 80), pointing to IL-8 as a good inflammatory marker in the diagnosis for FM syndrome. In addition, although the serum concentration of IL-8 was significantly lower in the secondary FM group than in the primary FM group, it was also higher compared to HW. This confirms that IL-8 could be a plausible inflammatory marker in the diagnosis of FM syndrome, irrespective of the differences in the aetiology of the illness. On the other hand, previous studies have shown that primary FM patients present higher serum CRP concentrations compared to the expected normal range in healthy people (6, 47). However, to the best of our knowledge, the present investigation is the first study which compares circulating CRP concentrations between FM patients and age-matched control healthy women. Our results clearly suggest that CRP levels can also help in the FM diagnosis, irrespective of primary or secondary. It is important to note that in our study all FM patients showed higher circulating levels of IL-8 and CRP than HW (as well as higher than the expected range for healthy people). In addition, the serum concentration of IFNγ (mean values) was higher in the whole and primary group of FM patients, in accordance with Peter and Wallace (65). In line with other studies (27, 28, 79), no significant differences between FM patients and HW were determined in IL-1β, IL-6, IL-10, and TNFα. Pro-inflammatory cytokines, such as IL-8, can promote pain and can also alter the SNS and HPA axis, with potential importance in FM syndrome (6, 73, 77). Altered cortisol release and increased basal levels of NA have been reported among female FM patients compared with age-matched female controls (73), in accordance with the results presented here (Table 2), reflecting an abnormal regulation of the neuroimmunoendocrine interactions in this pathology. Nevertheless it is difficult to know whether altered HPA axis and SNS activities are the cause or the consequence of the high inflammatory status in FM syndrome. It is possible to

Table 2. Circulating concentrations of NA and Cortisol before (Pre-) and following (Post-) 4 months of the aquatic exercise program.

		WHOLE FM GROUP (n=14)		PRIMARY FM GROUP (n=10)		SECONDARY FM GROUP (n=4)	
	HW (n=13)	Pre-	Post-	Pre-	Post-	Pre-	Post-
NA (120-680 pg/ml)	137 ± 28	499 ± 72 ***	724 ± 121 ***+	456 ± 57 ***	623 ± 116 ***+	607 ± 99 ***	977 ± 207 ***
Cortisol (6-23 µg/dl)	9.4 ± 2.3	11.5 ± 1.4	8.7 ± 1.0 +	11.0 ± 1.8 *	8.7 ± 1.4 +	10 ± 0.3	8.7 ± 0.1

Results represent the mean concentration (in pg/ml for NA and µg/dl for Cortisol) ± SEM of a set of independent experiments performed in duplicate (one experiment per volunteer). HW column shows the mean values found in age-matched control healthy women group. The expected range of concentrations in healthy people are shown in parentheses. * P<0.05; *** p<0.001 compared with HW values (unpaired *t*-test); +p<0.05 compared with Pre- values (paired *t*-test).

think that the high levels in cortisol and NA in FM women could be a physiological adaptation in these patients to restore the altered homeostasis by their greater inflammatory status. The activation of the stress system stimulates a negative feedback mechanism which protects the organism from an "overshoot" of pro-inflammatory cytokines which could end in tissues damage. Conversely, the increased levels of cortisol and NA would also be interpreted as being the cause of increased levels of pro-inflammatory cytokines, since it has also been reported that under certain conditions and in certain local response stress hormones actually can stimulate regional immune responses through induction of pro-inflammatory cytokines such as IL-8 (17).

It seems clear that an unbalanced neuroimmunendocrine status is closely associated with the FM syndrome. Strategies, such as exercise, directed to regulate the activity of HPA axis and SNS, as well as the inflammatory status in FM patients might therefore improve their quality of life. As recently reviewed by Woods and co-workers (84), and Pedersen and Saltin (64), most longitudinal studies have shown that exercise training has an anti-inflammatory effect for people suffering from chronic diseases, such as heart disease, metabolic syndrome or rheumatoid arthritis. The question now was to know whether the "anti-inflammatory effects" can be one of the mechanisms involved in the exercise-induced benefits in FM patients. This is the first study to this respect, and the results have been positively surprising, since the three inflam-

Table 3. Summary results of the HRQoL scores (SF-36 and FIQ) before (Pre-) and following (Post-) 4 months of the aquatic exercise program.

	WHOLE FM GROUP (n=14)		PRIMARY FM GROUP (n=10)		SECONDARY FM GROUP (n=4)	
	Pre-	Post-	Pre-	Post-	Pre-	Post-
Physical function	53 ± 4	52 ± 5	54 ± 5	58 ± 5	50 ± 5	35 ± 3 **+**
Role-physical	7 ± 7	6 ± 3	6 ± 4	3 ± 3	10 ±10	17± 8
Bodily pain	31 ± 3	31 ± 2	29 ± 4	33 ± 3 **+**	31 ± 6	28 ± 3
General health	34 ± 6	32 ± 6	28 ± 5	31 ± 7 **+**	39 ± 21	35 ± 5
Vitality	28 ± 3	28 ± 3	26 ± 2	27 ± 3	38 ± 13	31 ± 11
Social function	49 ± 6	43 ± 5	55 ± 5	46 ± 7	17 ± 11	33 ± 4 **+**
Role-emotional	30 ± 13	22 ±8	25 ± 12	25 ± 9	50 ± 28	11 ± 8
Mental health	44 ± 4	48 ± 4	45 ± 5	47 ± 5	42 ± 6	51 ± 7 **+**
Total FIQ	57±3	56±4	58±4	53±5	55±7	68±5

Results represent the mean value ± SEM of the scores determined in each volunteer from each FM group. **+** $p<0.05$ compared with Pre- values (paired *t*-test)

Table 4. Summary results of the 6-min walk test before (Pre-) and following (Post-) 4 months of the aquatic exercise program.

	Pre-	Post-
Whole FM group (n=14)	496 ± 13	522 ± 16 +
Primary FM group (n=10)	508 ± 15	537 ± 20 +
Secondary FM group (n=4)	460 ± 20	486 ± 14 +

Results represent the mean value (in metres) ± SEM of the distance walked by the volunteers from each FM group. $p<0.05$ compared with Pre- values (paired *t*-test)

matory markers (IL-8, CRP, and IFNγ) which, in FM patients, were clearly and significantly elevated above the range of healthy controls, decreased significantly after the exercise training program. This behaviour was found in the all patients for CRP and IL-8, and also for IFNγ in those who presented elevated basal levels of this cytokine (40% in primary FM group and one patient from the secondary FM group). This anti-inflammatory effect of the aquatic exercise program was paralleled by a significant decrease in the circulating cortisol concentration and a significant increase in NA (mainly in the primary FM group). A paradoxical fall in the circulating concentrations of cortisol after exercise had been previously described in FM patients (73), suggesting that a decrease in the concentration of pro-inflammatory cytokines reduces the activation of HPA axis. The increase in the circulating levels of NA has also been reported after moderate exercise in sedentary healthy women, which may contribute to improve their innate immune mechanisms and their circulating balance of pro-/anti- inflammatory response (22, 59), but unfortunately there was no information given on sedentary women with FM as compared with healthy controls. Nevertheless, the present results on women with FM also agree with the finding that NA can reduce the IL-8 levels in specific conditions such as inflammatory diseases (17), and this decrease in IL-8 levels may contribute to improve the bodily pain, as we have also found in this study in the primary FM group. The anti-inflammatory effect of exercise on the FM patients is also corroborated by an important decrease in their circulating CRP concentration after 4 months of the aquatic exercise program. Although to the best of our knowledge no previous studies with this focus have been performed in women with FM, several investigations have reported that regular exercise training induces a reduction of circulating CRP concentration in healthy sportspeople (19, 40) and in patients with coronary disease (24). Cross-sectional studies have also demonstrated an inverse relationship between regular physical activity and the serum concentration in CRP in both healthy athletes and sedentary people (32), reflecting the beneficial anti-inflammatory effects of habitual exercise. Although hepatic CRP production is generally stimulated by IL-6, and to a lesser extent by IL-1 and TNFα, the exercise-induced decrease in CRP in our investigation on FM patients occurred irrespective of any significant changes in the circulating levels of these cytokines.

Finally, the benefits of exercise training on the quality of life of primary FM patients have been also confirmed in this investigation (Table 3), since after 4 months of the pool-aquatic habitual exercise an improvement in several scores relative to the health-related quality of life was observed, with significant differences in the scores for bodily pain and general health of the SF-36 questionnaire. On the contrary, and probably as a consequence of additional underlying diseases,

in the secondary FM patients, an improvement was only observed in social function and mental health. Due to these different and partly opposite trends in the two groups, no significant differences induced by the exercise program were found when evaluating the whole group of FM patients. The different behaviour between primary and secondary FM patients in the subjective perception of their quality of life was also found after analyzing the total FIQ scores (indicative of the impact of FM in the quality of life), since post-exercise FIQ scores improved after the exercise program in the primary group but got worse in the secondary one. It is possible that the exercise was too intense for the secondary group of patients. On the other hand, as expected, the whole group of FM patients improved their aerobic capacity measured by the 6-min walk test (Table 4). In general, our results confirm that habitual aquatic exercise can improve the physical fitness and well-being in FM patients, above all when they do not have other underlying illnesses.

SUMMARY AND FUTURE DIRECTIONS

It is now well known that physically active people are at a lower risk of illnesses, and the accumulated evidence shows that habitual exercise is an effective means of preventing or delaying chronic diseases in healthy people. Thus, today, exercise is recommended in the treatment and prevention of a large number of diseases. Based on the hypothesis of the anti-inflammatory effects of exercise, habitual training is especially considered as a good therapeutic help for inflammatory pathologies. Although the aetiology of FM remains elusive, the current hypothesis suggests that inflammatory disorders accompanied by changes in the neuro-immune-endocrine system are underlying in this syndrome. As a consequence of the widespread musculoskeletal pain, stiffness and fatigue, women suffering from FM display reduced physical performance capacity and they have more difficulty in performing everyday physical activities compared with healthy women. Habitual exercise programs are the main non-pharmacological interventions for these patients, being an effective tool for reducing pain, stiffness and fatigue, improving their quality of life, function and independence. However, the mechanisms underlying the benefits of exercise in the FM syndrome remain unknown. Bearing this in mind, the objective of the investigation presented in this article was to establish if the benefits of exercise in FM patients may be mediated via anti-inflammatory effects. The main conclusion of the study is that primary FM patients (who do not have any other diagnosed inflammatory disease triggering the syndrome) present a neuro-immune-endocrine deregulation (reflected by high circulating levels of the pro-inflammatory markers IL-8, IFNγ, and CRP, as well as by higher levels of cortisol and NA compared with healthy women) which clearly improved after 4 months of pool-aquatic habitual exercise in warm water. In general, the exercise program also improved the pain and the health-related quality of life in this group of FM diagnosed patients. These results confirm the high "inflammatory status" in FM women, and further confirm that the benefits of exercise may well be mediated, at least in part, by its anti-inflammatory effects. A similar behaviour was observed in the secondary group of FM patients (who have been also diagnosed with other underlying pathologies) with respect to stress and inflammatory markers, but they did not improve pain and the health-related quality of life in general.

This reflects the importance to take into account the biophysiology in each FM patient when exercise would be applied as therapeutic help in this syndrome, as well as for the interpretations of the results in this line of research.

Woods and co-workers reviewed (84) the mechanisms responsible for the observed anti-inflammatory effects of regular exercise, and they conclude that more investigations are needed to confirm or reject the anti-inflammatory hypothesis of exercise that allow better applications of exercise therapy in the treatment of inflammation-associated diseases. We agree with them, moreover taking into account that most of the studies on the anti-inflammatory effects of exercise have been performed in healthy sportsmen. Even if we think it is highly likely, it is not formally proven whether an induced anti-inflammatory effect of exercise in healthy people, with an optimal neuroendocrine and inflammatory feedback, is good or not for an optimal regulation of homeostasis. It is at least possible that the anti-inflammatory effects of exercise are mainly or only positive for people with an unhealthy high inflammatory status, such as individuals suffering diseases associated with chronic inflammation. Although in our opinion the results observed in this investigation are enlightening and could be the starting point for further studies focused on the benefits of exercise in FM syndrome, there are still many questions to answer, moreover having in mind the fact that FM patients are usually sedentary women or non-physically active women who could present higher inflammatory response to exercise than men. Although no contraindications have been found for exercise in FM patients (64), further studies are necessary to define the duration and intensities of exercise programs in order to get anti-inflammatory responses that restore an optimal cytokine-HPA axis feedback circuit, and to avoid unhealthy pro-inflammatory responses, since alteration in the cytokine-glucocorticoid feedback circuit can itself provoke FM (7).

It will be also important to unify the criteria for grouping FM patients according to the published classifications (mainly primary and secondary FM patients), since FM patients underlying other pathologies that contribute to trigger the syndrome can encumber the interpretation of the results when FM patients are considered as a whole. Also, patients with severe depression should be considered as a separate group. Bearing in mind that most FM patients are women, and their inflammatory response to exercise can be different at different stages of the menstrual cycle (50), it would be also important to consider their pre- or postmenopause status, as well as the phase of their menstrual cycle. Up to now, we do not yet know whether exercise alone (without pharmacological interventions) improves the pain and the quality of life of patients. So, further research focused on the anti-inflammatory effects of exercise in the FM syndrome should be carried out in order to develop exercise training programs which could become a therapeutic intervention in the absence of pharmacological treatment.

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