Article

Nitric Oxide, Inflammation, Lipid Profile, and Cortisol in Normal- and Overweight Women With Fibromyalgia

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Abstract

Objectives: Research has identified many factors associated with fibromyalgia (FM), but findings have been inconsistent. This study aimed to investigate changes in levels of nitric oxide (NO), inflammatory markers, lipid profile, and cortisol in normal- and overweight patients with FM and controls. Since most patients with FM are overweight, we explored possible changes in these markers according to body mass index (BMI). **Methods:** This preliminary study was performed on serum samples of women with FM and age-matched controls, grouped according to their BMI: 12 normal-weight patients and 12 controls and 13 overweight patients and 8 controls. Ozone-based chemiluminescence assay was used to measure NO. Inflammatory mediators and cortisol were determined by immunoassay. Lipid profile was measured by a spectrophotometric procedure. Functional capacity was assessed by the fibromyalgia impact questionnaire (FIQ). **Results:** Normal-weight patients showed higher levels of C-reactive protein (CRP) and apolipoprotein B compared to controls (both $p < .05$). CRP, apolipoprotein B, and triglycerides were higher in overweight patients versus overweight controls (all $p < .05$) and in overweight versus normal-weight patients (CRP $p < .01$; apolipoprotein B, triglycerides $p < .05$). The other markers were unaffected. Apolipoprotein B ($r = .762$; $p < .05$) and NO ($r = -.921$; $p < .05$) levels correlated with FIQ score in normal-weight patients. CRP level correlated with FIQ ($r = .912$; $p < .05$) in overweight patients. **Conclusions:** CRP and apolipoprotein B, biomarkers linked to cardiovascular events, may be associated with FM-related dysfunction in normal- and overweight women with FM. Their increased levels in these patients may indicate an increased risk of cardiovascular disease.

Keywords

fibromyalgia, C-reactive protein, apolipoprotein B, overweight

Fibromyalgia (FM) is a syndrome that produces musculoskeletal pain and functional disability. The chronic widespread pain of FM can lead to physical and mental distress, decreased quality of life, loss of employment, and elevated health care costs. FM is related to a higher prevalence of overweight and obesity than that which occurs in the general population (Arranz, Canela, & Rafecas, 2012; Cordero et al., 2014; Neumann et al., 2008; Okifuji, Bradshaw, & Olson, 2009). In Spain, the estimated prevalence of FM is 4.2% in females and 0.2% in males (Mas, Carmona, Valverde, Ribas, & EPISER Study Group, 2008).

In spite of recent research, the etiology of FM remains unclear. Currently, it can take up to 5 years for a patient to receive a diagnosis of FM, making it a priority to find markers that help in the diagnosis (Brown et al., 2012). In a recent study, we found an imbalance between oxidants and antioxidants in women with FM (La Rubia, Rus, Molina, & Del Moral, 2013). In addition to oxidative stress, research has identified several conditions that are associated with this syndrome including (1) microcirculation abnormalities (Kasikcioglu, Dinler, & Berker, 2006; Katz, Greene, Ali, & Faridi, 2007), (2) inflammation (Bote, García, Hinchado, & Ortega, 2012; Menzies & Lyon, 2010), (3) alterations in the lipid profile (Gurer, Sendur, & Ay, 2006; Ozgocmen & Ardicoglu, 2000), and (4) neuroendocrine disturbances (Crofford et al., 2004; Gur, Cevik, Sarac, Colpan, & Em, 2004). These conditions are related to altered levels of nitric oxide (NO), cytokines, lipids, and cortisol, respectively.

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NO is a key molecule because it is implicated in pain pathways and in the regulation of inflammation, lipidaemia and the hypothalamic–pituitary–adrenal (HPA) axis (Esch, Stefano, Fricchione, & Benson, 2002; Kasikcioglu et al., 2006; Pall, 2007; Sadeghi-Hashjin, Abuhosseini, & Asri-Rezaei, 2009). It is an intercellular messenger that plays an important role in many biochemical processes such as vasodilatation, immune response, neurotransmission, and the modulation of nociception. Due to its action as a vasodilator, changes in the levels of NO may cause alterations in the microcirculation in tissues. Researchers have reported conflicting results regarding NO levels in patients suffering from FM (Akkus et al., 2009; Bradley et al., 2000; Kim, Kim, Lee, Park, & Choe, 2010; Ozgocmen et al., 2006). Moreover, some authors have proposed that an increased synthesis of NO may be involved in the onset of FM and pain (Larson, Giovengo, Russell, & Michalek, 2000; Pall, 2007), whereas others have suggested that decreased NO production in patients with FM may cause vasoconstriction in the muscles, leading to muscular fatigue (Kasikcioglu et al., 2006) and pain (Katz et al., 2007).

Inflammation is characterized by interplay between pro- and anti-inflammatory cytokines. Major proinflammatory cytokines include interleukin (IL)-1, -12, -18, tumor necrosis factor α (TNF-α), and γ-interferon (IFN-gamma). Some anti-inflammatory cytokines are IL-1 receptor antagonist, IFN-α, and IL-4, -10, -11, and -13. IL-6 can behave as both a pro- and an anti-inflammatory cytokine. NO can act as an anti-inflammatory molecule by inhibiting the activity of the transcription factor nuclear factor kb (Esch et al., 2002). On the other hand, C-reactive protein (CRP) is an acute-phase protein whose levels increase during inflammatory processes. The acute-phase response, mediated by the synthesis of acute-phase proteins, occurs as a result of a rise in the concentration of cytokines such as IL-6 in response to a wide range of acute and chronic inflammatory conditions. Although researchers have observed a dysregulation of the inflammatory response in FM, they have reported conflicting results regarding levels of inflammatory markers in these patients (Bote et al., 2012; Menzies & Lyon, 2010). Studies have also identified associations between cytokines such as TNF-α and IL-1, -6, and -8 and symptoms such as fatigue, pain, and stress response (Gur & Oktayoglu, 2008; Wallace et al., 2001).

Several studies have found an association between hyperlipidemia and FM, though their results have not been conclusive (Cordero et al., 2014; Gurer et al., 2006; Ozgocmen & Ardicoglu, 2000). NO has been related to the regulation of the lipid profile because the use of NO donors, practiced routinely in cardiovascular disease therapy, may decrease the risk of hypercholesterolemia and atherosclerosis (Sadeghi-Hashjin et al., 2009). Cytokines may also alter lipid metabolism (Feingold & Grunfeld, 1992).

Previous findings have also shown that inflammatory disorders accompanied by changes in the neuroendocrine-immune system are associated with FM (Bote et al., 2012). Psychological stress can affect the production of cytokines. In turn, cytokines influence the HPA axis, one of the principal systems of stress response in the body. The HPA axis is responsible for stimulating the release of stress hormones such as cortisol, epinephrine, and norepinephrine in response to stress. IL-1, IL-6, and TNF-α activate the axis, and IL-2 and interferon-α downregulate it (Thompson & Barkhuizen, 2003). NO may also influence the HPA axis by neutralizing norepinephrine activity (Esch et al., 2002). Though investigators have observed alterations in the HPA axis along with altered response to stress in FM (Crofford et al., 2004; Gur et al., 2004), presently there are conflicting data as to the activity of the HPA axis in these patients.

In view of these reported findings, it seems unlikely that FM is caused exclusively by the dysregulation of a single factor. The purpose of the present study was to investigate changes in serum levels of factors previously suggested to be associated with FM, including NO, inflammatory markers, lipid profile, and cortisol, in women with FM and healthy controls to identify a pattern of differentially expressed markers. Given the high prevalence of overweight in these patients, we examined the possible changes in the levels of the above-mentioned markers according to the body mass index (BMI) of the patients. For this purpose, we compared normal- and overweight women with FM to healthy women of similar weight. The results of this study represent an advance in the knowledge about FM and provide a basis for further research into this complex syndrome.

Method

Design

The present study was a correlational, cross-sectional, case–control study.

Subjects

The Ethics Committees of the University of Jaén and of the Ciudad de Jaén Hospital (Spain) approved the study according to the guidelines of the European Community Council Directives and the Declaration of Helsinki, and all participants provided written informed consent. Patient participants comprised 25 women diagnosed with FM who belonged to a local association focused on FM (AFIXA, Jaén, Spain). We recruited age- and BMI-matched healthy women from the University of Jaén (Spain) to serve as controls. Participants were divided into four groups according to their BMI and condition (patient or control): (1) 12 normal-weight patients with FM (BMI 18.50–24.9), (2) 12 normal-weight controls (BMI 18.50–24.9), (3) 13 overweight patients with FM (BMI 25–29.9), and (4) 8 overweight controls (BMI 25–29.9).

The criterion for inclusion in the FM group was that patients meet the 1990 American College of Rheumatology criteria for classification of primary FM. For this purpose, women with FM had to have been diagnosed by a rheumatologist of the Public Health System of Andalucía (Spain). Exclusion criteria for both groups included the presence of any other chronic disease (diabetes mellitus, hypertension, cancer, or ischemic heart disease) or pregnancy or lactation. None of the participants were

being treated with corticosteroids, lipid-lowering drugs, oestrogens, analgesics, or anti-inflammatory drugs, and women were only included if they had stopped using these medications at least 2 months before the study. All the participants were nonsmokers and had a sedentary lifestyle.

We obtained demographic and clinical data from patient interviews and questionnaires. The same practitioner carried out all the measurements and tests throughout the study. The questionnaires were completed immediately after the blood was drawn. In patients with FM, we used the fibromyalgia impact questionnaire (FIQ) to evaluate functional capacity in daily living activities and a visual analog scale (VAS; 10 cm) to score musculoskeletal pain. We assessed the physical (physical component summary [PCS-12]) and mental (mental component summary [MCS-12]) health status of patients and controls using the Short Form 12 Health Survey (SF-12) Health Survey, with total score ranging from 0 to 100 and lower values reflecting worse health status.

Blood Collection and Preparation of Blood Samples

We drew total blood samples by venipuncture from the antecubital vein into Ethylenediaminetetraacetic acid, (EDTA-free tubes) (Becton-Dickinson, UK), in the morning after a requested 12-hr fast. We collected blood at the same time of day for all participants to avoid the effects of daily variations in the level of NO and cortisol (Fatima et al., 2013; Kanabrocki et al., 2001). After blood collection, we allowed the tubes to stand at room temperature for 30 min until the blood clotted. We then centrifuged the blood at 3,500 rpm (Avanti J-30I; Beckman Coulter, California (CA), USA) for 5 min at 4°C to obtain serum samples. Lipid profile and cortisol level were measured in serum samples following blood collection. The remaining serum samples were kept at −80°C until their later use for determination of levels of NO and inflammatory markers.

NO Measurement

The reaction of NO with ozone results in the emission of light, and this light, emitted in proportion to the NO concentration, is the basis for the assay that we used in the present study, one of the most accurate NO assays available. NO production was indirectly quantified by measuring nitrate/nitrite and S-nitrose compounds (NOx) using an ozone chemiluminescence-based method. The thawed serum aliquots were deproteinized with NaOH 0.8 N and $ZnSO_4$ 16% solutions. The total amount of NOx was determined as previously described (Rus, del Moral, Molina, & Peinado, 2011) using the purge system of Sievers Instruments, model NOA 280i (GE Analytical Instruments, Colorado (CO), USA). NOx values were referred to the total protein concentration in the initial samples.

Determination of Inflammatory Markers

The IL-6 level was determined by a chemiluminescent immunoassay using the Access Immunoassay Systems (Beckman Coulter). Levels of IL-10 were measured by a chemiluminescent immunoenzymatic assay using an MLX™ luminometer (Dynex Technologies, Chantilly, VA). CRP was measured using an AU 5800 analyzer (Beckman Coulter). Normal laboratory values for CRP are 0.0–5.0 mg/L.

Cortisol Measurement

Cortisol level was determined in serum samples by a fluorescence polarization immunoassay using an AxSYM analyzer (Abbott Laboratories, Illinois (IL), USA). Normal laboratory values for cortisol are 6.0–22.6 μg/dL.

Determination of Lipid Profile

Serum lipid profile (total cholesterol, high-density lipoprotein [HDL]-cholesterol, triglycerides, apolipoprotein A1, and apolipoprotein B) was measured by a spectrophotometric procedure using an OLYMPUS AU 5400 analyzer (Beckman Coulter). Low-density lipoprotein (LDL)-cholesterol levels were estimated indirectly with the Friedewald equation. The level of homocysteine was determined by a fluorescence polarization immunoassay using an AxSYM analyzer (Abbott Laboratories).

Measurement Reliability of Assays

All markers, with the exception of NO, were determined by standard procedures according to the manufacturer's instructions using automated analyzers (AxSYM, Access Immunoassay Systems, AU 5800, OLYMPUS AU 5400) at the Department of Clinical Analyses of the Ciudad de Jaén Hospital. NO level was indirectly quantified using the Nitric Oxide Analyzer 280i at the Scientific and Technical Instrumentation Centre of the University of Jaén (Spain). The reliability of the assays performed was confirmed by reference to the accurate specificity and sensitivity of each analyser used.

Statistical Analysis

Data for continuous variables are expressed as mean ± standard deviation. We performed data management and analysis using the statistical package SPSS for Windows, Version 19.0 (SPSS, Inc., Chicago, IL). The Kolmogorov-Smirnov test (α-value = .05) and Levene's test (α-value = .05) were performed to test normality and homoscedasticity, respectively. Data that followed a normal distribution and the principle of homoscedasticity of variances were tested by an unpaired Student's *t*-test to compare differences between the means. The degree of statistical significance in data that did not follow a normal distribution or the principle of homoscedasticity was established by applying the Mann-Whitney *U* test. Pearson's correlation was used to assess the relation between continuous variables (biochemical markers and FM clinical parameters). Linear regression was used to test interactions between BMI and the study variables. The level of statistical significance was set at $p < .05$.

Table 1. 1. Demographic and Clinical Characteristics of Women Diagnosed With Fibromyalgia (FM) and Healthy Controls.

Variable	Group 1 (FM, Normal Weight) $n = 12$	Group 2 (Control, Normal Weight) $n = 12$	95% CI	Group 3 (FM, Overweight) $n = 13$	Group 4 (Control, Overweight) $n = 8$	95% CI
Age (years)	48.71 ± 9.65	47.14 ± 6.20	[−11.02, 7.87]	55.25 ± 4.92	57.00 ± 9.84	[−7.98, 11.48]
FIQ score	51.74 ± 20.05	NR	NR	62.55 ± 14.95	NR	NR
VAS score	5.41 ± 2.76	NR	NR	5.60 ± 1.50	NR	NR
PCS-12 score	37.36 ± 10.69	52.21 ± 8.27	[3.71, 25.98*]	32.16 ± 7.27	54.10 ± 1.53	[15.71, 28.16†]
MCS-12 score	30.27 ± 10.02	48.60 ± 5.93	[8.46, 28.19**]	33.55 ± 10.87	52.10 ± 5.64	[2.37, 34.71†]
BMI (kg/m^2)	23.29 ± 1.66	21.43 ± 1.94	[−3.96, 0.24]	26.24 ± 0.54	25.41 ± 0.53	[−1.65, 0.03]

Note. Data are expressed as mean ± standard deviation. Normal weight: BMI between 18.50 and 24.9 kg/m^2; overweight: BMI between 25 and 29.9 kg/m^2. 95% CI = 95% confidence interval; BMI = body mass index; FIQ = fibromyalgia impact questionnaire; MCS-12 = mental health status; NR = not reported; PCS-12 = physical health status; VAS = visual analog scale.
*$p < .05$ and **$p < .01$ for differences between Groups 1 and 2. †$p < .05$ for differences between Groups 3 and 4.

Table 2. Nitric Oxide, Inflammatory Markers, and Cortisol Levels in Women Diagnosed With Fibromyalgia (FM) and Healthy Controls.

Marker	Group 1 (FM, Normal Weight) $n = 12$	Group 2 (Control, Normal Weight) $n = 12$	95% CI	Group 3 (FM, Over-weight) $n = 13$	Group 4 (Control, Over-weight) $n = 8$	95% CI
NOx (μmol/mg protein)	36.97 ± 16.19	22.06 ± 11.02	[−35.11, 5.29]	20.54 ± 11.15	20.29 ± 7.01	[−19.32, 18.82]
IL-6 (pg/ml)	1.00 ± 0.69	1.06 ± 0.51	[−0.67, 0.79]	1.37 ± 0.77	1.27 ± 0.61	[−1.27, 1.08]
IL-10 (pg/ml)	1.54 ± 0.56	1.56 ± 0.69	[−0.84, 0.87]	1.47 ± 0.74	0.69 ± 0.53	[−2.15, 0.58]
CRP (mg/l)	1.01 ± 0.75*	0.35 ± 0.25	[−1.28, −0.04*]	3.11 ± 1.38†	0.45 ± 0.35	[−5.20, −0.13†]
Cortisol (μg/dl)	10.16 ± 3.76	11.45 ± 5.08	[− 4.26, 6.84]	10.75 ± 2.96	10.66 ± 8.50	[−7.41, 7.24]

Note. Data are expressed as mean ± standard deviation. Normal values for CRP = 0.0–5.0 mg/L; normal values for cortisol = 6.0–22.6 μg/dL. 95% CI = 95% confidence interval; CRP = C-reactive protein; IL = interleukin; NOx = nitric oxide metabolites.
*$p < .05$ for differences between Groups 1 and 2. †$p < .05$ for differences between Groups 3 and 4.

Results

Demographic and Clinical Data

Table 1 lists the demographic and clinical data for the patients with FM and the controls. The values for the physical (PCS-12) and mental (MCS-12) health status of normal-weight women with FM were lower than those of the normal-weight controls ($p < .05$, $p < .01$, respectively). Similarly, the PCS-12 and MCS-12 scores of overweight patients were lower than those of the overweight healthy women ($p < .05$, $p < .05$, respectively).

Marker Measurement

Table 2 shows the results obtained from the determination of biomarkers in each group. There were no significant differences in serum NOx levels between the patients with FM and the healthy volunteers. Serum levels of the proinflammatory cytokine IL-6 and the anti-inflammatory cytokine IL-10 remained unaltered in patients with FM versus controls. We found higher levels of CRP in normal- ($p < .05$) and overweight ($p < .05$) women with FM compared to normal- and overweight control subjects, respectively. There were no differences in levels of the neuroendocrine stress biomarker cortisol between women with FM and the controls.

Determination of Lipid Profile

Most parameters of the serum lipid profile (total cholesterol, HDL cholesterol, LDL cholesterol, apolipoprotein A1, and homocysteine) were similar in patients with FM versus controls. Apolipoprotein B levels ($p < .05$) were higher in normal-weight women with FM in comparison to healthy women, but triglycerides did not differ. However, we detected high levels of triglycerides ($p < .05$) and apolipoprotein B ($p < .05$) in the overweight patients compared to the overweight controls (Table 3).

Comparative Study Between Normal- and Overweight Women With FM

CRP ($p < .01$), apolipoprotein B ($p < .05$), and triglycerides ($p < .05$) were higher in normal-weight women diagnosed with FM in comparison to overweight patients (Table 4). The other parameters were similar between groups, with the exception of BMI ($p < .01$).

Correlations Between Study Variables in Women Diagnosed With FM

We observed a significant negative association between NO level and functional capacity determined by FIQ (Pearson correlation coefficient, $r = -0.921$; $p < .05$) in normal-weight

Table 3. Lipid Profiles in Women Diagnosed With Fibromyalgia (FM) and Healthy Controls.

Marker	Group 1 (FM, normal weight) $n = 12$	Group 2 (control, normal weight) $n = 12$	95% CI	Group 3 (FM, overweight) $n = 13$	Group 4 (control, overweight) $n = 8$	95% CI
Total cholesterol (mg/dl)	213.00 ± 20.36	214.71 ± 35.53	[−34.55, 37.98]	233.42 ± 38.23	189.66 ± 24.11	[−99.83, 12.30]
HDL cholesterol (mg/dl)	71.50 ± 15.79	63.57 ± 13.10	[−25.54, 9.68]	63.14 ± 6.38	68.00 ± 13.74	[−9.18, 18.89]
LDL cholesterol (mg/dl)	127.66 ± 13.66	132.14 ± 27.91	[−23.17, 32.12]	146.14 ± 44.00	107.33 ± 20.25	[−101.56, 23.94]
Triglycerides (mg/dl)	71.00 ± 19.51	70.66 ± 29.87	[−35.65, 34.99]	104.16 ± 18.19	70.66 ± 6.02	[−59.76, −7.23†]
Apolipoprotein A1 (mg/dl)	155.25 ± 10.68	153.14 ± 19.09	[−25.87, 21.66]	161.42 ± 12.52	159.66 ± 17.61	[−24.00, 20.47]
Apolipoprotein B (mg/dl)	93.28 ± 8.11	82.66 ± 7.65	[−20.30, −0.92*]	115.20 ± 20.52	75.00 ± 7.93	[−65.80, −14.59†]
Homocysteine (μmol/L)	9.96 ± 3.73	9.22 ± 1.72	[−4.27, 2.80]	10.97 ± 1.88	9.66 ± 2.55	[−4.60, 1.99]

Note. Data are expressed as mean ± standard deviation. 95% CI = 95% confidence interval; HDL = high-density lipoprotein; LDL = low-density lipoprotein.
*$p < .05$ for differences between Groups 1 and 2. †$p < .05$ for differences between Groups 3 and 4.

Table 4. Comparative Study Between Normal-Weight (Group 1) and Overweight (Group 3) Women With Fibromyalgia (FM).

Variable	Group 1 $n = 12$	Group 3 $n = 13$	95% CI
Age (years)	48,71 ± 9,65	55.25 ± 4.92	[−14.90, 1.83]
FIQ score	51.74 ± 20.05	62.55 ± 14.95	[−30.36, 8.75]
VAS score	5.41 ± 2.76	5.60 ± 1.50	[−2.62, 2.25]
PCS-12 score	37.36 ± 10.69	32.16 ± 7.27	[−4.87, 15.28]
MCS-12 score	30.27 ± 10.02	33.55 ± 10.87	[−16.73, 10.17]
BMI	23.29 ± 1.66	26.24 ± 0.54	[−4.28, −1.60**]
NOx (μmol/mg protein)	36.97 ± 16.19	20.54 ± 11.15	[−6.18, 39.04]
IL-6 (pg/ml)	1.00 ± 0.69	1.37 ± 0.77	[−1.27, 0.53]
IL-10 (pg/ml)	1.54 ± 0.56	1.47 ± 0.74	[−0.75, 0.89]
CRP (mg/dl)	1.01 ± 0.75	3.11 ± 1.38	[−3.43, −0.77**]
Cortisol (μg/dl)	10.16 ± 3.76	10.75 ± 2.96	[−4.49, 3.32]
Total cholesterol (mg/dl)	213.00 ± 20.36	233.42 ± 38.23	[−58.87, 18.01]
HDL cholesterol (mg/dl)	71.50 ± 15.79	63.14 ± 6.38	[−8.24, 24.95]
LDL cholesterol (mg/dl)	127.66 ± 13.66	146.14 ± 44.00	[−59.84, 22.89]
Triglycerides (mg/dl)	71.00 ± 19.51	104.16 ± 18.19	[−58.91, −7.42*]
Apolipoprotein A1 (mg/dl)	155.25 ± 10.68	161.42 ± 12.52	[−23.11, 10.76]
Apolipoprotein B (mg/dl)	93.28 ± 8.11	115.20 ± 20.52	[−40.73, −3.09*]
Homocysteine (μmol/L)	9.96 ± 3.73	10.97 ± 1.88	[−4.63, 2.60]

Note. Data are expressed as mean ± standard deviation. 95% CI = 95% confidence interval; CRP = C-reactive protein; FIQ = Fibromyalgia Impact Questionnaire; HDL = high-density lipoprotein; IL = interleukin; LDL = low-density lipoprotein; MCS-12 = mental health status; NOx = nitric oxide metabolites; PCS-12 = physical health status; VAS = visual analog scale; BMI = body mass index.
*$p < .05$. **$p < .01$ for differences between Groups 1 and 2.

patients. The FIQ score correlated positively with apolipoprotein B ($r = 0.762$; $p < .05$) in normal-weight women with FM and with CRP ($r = 0.912$; $p < .05$) in overweight women with FM. Linear regression analyses showed interactions of BMI with CRP (standardized or β coefficients = .541; $p < .05$) and triglycerides (standardized or β coefficients = .638; $p < .05$) in women diagnosed with FM.

Discussion

FM is among the most common causes of musculoskeletal pain and disability. Although research has revealed many factors that are related to FM, the syndrome's etiology is not yet understood. Our results from the present study showed increased levels of CRP and apolipoprotein B in normal-weight women with FM compared to the healthy normal-weight controls. CRP, apolipoprotein B, and triglycerides were higher in overweight patients versus overweight controls and in overweight versus normal-weight patients. Apolipoprotein B and NO correlated with FIQ in normal-weight patients, while CRP correlated with FIQ in overweight women with FM. Finally, we found interactions of BMI with CRP and triglycerides in women diagnosed with FM.

In recent years, authors have proposed that NO may be implicated in the pathophysiology of FM. Nonetheless, previous findings regarding NO levels in patients with FM have been inconsistent. Our results in the present study showed no significant differences in NO between either normal-weight or overweight women with FM and their age- and weight-matched controls, which is in accord with the results of a number of previous studies (Akkuş et al., 2009; Kim et al., 2010; Sendur, Turan, Tastaban, Yenisey, & Serter, 2009). However, we did note a pattern of increasing NO in normal-weight patients in comparison to the controls. In contrast to our data,

some authors have reported diminished NO in patients with FM in comparison to healthy subjects (Ozgocmen et al., 2006), and others have found statistically significant increases in NO levels in FM patients (Bradley et al., 2000). These conflicting results could be due to interpersonal variability or to the use of different methods of determining NO.

Likewise, the findings on the effects of NO on FM clinical parameters have not been conclusive. While some authors have found no correlations between NO level and FM symptoms (FIQ-pain and FIQ-fatigue; Ozgocmen et al., 2006), others have observed a positive correlation between NO and pain as measured by VAS (Sendur et al., 2009). Our data revealed a negative association between NO, an important vasodilator, and the FIQ score. This finding may provide support to the hypothesis that associates FM pain and the muscle vasoconstriction induced by regional vasomotor dysregulation (Katz et al., 2007). Decreased production of NO may cause impaired microcirculation in muscles of patients with FM, which might lead to fatigue (Kasikcioglu et al., 2006), one of the main symptoms of this syndrome.

Research has also found an association between FM and dysregulation of inflammatory markers (Bote et al., 2012; Menzies & Lyon, 2010). Our results in the present study indicate increased levels of serum CRP in normal- and overweight women with FM versus healthy subjects, which is in agreement with published results (Bote et al., 2012, Xiao, Haynes, Michalek, & Russell, 2013). CRP positively correlated with FIQ score in overweight patients, suggesting that the regulation of this inflammatory marker may be associated with FM. Our results also reveal an interaction between CRP and BMI in women with FM. Obesity has been associated with syndromes that produce local and widespread pain, including FM (Neumann et al., 2008; Okifuji et al., 2009). Moreover, patients with FM have improved symptoms after losing weight (Saber et al., 2008). In addition to its role as a mediator of inflammation, CRP has been related to obesity (Visser, Bouter, McQuillan, Wener, & Harris, 1999; Xiao et al., 2013) and cardiovascular disease (Ridker, Hennekens, Buring, Rifai, 2000). Higher BMI has been associated with higher CRP concentrations, implying a state of low-grade systemic inflammation in overweight and obese people (Visser et al., 1999). Low-grade systemic inflammation due to increased CRP levels enhances the risk of cardiovascular disease. In fact, CRP is a significant predictor of the risk of cardiovascular events in women, suggesting that the measurement of CRP may improve the predictive value of models of cardiovascular-disease risk based only on standard lipid screening (Ridker et al., 2000). Therefore, the increased levels of CRP found in the patients with FM in the present study may be related to their risk of suffering a cardiovascular event. On the other hand, our results also show that levels of IL-6 and IL-10 did not differ between patients and controls. This finding is in accordance with those of previous studies, which similarly failed to find differences in IL-6 (Geiss, Rohleder, & Anton, 2012; Kim et al., 2010; Wang, Moser, Schiltenwolf, & Buchner, 2008; Xiao et al., 2013) and IL-10 (Wallace et al., 2001, Wang et al., 2008) levels between patients with FM and healthy subjects. In contrast, researchers have reported elevated levels of IL-6 (Hernandez et al., 2010) and IL-10 (Bazzichi et al., 2007) in patients with FM in comparison to healthy controls. NO is an important anti-inflammatory agent that inhibits the activity of the nuclear factor kb, a stress-related transcription factor (Esch et al., 2002) that induces cells to release inflammatory cytokines. It is plausible that the physiological levels of NO might have regulated the levels of cytokines in our patients with FM.

Most parameters of the lipid profiles of our patients were similar to those of the controls. Only apolipoprotein B levels were increased in normal-weight women with FM in comparison to healthy volunteers. In addition, we detected high levels of triglycerides and apolipoprotein B in overweight patients compared to overweight controls. To the best of our knowledge, no previous studies have investigated the levels of apolipoproteins A and B in patients with FM compared to healthy controls. Apolipoproteins not only play a critical role in maintaining the structure of the lipoproteins, they are also involved in their metabolism, acting as activators and inhibitors of enzymes and receptor ligands and transferring lipids among lipoproteins. In the present study, apolipoprotein B correlated positively with the FIQ score in normal-weight women diagnosed with FM, suggesting that its regulation may be associated with FM. A previous study did not report any relation between the lipid profile and the FIQ and VAS scores in patients with FM (Gurer et al., 2006), but other authors have reported that total cholesterol, triglycerides, and BMI correlate with FIQ and VAS data, implying that overweight and lipid profile may be related to FM symptoms (Cordero et al., 2014). Supporting this suggestion, authors have reported that the apolipoprotein E4 genotype may contribute to the risk of developing FM (Reeser, Payne, Kitchner, & McCarty, 2011). Moreover, researchers have suggested that high apolipoprotein B levels are the primary predictor of coronary heart disease in women (Onat et al., 2007). Accordingly, the augmented levels of apolipoprotein B and CRP in participants with FM in the present study may be contributing to the risk of cardiovascular events in these patients. Authors have recently proposed a relationship between FM and cardiovascular disease because patients with FM are susceptible to increased platelet activation and mean platelet volume values, which contribute to an increased risk for cardiovascular disease (Haliloğlu, Carlioglu, Sahiner, Karaaslan, & Kosar, 2014).

We assessed function of the HPA axis in participants of the present study by measuring cortisol levels. Cortisol plays a major role in the stress response. A healthy cortisol circadian rhythm is characterized by high morning and low evening levels. In this study, we collected all blood samples in the early morning to avoid the effects of the daily variations in levels. At present, there is no consensus concerning the activity of the HPA axis in FM. In accord with several previous studies (Freitas, Lemos, Spyrides, & Sousa, 2012; Lentjes, Griep, Boersma, Romijn, & de Kloet, 1997), we did not find any significant differences in cortisol levels between the FM patients and the healthy controls. Conversely, investigators have reported both

reduced (Gur et al., 2004) and elevated (Bote et al., 2012; Crofford et al., 2004) levels of cortisol in earlier studies in patients with FM versus controls. Research has shown that patients with FM have normal morning, afternoon, and evening serum cortisol levels compared to healthy subjects but elevated night levels (Fatima et al., 2013). These conflicting results may be related to the time of the day in which the level of cortisol was measured, given that there are 8–9 peaks of cortisol during a 24-hr period. The HPA axis may be influenced by cytokines and NO. Stress may affect the production of cytokines, and cytokines may regulate the activity of the HPA axis (Thompson & Barkhuizen, 2003). NO is reportedly involved in stress physiology and stress-related diseases, as it may counteract the activity of the stress hormone norepinephrine and the sympathetic response (Esch et al., 2002). Our data in this study show that levels of cortisol, cytokines, and NO did not differ in women with FM in comparison with the controls. Consequently, the HPA axis does not appear to be disturbed in our sample of patients, as cortisol levels remained unaltered and we found no correlation between cortisol and FM clinical markers.

The main limitation of the present study was the small sample size. Therefore, additional research in a larger sample is needed to confirm the increase in serum levels of CRP and apolipoprotein B in patients with FM versus controls before proposing routine screening for these cardiovascular risk markers in such patients. In addition, although the practitioner responsible for administering the questionnaires (FIQ, VAS, and SF-12) sought to minimize the time from blood collection until the patients completed the questionnaires, occasionally there were circumstances that delayed the completion of the questionnaires (e.g., the need for breakfast immediately after blood collection or an urgent appointment with the doctor). Nevertheless, in no case was the delay more than 2 hr.

Conclusions

The findings of this study suggest that the regulation of CRP and apolipoprotein B may be associated with FM-related dysfunction in both normal- and overweight women with FM, as reflected by their increased serum levels and their correlation with FM clinical parameters. Given that both CRP and apolipoprotein B are related to cardiovascular events, our results propose that women diagnosed with FM may be at increased risk of cardiovascular disease. The serum levels of other markers that prior research had suggested were associated with this syndrome, such as NO, cytokines, and cortisol, were not altered in our cohort of patients with FM in comparison to the controls. These findings contribute to nursing science by providing evidence that may ultimately help to improve the practice guidelines for the care of patients with FM.

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Author Contribution

Rus, A. contributed to conception, design, data acquisition, data analysis, and interpretation of data; drafted the manuscript; critically revised manuscript; gave final approval; agreed to be held accountable for all aspects of work, ensuring integrity and accuracy. Molina, F. contributed to conception, design, data analysis, and interpretation of data; critically revised the manuscript; gave final approval; agreed to be held accountable for all aspects of work, ensuring integrity and accuracy. Gassó, M. contributed to data acquisition and interpretation of data; critically revised the manuscript; gave final approval; agreed to be held accountable for all aspects of work, ensuring integrity and accuracy. Camacho, M. V. contributed to data acquisition and interpretation of data; critically revised the manuscript; gave final approval; agreed to be held accountable for all aspects of work, ensuring integrity and accuracy. Peinado, M. A. contributed to design and interpretation of data; critically revised the manuscript; gave final approval; agreed to be held accountable for all aspects of work, ensuring integrity and accuracy. Del Moral, M. L. contributed to conception, design, and interpretation of data; critically revised the manuscript; gave final approval; agreed to be held accountable for all aspects of work, ensuring integrity and accuracy.

Declaration of Conflicting Interests

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