*Article*

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# Extra Virgin Olive Oil Improves Oxidative Stress, Functional Capacity, and Health-Related Psychological Status in Patients With Fibromyalgia: A Preliminary Study

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## Abstract

**Objectives:** Fibromyalgia (FM) is a chronic disease that imposes physical, psychological, and social limitations. We have reported that oxidative stress may play a role in the pathophysiology of FM. Olive oil has been shown to be effective treatment against the oxidative stress associated with several diseases. The aim of this study was to investigate the effect of olive oil on oxidative stress and health-related parameters in FM. **Methods:** This preliminary study was performed on blood samples of 23 women diagnosed with FM who consumed 50 ml of organic olive oil daily for 3 weeks. Subjects were randomized into two groups: one ingested extra virgin olive oil (EVOO) and the other refined olive oil (ROO), which have different antioxidant content. The patients' oxidative (lipid, protein, and DNA oxidation) and antioxidative (antioxidant enzyme activities and compounds) profiles were examined before and after the treatment period. Functional capacity and physical and mental health status were assessed using the Fibromyalgia Impact Questionnaire (FIQ) and the Physical Component (PCS-12) and Mental Component Summaries (MCS-12) of the Short Form-12 Health Survey, respectively. **Results:** Significant differences were found in pre–post change between the EVOO and ROO groups for protein carbonyls, lipid peroxidation, and FIQ and MCS-12 scores. Differences between groups approached statistical significance for oxidative DNA damage and levels of the antioxidant compound zinc. **Conclusions:** EVOO may protect women with FM against oxidative stress in addition to improving functional capacity and health-related psychological status. Findings suggest that olive oil may be a valuable therapeutic support in FM.

## Keywords

fibromyalgia, olive oil, oxidative stress, antioxidants

The syndrome of fibromyalgia (FM) is characterized primarily by chronic, widespread musculoskeletal pain. The estimated prevalence of FM in the general population is 1.7–5.4%, with a ratio of females to males of 2.3–13.7 to 1 according to the different classification criteria sets available (Jones et al., 2015). The pathophysiology of FM is not completely known; therefore, no effective treatment is available. Research has shown many factors to be related to FM, including oxidative stress, central sensitization, genetic factors, impaired hypothalamic–pituitary–adrenal axis function, and altered levels of neurotransmitters, C-reactive protein (Rus et al., 2016), and apolipoprotein B (Rus et al., 2016). In particular, several studies, including one from our own laboratory, have implicated oxidative stress in the etiology of FM (La Rubia, Rus, Molina, & Del Moral, 2013; Ozgocmen, Ozyurt, Sogut, & Akyol, 2006). Oxidative stress is the result of imbalance between free radicals, such as reactive oxygen species (ROS), and antioxidant defenses in the cells. Since oxidative stress seems to be involved in the pathophysiology of FM, supplementation with antioxidants may help to counteract the adverse effects in these patients.

Several studies in patients with FM have shown that supplementation with antioxidants such as vegetables, melatonin or coenzyme $Q_{10}$ ($CoQ_{10}$) alleviated pain and improved the quality of life (Cordero et al., 2013; Donaldson, Speight, & Loomis, 2001; Wilhelmsen, Amirian, Reiter, Rosenberg, & Gögenur, 2011). Most of these studies did not analyze oxidative stress markers, focusing only on clinical parameters.

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The administration of $CoQ_{10}$ ameliorated lipid peroxidation and restored mitochondrial dysfunction in patients with FM (Cordero et al., 2013). On the other hand, vitamins C and E, alone or in combination with physical exercise, did not improve symptoms in patients with FM but showed a protective effect against oxidative stress by decreasing lipid peroxidation and augmenting glutathione peroxidase activity (Naziroğlu et al., 2010).

Olive oil, the main source of fat in the Mediterranean diet, is characterized by a high concentration of antioxidant compounds, including phenolic compounds. The concentration of antioxidants in olive oil depends on the extraction mechanisms. Extra virgin olive oil (EVOO), produced exclusively by physical processes, contains high concentrations of antioxidants, mainly phenolic compounds. However, these compounds are lost when olive oil is submitted to chemical extraction mechanisms such as refinement. The health benefits of the phenolic compounds in olive oil include their antioxidant activity, which is related to the capacity to protect DNA, proteins, and lipids from damage caused by exposure to ROS (Fki, Sahnoun, & Sayadi, 2007). Several epidemiological studies have demonstrated beneficial effects of olive oil ingestion on oxidative stress–related diseases, including cardiovascular diseases (Guasch-Ferré et al., 2014), cancer (Pelucchi, Bosetti, Negri, Lipworth, & La Vecchia, 2011), and rheumatoid arthritis (Berbert, Kondo, Almendra, Matsuo, & Dichi, 2005).

No available studies examine the antioxidant effect of olive oil in women with FM. Therefore, the goal of this work is to investigate the effect of olive oil consumption on oxidative stress markers and health-related parameters in patients with FM.

## Method

### Participants

A total of 23 women with FM who belonged to the AFIXA (Association of Fibromyalgia of Jaén, Spain) participated in the study after providing written informed consent. Participants had to meet the 1990 American College of Rheumatology criteria for classification of primary FM. Exclusion criteria included the presence of any other chronic disease (diabetes mellitus, hypertension, cancer, and ischemic heart disease), pregnancy, lactation, digestive intolerance to olive oil, or Grade II obesity (body mass index [BMI] $\geq$ 35 kg/m$^2$). Participants were not consuming any medication that affects antioxidative status. Nor were they taking corticosteroids, estrogens, analgesics, or anti-inflammatory drugs and could be included only after they had stopped using such medications for at least 2 months before the study. None consumed alcohol, and all were nonsmokers. All the participants had a sedentary lifestyle.

### Study Design

We performed this randomized, controlled, double-blind nutritional trial in accordance with the Declaration of Helsinki (2008) of the World Medical Association. The Ethics Committee of the University of Jaén (Spain) approved the study. The trial consisted of the consumption of one of the two organic olive oils with different antioxidant content, EVOO or refined olive oil (ROO), over 3 weeks. We randomly assigned participants to one of the two groups: (1) EVOO group ($n = 11$ women with FM consuming 50 ml/day of organic EVOO) and (2) ROO group ($n = 12$ women with FM consuming 50 ml/day of organic ROO). Our randomization process was as follows: We assigned each participant a number as we called on her in the office of AFIXA. We subsequently used these numbers for randomization using the library ''random'' for R (see free-GNU R webpage). This method uses the true random number service provided by RANDOM.ORG, and the randomness comes from atmospheric noise. Before beginning the 3-week dietary trial, participants conducted a 2-week washout period during which they consumed ROO (50 ml daily for 2 weeks) because they were part of a population that consumes olive oil regularly. We measured oxidative stress markers and clinical parameters in each participant before and after the 3-week treatment period.

We obtained organic olive oils from Olifarma S.L. (Granada, Spain). The olive oils were similarly packaged, and participants were blinded as to the type of olive oil they consumed. Participants consumed the treatment olive oil raw but used ROO for cooking in order to maintain their ingestion of antioxidants unchanged. We supplied ROO for cooking in sufficient quantity for the whole family. We estimated the participants' intake by means of a 24-hr recall that they completed for 3 days (2 working days and a day off) at the beginning of the trial. We used the average of the values obtained over the 3 days to calculate participants' energy intake (kcals/day) and intake of macronutrients (carbohydrates [g/day], lipids [g/day], and protein [g/day]) and micronutrients, especially those that may have an antioxidant effect, including vitamin C, vitamin A, copper, zinc, and selenium. Based on this analysis, we provided all participants with dietary recommendations, focusing on the aspects that each should improve. We also checked the weight of the participants at the beginning and end of the trial. We included this dietary recall and analysis (1) so that we could make dietary recommendations for normalizing each participant's intake of antioxidants during the trial and (2) to avoid an excess of calories and/or lipid intake due to the extra intake of olive oil that could produce a significant dietary imbalance and weight gain during the trial. We requested that participants return all the treatment olive oil containers (the consumed and unconsumed containers) at the end of the trial for control of the olive oil consumption (i.e., a count of the empty and full containers each participant returned).

### Clinical Characteristics of Participants

We determined participants' clinical characteristics from interviews and questionnaires administered before and after the nutritional trial. The same specialist carried out all the measurements and tests throughout the study. We evaluated

functional capacity in activities of daily living using the Fibromyalgia Impact Questionnaire (FIQ) and self-reported musculoskeletal pain using a Visual Analogue Scale (VAS). We assessed the self-reported physical and mental health status of participants using the Physical Component Summary (PCS-12) and Mental Component Summary (MCS-12) of the Short Form-12 Health Survey (SF-12), respectively. FIQ and SF-12 scores range from 0 to 100, with 50 as the average value. High scores on the FIQ and low scores on the SF-12 reflect worse health status. VAS scores range from 0 to 10, with 7 as the average value in the FM population. High values on the VAS reflect higher levels of pain.

## Blood Collection and Preparation of Blood Samples

Venous blood was drawn from the antecubital vein into two ethylenediaminetetraacetic acid (EDTA) tubes and one EDTA-free tube in the early morning after an overnight fast. We collected blood before and after the trial period from all participants and at the same time of the day to prevent daily variations in the level of antioxidants. We prevented daily variations in the level of antioxidants by collecting the blood at the same time of the day. We determined antioxidant enzyme activity and 8-hydroxy-2′-deoxyguanosine (8-oxo-dG) level in lymphocytes, separated from whole blood (one EDTA tube) by differential centrifugation using Histopaque-1077 (Sigma-Aldrich). We allowed the blood to clot in the EDTA-free tube for 30 min at room temperature. We then centrifuged one EDTA tube and the EDTA-free tube at 3,500 rpm for 5 min at 4°C to obtain plasma and serum samples, respectively. We measured antioxidant compounds in serum samples and oxidative and antioxidative markers (total antioxidant capacity [TAC], lipid peroxidation, and protein carbonyl content) in plasma samples.

## Determination of Oxidative Status

*Thiobarbituric acid reactive substances (TBARS).* TBARS are a good indicator of lipid peroxidation. We determined TBARS levels spectrophotometrically following the manufacturer's recommendations (Catalog # 0801192, TBARS Assay Kit, OXItek).

*Protein carbonyl content.* Protein carbonyl content is the most general indicator of protein oxidation. We performed the protocol following the assay kit manufacturer's instructions (Item No. 10005020, Protein Carbonyl Assay Kit, Cayman).

*8-oxo-dG.* 8-oxo-dG is a frequently used biomarker of oxidative DNA damage and oxidative stress. We isolated and digested nuclear DNA following Espinosa et al.'s (2007) method and then separated the 8-oxo-dG by high-performance liquid chromatography. We measured the amount of 8-oxo-dG and deoxyguanosine (dG) in the DNA digest by electrochemical and Ultraviolet (UV) absorbance detection, respectively.

## Determination of Antioxidative Status

*Superoxide dismutase (SOD).* We determined SOD activity spectrophotometrically using the Bioxytech SOD-525 kit (Catalog No. 21010, OxisResearch) and expressed it relative to protein content.

*Glutathione peroxidise (GPx).* We measured GPx activity according to the assay kit manufacturer's recommendations (Item No. 703102, Glutathione Peroxidase Assay Kit, Cayman) and normalized it to protein content.

*Catalase.* We determined catalase activity spectrophotometrically following the assay kit manufacturer's instructions (Catalog No. 21042, Bioxytech Catalase-520, OxisResearch) and referred it to total protein.

*TAC.* We measured TAC in plasma following the assay kit manufacturer's instructions (Item No. 709001, Antioxidant Assay Kit, Cayman).

*Antioxidant compounds.* We measured antioxidant compounds during routine blood sampling for biochemical analyses. The analyzers we used were Olympus AU5400 (Beckman Coulter; copper, iron, bilirubin, uric acid, albumin, zinc, transferrin, ferritin) and BN II System (Siemens Healthcare Diagnostics; ceruloplasmin).

## Statistical Analysis

We performed all statistical analyses using the free-GNU R software, R version 3.2.3 (R Foundation for Statistical Computing, http://www.r-project.org/) with Wilcox's "Rallfun-v30.txt" library (http://www-rcf.usc.edu/∼rwilcox/. Ask for the functions "t1wayv2," "lincon," "pball," "yuenv2," and "msmedse"). In a study with clinical samples, it is possible to find outliers or to violate the assumptions of normality or homoscedasticity, making it inappropriate to use conventional statistical techniques (e.g., analysis of variance [ANOVA] or *t*-test). We analyzed variability and central tendency indexes in box plot graphs according to robust statistics based on the median (Wilcox, 2012) in order to explore these assumptions while summarizing the data. Figures 1–3 did not show significant fluctuations of variability but revealed the presence of outliers (in all variables except for one), which is the reason we opted for robust analysis. We conducted the robust variant of ANOVA using a statistical method based on Yuen-Welch logic applied on trimmed means ($V_W$ onward; see Wilcox, 2012, for an explanation of the superiority of this robust test in the context of between-group designs). The robust ANOVA focused on the pre–post differences in order to gain sensitivity, thus avoiding a potential ceiling effect. We analyzed correlations between biochemical markers and clinical parameters using a robust variant, the percentage bend correlation ($r_{PB}$ onward). For all the analyses, we added the estimated robust effect size from Wilcox (2012) "Explanatory Measure"

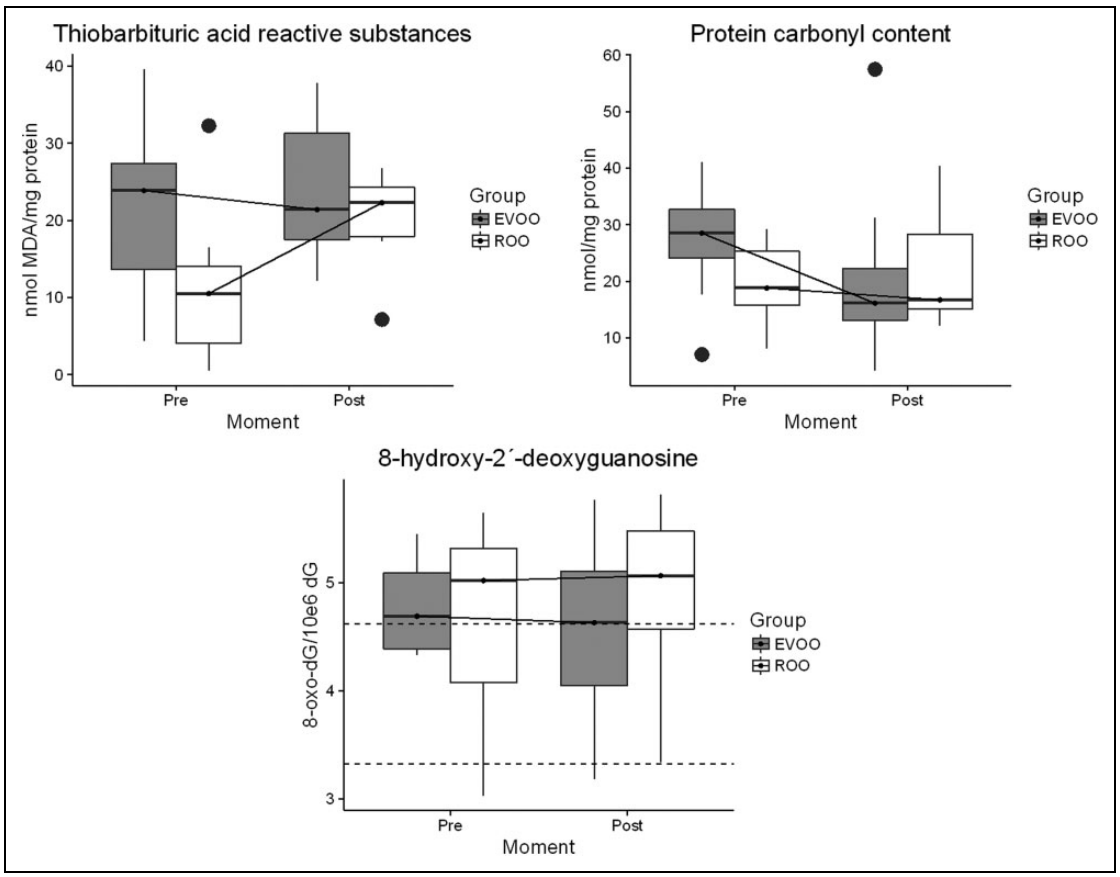


**Figure 1.** Oxidative stress markers in patients with fibromyalgia from before 3-week dietary trial to after. The horizontal dashed lines reflect the range of normal values for the parameter. The dots show outliers. EVOO = patients with fibromyalgia who consumed extra virgin olive oil ($n = 11$); ROO = patients with FM who consumed refined olive oil ($n = 12$); MDA = malondialdehyde; 8-oxo-dG = 8-hydroxy-2′-deoxyguanosine.

(Ef. size onward). Values of .15, .35, and .50 correspond to the three bands of interpretation of the effect size as small, moderate, and large, respectively, from Wilcox robust interpolation of classical .2, .5, and .8 Cohen bands. We set statistical significance at $p \leq .05$ and adjusted the probabilities found with the robust statistics for multiple testing logic ($p$.Adj onward) for each of the sets of variables (clinical, oxidative, antioxidative, and health related). We used the Benjamini–Hochberg test, a less stringent condition than the usual Bonferroni variant, to provide a gain in statistical power (see Peña, Habiger, & Wu, 2011, for a review of the methods of multiple comparisons).

## Results

### Participants

Table 1 summarizes the data for all the study variables, including clinical, oxidative, antioxidative, and health-related parameters, together with robust 95% confidence intervals of difference between pre- and post measures, $p$ value (robust statistic), adjusted $p$ value for multiple tests ($p$.Adj), and the effect size of the robust ANOVA. We randomized participants into two groups of similar age (mean age of 53.63 $\pm$ 5.50 years for the EVOO group and 48.16 $\pm$ 7.96 years for the ROO group) and found no significant differences in the other parameters between groups, including BMI, systolic blood pressure, diastolic blood pressure, and cardiac frequency, whether pre, post, or pre–post differences. All these clinical parameters showed small values (below .15) for effect size (Table 1).

### Effects of Type of Olive Oil on the Oxidative Profile in FM

There were statistically significant differences in levels of TBARS, difference, diff $= -10.763$, robust omnibus Wilcox test, $V_W\,(10.353) = 6.34$, $p$.Adj $< .047$, and protein carbonyls, diff $= -13.312$, $V_W(9.729) = 11.175$, $p$.Adj $< .023$, between the EVOO and ROO groups. TBARS and protein carbonyl levels declined significantly posttreatment in the EVOO group in comparison to the ROO group. Difference in the pre–post change in 8-oxo-dG levels approached statistical significance when the two groups were compared, diff $= -0.522$, $V_W\,(7.685) = 4.434$, $p$.Adj $< .070$ (Figure 1 and Table 1). All these markers showed large values (above .50) for effect size (Table 1).

### Effects of Type of Olive Oil on the Antioxidative Profile in FM

Pre–post changes in the enzyme activities of SOD, GPx, and catalase were statistically equivalent in the two groups,

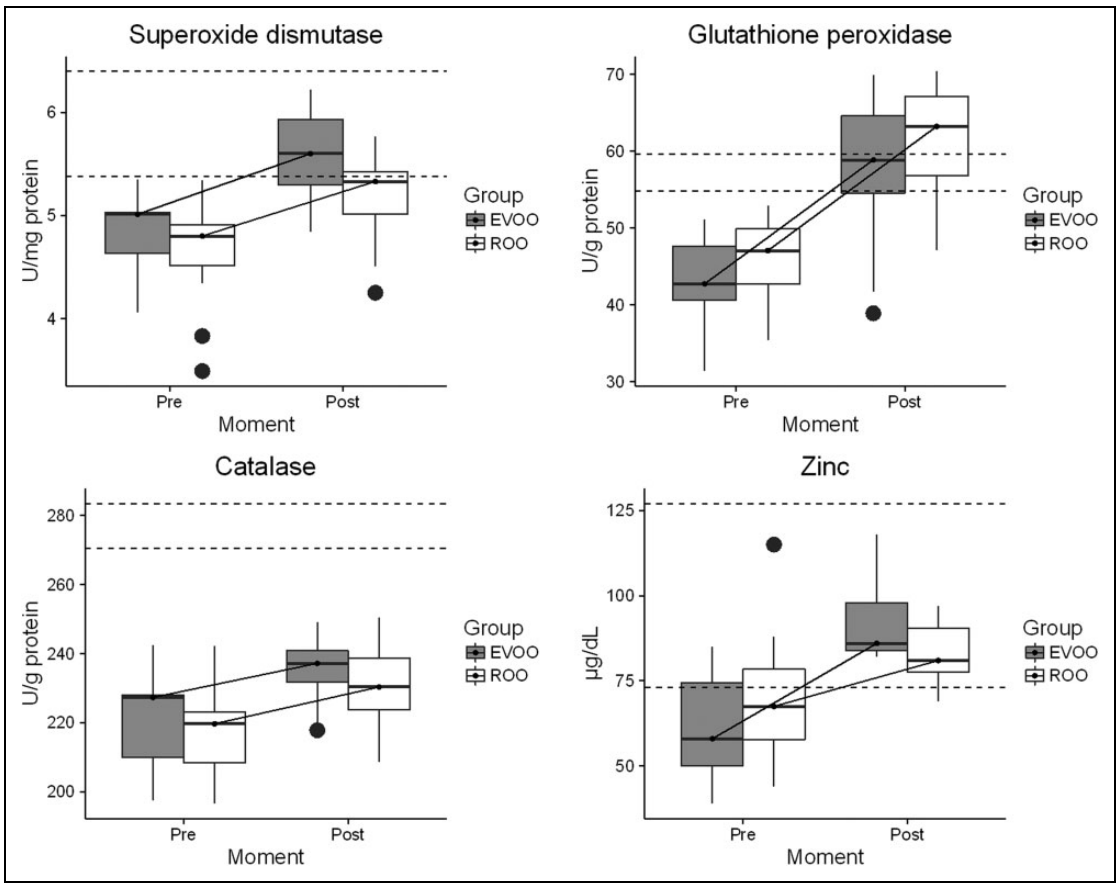


**Figure 2.** Antioxidative markers in patients with fibromyalgia from before 3-week dietary trial to after. The horizontal dashed lines reflect the range of normal values for each parameter. The dots show outliers. EVOO = patients with fibromyalgia who consumed extra virgin olive oil ($n = 11$); ROO = patients with FM who consumed refined olive oil ($n = 12$).

for SOD, the variable with the highest statistically nonsignificant value of effect size: diff = 0.145, $V_W$ (6.184) = 1.433, $p$.Adj < .706 (Figure 2 and Table 1). Concerning the antioxidative markers, only zinc approached statistical significance in difference in pre–post change between groups, but the adjusted probability proved far from significance, diff = 17.00, $V_W$ (11.996) = 4.326, $p < .060$, $p$.Adj < .596. The other markers remained unaltered and thus are not shown in the figure, for transferrin, the variable with the highest statistically nonsignificant value of effect size: diff = −4.143, $V_W$ (9.372) = 0.745, $p$.Adj < .946. Zinc showed a large value (above .50) for effect size and SOD a moderate-to-large value (.397). The other markers registered small values (below .15) for effect size, except for copper, ceruloplasmin, ferritin, and transferrin, which showed small-to-moderate values (Table 1).

### *Effects of Type of Olive Oil on Health-Related Parameters in FM*

FIQ, diff = −19.619, $V_W$ (12.191) = 9.04, $p$.Adj < .035, and MCS-12, diff = 11.228, $V_W$ (12.059) = 7.582, $p$.Adj < .035, scores exhibited significant differences in pre–post changes between the two groups. The FIQ score declined in the EVOO group but did not change in the ROO group, whereas the MCS-12 score increased in the EVOO group and decreased in the ROO group. There were no statistically significant differences for pre–post changes in either VAS, Diff = −1.071, $V_W$ (9.961) = 1.309, $p$.Adj < .279, or PCS-12, diff = −2.824, $V_W$ (10.641) = 2.652, $p$.Adj < .177, scores between groups (Figure 3 and Table 1). FIQ and MCS-12 scores showed large values (above .50) for effect size, while VAS and PCS-12 presented moderate-to-large values (.384 and .453, respectively).

### *Correlations Between Biochemical Markers and Health-Related Parameters*

Lipid peroxidation positively correlated with FIQ ($r_{PB} = .769$, $p < .006$) and VAS ($r_{PB} = .749$, $p < .008$) scores in the EVOO group. There were no other significant correlations between biochemical markers and health-related parameters in the EVOO group and none at all in the ROO group.

## Discussion

FM is a pathologically complex syndrome without a known cure, so any effort to find possible strategies to improve the

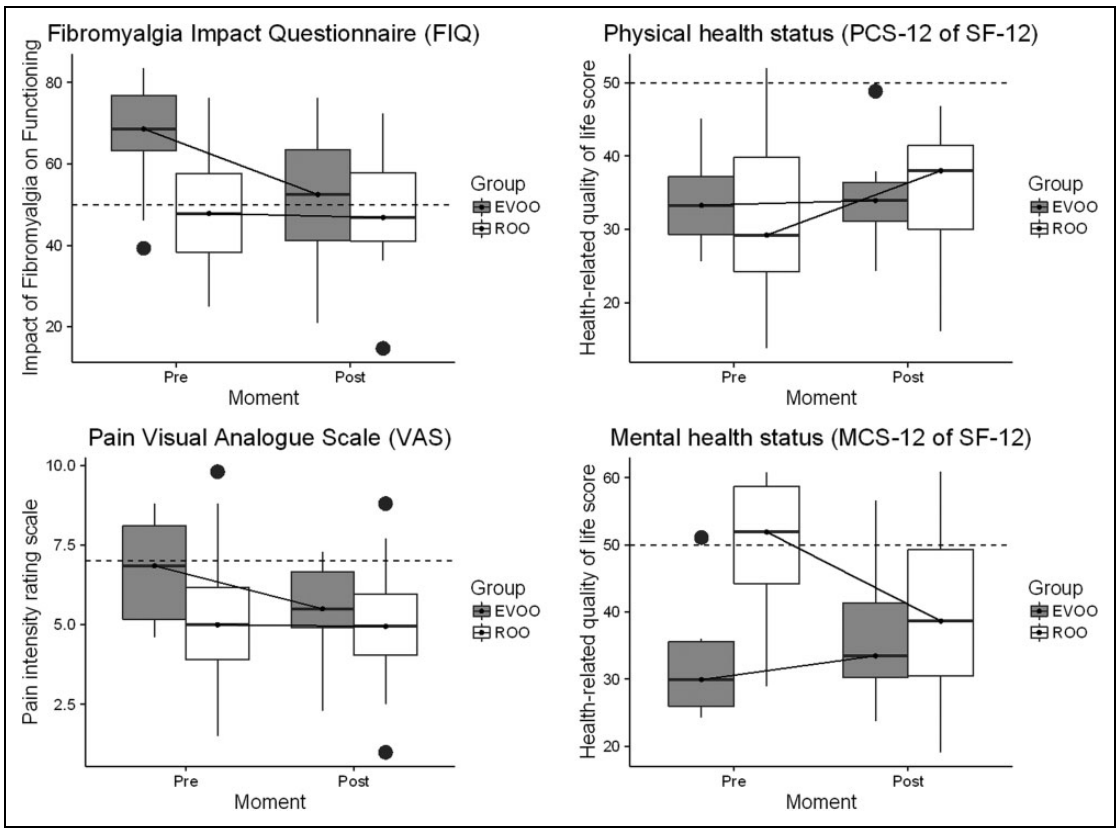


**Figure 3.** Health-related parameters in patients with fibromyalgia from before 3-week dietary trial to after. The horizontal dashed lines reflect the range of normal values for each parameter. The dots show outliers. EVOO = patients with fibromyalgia who consumed extra virgin olive oil ($n = 11$); ROO = patients with FM who consumed refined olive oil ($n = 12$). MCS-12 = Mental Component Summary; PCS-12 = Physical Component Summary; SF-12 = Short Form-12 Health Survey.

health-related parameters and biochemical markers altered in these patients is noteworthy. The aim of the present study was to investigate the effects of two organic olive oils (EVOO and ROO) with different antioxidant content on oxidative stress markers and health-related parameters in women with FM.

The results revealed, for the first time, that EVOO may protect patients against FM-induced oxidative stress by diminishing protein, lipid, and DNA oxidation and raising zinc levels as well as improve functional capacity and health-related psychological status. All these variables showed a large magnitude of effect size. Moreover, SOD activity, pain (VAS), and physical health status (PSC-12) may also warrant further investigation since they presented moderate-to-large values for effect size, though they did not reach statistical significance in this study. The clinical characteristics of participants in the present study showed a small effect size, confirming that differences between groups concerning these parameters were negligible.

## *Effects of Olive Oil on the Oxidative Profile in FM*

Our data showed notable differences in changes in the levels of protein carbonyls, TBARS, and 8-oxo-dG between the two experimental groups from before to after the 3-week dietary trial period during which participants consumed either EVOO or ROO, suggesting that nutrition may modulate oxidative stress in these patients. Participants who consumed EVOO showed a greater reduction in levels of protein carbonyls than those who consumed ROO, suggesting that EVOO may diminish oxidative damage in women with FM. In a previous study, we reported that protein carbonyls were augmented in women with FM compared to healthy women (La Rubia et al., 2013). Since no other data have been published on the effect of olive oil on oxidative stress in patients diagnosed with FM, we will discuss the results of the current study in relation to results from studies exploring other experimental situations or pathologies. In support of our results, previous researchers found that olive oil diminished protein carbonyl content in rats submitted to oxidative stress (Amamou et al., 2015) and in the hearts of senescence-accelerated mice (Bayram et al., 2012). Conversely, other researchers found that plasma protein carbonyls were not affected in healthy individuals after consumption of a phenol-rich olive oil (Vissers, Zock, Wiseman, Meyboom, & Katan, 2001).

Regarding TBARS, our data showed that consumption of organic EVOO lowered TBARS levels in patients with FM, while consumption of ROO raised these levels. One possible explanation for the increase in this oxidative stress marker after ROO intake may be that the women from this southern Spanish

**Table 1.** Clinical, Oxidative, Antioxidative, and Health-Related Parameters in Women Diagnosed With Fibromyalgia.

| | EVOO Group ($n = 11$) | | ROO Group ($n = 12$) | | | | | |
|---|---|---|---|---|---|---|---|---|
| Parameter | Pre | Post | Pre | Post | 95% CI | *p* | *p*.Adj | Ef. Size |
| Clinical characteristics | | | | | | | | |
| Body mass index (kg/m$^2$) | 26.71 (2.95) | 26.06 (2.66) | 27.29 (1.23) | 27.11 (1.32) | [−0.50, 0.62] | .816 | .915 | 0.021 |
| Systolic blood pressure (mmHg) | 120.00 (5.82) | 120.00 (6.79) | 117.50 (5.82) | 110.00 (7.76) | [−8.46, 10.78] | .788 | .915 | 0.081 |
| Diastolic blood pressure (mmHg) | 70.00 (3.88) | 70.00 (3.88) | 70.00 (3.88) | 70.00 (3.88) | [−7.49, 6.78] | .915 | .915 | 0.000 |
| Cardiac frequency (bpm) | 70.00 (2.72) | 72.00 (3.49) | 68.00 (2.52) | 72.50 (4.66) | [−6.65, 11.97] | .541 | .915 | 0.138 |
| Oxidative stress markers | | | | | | | | |
| Thiobarbituric acid reactive substances (nmol MDA/mg protein) | 23.88 (4.63) | 21.40 (4.40) | 10.52 (3.04) | 22.30 (1.63) | [−20.24, −1.28] | .030* | .045 | 0.558 |
| Protein carbonyl content (nmol/mg protein | 28.52 (3.51) | 16.14 (4.06) | 18.81 (2.76) | 16.70 (3.71) | [−22.22, −4.41] | .008* | .023 | 0.607 |
| 8-hydroxy-2′-deoxyguanosine (8-oxo-dG/10e6 dG) | 4.69 (0.22) | 4.63 (0.45) | 5.02 (0.47) | 5.07 (0.40) | [−1.10, 0.05] | .070† | .070 | 0.751 |
| Antioxidant enzyme activities | | | | | | | | |
| Superoxide dismutase (U/mg protein) | 5.01 (0.13) | 5.60 (0.20) | 4.80 (0.27) | 5.33 (0.20) | [−0.15, 0.44] | .275 | .706 | 0.397 |
| Glutathione peroxidase (U/g protein) | 42.73 (1.79) | 58.88 (5.09) | 47.07 (2.41) | 63.24 (3.55) | [−3.05, 2.13] | .706 | .706 | 0.120 |
| Catalase (U/g protein) | 227.31 (5.71) | 237.18 (5.31) | 219.67 (6.26) | 230.32 (4.62) | [−17.50, 25.15] | .704 | .706 | 0.112 |
| Antioxidative markers | | | | | | | | |
| Total antioxidant capacity (μmol/L) | 247.10 (6.21) | 250.60 (5.65) | 238.85 (6.81) | 244.15 (4.91) | [−16.23, 25.72] | .630 | .946 | 0.130 |
| Copper (μg/dl) | 108.30 (7.47) | 95.00 (5.94) | 97.95 (8.99) | 83.00 (6.58) | [−7.80, 10.31] | .756 | .946 | 0.161 |
| Zinc (μg/dl) | 58.00 (7.57) | 86.00 (5.82) | 67.50 (6.79) | 81.00 (3.49) | [−0.81, 34.81] | .060† | .596 | 0.609 |
| Ceruloplasmin (mg/dl) | 30.60 (2.33) | 30.70 (1.86) | 26.80 (2.39) | 26.90 (2.25) | [−1.64, 3.07] | .522 | .946 | 0.179 |
| Iron (μg/dl) | 76.00 (15.72) | 84.50 (15.53) | 79.00 (24.07) | 72.00 (19.22) | [−25.30, 39.73] | .634 | .946 | 0.150 |
| Ferritin (μg/L) | 74.00 (22.91) | 47.00 (13.01) | 49.50 (24.26) | 36.00 (10.48) | [−22.87, 33.39] | .683 | .946 | 0.179 |
| Transferrin (mg/dl) | 272.00 (27.76) | 258.00 (25.82) | 282.00 (29.7) | 269.00 (26.79) | [−14.94, 6.65] | .410 | .946 | 0.239 |
| Uric acid (mg/dl) | 3.90 (0.12) | 4.00 (0.19) | 3.60 (0.27) | 4.10 (0.43) | [−0.54, 0.40] | .747 | .946 | 0.102 |
| Albumin (g/dl) | 4.04 (0.16) | 4.20 (0.10) | 4.35 (0.01) | 4.10 (0.06) | [−0.17, 0.20] | .867 | .964 | 0.055 |
| Bilirubin (mg/dl) | 0.51 (0.08) | 0.49 (0.04) | 0.55 (0.10) | 0.56 (0.05) | [−0.15, 0.15] | 1.000 | 1.000 | 0.000 |
| Health-related parameters | | | | | | | | |
| Fibromyalgia Impact Questionnaire | 68.61 (7.17) | 52.47 (9.68) | 47.84 (7.47) | 46.83 (4.87) | [−33.81, −5.43] | .011* | .035 | 0.644 |
| Pain Visual Analogue Scale | 6.85 (0.82) | 5.50 (0.83) | 5.00 (1.40) | 4.95 (1.01) | [−3.16, 1.02] | .279 | .279 | 0.384 |
| Physical health status (PCS-12) | 33.30 (2.46) | 33.93 (1.77) | 29.22 (4.50) | 37.98 (4.59) | [−6.66, 1.01] | .133 | .177 | 0.453 |
| Mental health status (MCS-12) | 31.62 (2.35) | 36.20 (2.94) | 49.10 (3.42) | 39.46 (3.96) | [3.19, 25.24] | .017* | .035 | 0.654 |

*Note.* Data are expressed as median (standard error of median). 95% CI = 95% robust confidence interval of pre–post difference; bpm = beats per minute; Ef.Size = effect size of robust analysis of variance; EVOO = participants consumed extra virgin olive oil; MCS-12 = Mental Component Summary of the Short Form-12 Health Survey; MDA = malondialdehyde; ROO = participants consumed refined olive oil; *p* = probability obtained with the robust statistic; *p*.Adj = adjusted *p* value for multiple tests; PSC-12 = Physical Component Summary of the Short Form-12 Health Survey.

*Statistically significant difference between groups ($p < .05$).

†Approaching significance for robust statistic ($p \leq .10$).

population are likely used to consuming EVOO, so that the exclusive consumption during the trial of ROO, which is free of antioxidant compounds, may be responsible for the observed increase in lipid peroxidation levels. In support of our findings, a number of research teams have reported that olive oil decreased lipid peroxidation in different situations, including rats submitted to oxidative stress (Amamou et al., 2015, using olive oil obtained by cold extraction, which is typical of EVOOs), senescence-accelerated mice (Bayram et al., 2012, using two types of olive oil: one containing low [44 mg gallic acid/kg oil] and one high [532 mg gallic acid/kg oil] amounts of phenolics), and healthy men (Weinbrenner et al., 2004, using EVOO). In contrast, authors have also reported that plasma lipid peroxidation was not affected after intake of EVOO in healthy subjects (Vissers et al., 2001).

There are conflicting data in the literature regarding the effects of olive oil on 8-oxo-dG levels as well, which decreased slightly in the present study in the group that consumed EVOO, though the difference between the groups in the change in levels only approached significance. In previous studies, EVOO consumption lowered levels of 8-oxo-dG in patients with metabolic syndrome (an oxidative stress–related disease;

Mitjavila et al., 2013) and in healthy men (Weinbrenner et al., 2004). In contrast, a diet based on EVOO did not change levels of 8-oxo-dG in rats with experimental cancer (Solanas et al., 2010). Reasons for these differences in findings may be differences in the concentration of antioxidants in the olive oils used, the amount of oil consumed, the duration of the trial period, and/or the experimental model used.

To summarize, our data reveal that consumption of organic EVOO may improve oxidative stress in patients with FM, suggesting that its consumption contributes to the dietary intake of biologically active compounds. The improvement in oxidative stress may be related to the activities of the phenolic compounds (mainly oleuropein and hydroxytyrosol) of olive oil as free-radical scavengers and metal chelators. Oleuropein and hydroxytyrosol possess a catechol group, which is essential for their scavenging activity of hydroxyl radicals and superoxide anions (Visioli, Poli, & Galli, 2002). On the other hand, the phenolic compounds may prevent the generation of free radicals by chelating metal ions (copper and iron) that catalyze reactions of free radical generation (Andrikopoulos, Kaliora, Assimopoulou, & Papageorgiou, 2002).

### *Effects of Olive Oil on the Antioxidative Profile in FM*

Our results did not reveal statistically significant changes in the enzymatic activities of SOD, GPx, and catalase in women with FM after olive oil intake, although these enzymes tended to increase after consumption of both types of organic olive oils. Notably, although the activity levels of the antioxidant enzymes were below normal values at baseline, SOD and GPx activities recovered to normal levels after EVOO consumption (Figure 2). Moreover, SOD showed a moderate-to-large value for effect size, suggesting that it may have been significantly affected by the consumption of olive oil if the sample size had been larger. Previous researchers have reported contradictory results regarding the effect of olive oil on antioxidant enzymes. While olive oil significantly boosted SOD activity in rats submitted to oxidative stress (Amamou et al., 2015, using olive oil obtained by cold extraction, which is typical of EVOOs), investigators observed reduced SOD activity after EVOO ingestion in healthy elderly subjects (Oliveras-López et al., 2013). In addition, whereas consumption of olive oil increased GPx activity in healthy males in one study (Weinbrenner et al., 2004), consumption of EVOO reportedly reduced the activity of GPx in elderly individuals in another (Oliveras-López et al., 2013). Consumption of olive oil also augmented catalase enzymatic activity in elderly subjects (Oliveras-López et al., 2013) and in rats suffering from oxidative stress (Amamou et al., 2015) in prior studies. As we suggested previously, differences in the concentration of antioxidants in the olive oils used, the amount of oil consumed, the duration of the trial period, and the experimental model used may account for the differences in the results.

Our data also show that, though the adjusted probability for zinc levels was far from the significance criterion, the difference in pre–post changes between the two groups approached the criterion for statistical significance (robust statistic). Moreover, zinc levels registered a large value for effect size. Therefore, we may consider that EVOO raised the levels of zinc in females with FM in the present study. We previously reported a significant decline in the zinc level in women with FM in comparison to healthy women (La Rubia et al., 2013).

Overall, the reduced protein and lipid levels and DNA oxidation and the increased zinc levels in patients with FM who consumed EVOO in the present study suggest that the 3-week period of EVOO consumption had an antioxidant effect. However, EVOO consumption had no effect on either TAC or the rest of the antioxidant compounds analyzed. One reason for these results may be that the duration of the trial was insufficient to affect the antioxidant markers. On the other hand, a short-term nutritional trial, such as the present one, may offer certain advantages with respect to a long-term trial, as it allows patients to adhere to a stricter and more controlled diet in relation to the intake of other antioxidants. In this way, the possible interference of other antioxidants can be limited, as can interference from possible changes in the lifestyles of the patients, which could also alter the results (Weinbrenner et al., 2004).

### *Effects of Olive Oil on Health-Related Parameters in FM*

We assessed a number of health-related parameters in women with FM, including severity of pain (VAS), functional capacity (FIQ), and physical (PCS-12) and mental (MCS-12) health status. Our data show that consumption of EVOO significantly decreased the FIQ score in women with FM, while ROO did not change this score. Also, EVOO improved the MCS-12 score in these patients, whereas ROO may have worsened it. Therefore, nutritional supplementation with EVOO improved, not only oxidative stress in women with FM but also their functional capacity and health-related psychological status. These data suggest that EVOO may improve health in women with FM by decreasing oxidative stress. Further analyses revealed that FIQ score positively correlated with lipid peroxidation in the EVOO group, which suggests that EVOO may improve functional capacity in women with FM by decreasing lipid peroxidation levels. These findings reveal the positive effects of improving dietary habits on health in patients diagnosed with FM.

Once again, the absence of prior studies on the effect of olive oil on the symptomatology of patients with FM creates some difficulty for discussing our findings' place in the broader literature. However, in a study that examined the effect of an extract of olives in a mouse model of chronic fatigue syndrome (pathology with similar symptoms to FM), researchers found significant decreases in fatigue and hyperalgesia together with a reduction in lipid peroxidation (Gupta, Vij, & Chopra, 2010).

Finally, our results did not reveal statistically significant changes in pain in women with FM associated with consumption of EVOO or ROO, though there was a tendency for pain to decrease after consumption of EVOO. Similarly, in a previous study investigating the efficacy of a combination of oleanolic

acid (component of olive oil), rosemary extract, and reduced iso-alpha acids from hops on treating pain in patients with FM, investigators found a nonsignificant trend for a decrease in pain and a significant improvement in quality of life (Lukaczer et al., 2005). In another study, researchers found that patients with FM who followed the Mediterranean diet for 2 months reported no significant changes in pain (Michalsen et al., 2005).

### *Limitations*

The main limitation of the present study is the small sample size, which was appropriate for a preliminary study. Therefore, additional research in a larger sample may be useful to confirm the improvement in oxidative stress and health-related parameters in patients with FM after the intake of organic EVOO.

## Conclusion

Certain nutritional components, such as olive oil, exert biological effects that may have implications for disease processes and therefore can act as adjuvants in the treatment of numerous diseases. Scientific evidence of the antioxidant effects of consuming olive oil in FM has, thus far, been lacking. The results of the present study reveal, for the first time, the valuable role EVOO can play in improving oxidative stress and health-related parameters in patients with FM, as reflected by decreased protein and lipid levels and DNA oxidation, increased zinc levels, and improved functional capacity and health-related psychological status. Our findings also suggest that EVOO may improve the functional capacity of women with FM by lowering lipid peroxidation levels. These results are relevant for nursing science because diet supplementation with olive oil may offer a positive means of improving, not only health status but also a biological mediator of symptoms (oxidative stress) in patients with FM. Finally, these findings may ultimately help to improve guidelines for the care of patients with FM by suggesting a direction for the development of nutritional interventions for symptom management.

### Authors' Note

The blood samples of the patients with fibromyalgia used in this study are available and stored in building B3 of the University of Jaén (Spain). Code R for statistical analyses can be obtained by e-mailing a request to mramos@ujaen.es

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### Author Contribution

A. Rus contributed to conception, design, data acquisition, data analysis, and interpretation; drafted the manuscript; critically revised the manuscript; gave final approval; and agrees to be held accountable for all aspects of work, ensuring integrity and accuracy. F. Molina contributed to conception, design, data acquisition, data analysis, and interpretation; drafted the manuscript; critically revised the manuscript; gave final approval; and agrees to be held accountable for all aspects of work, ensuring integrity and accuracy. M. M. Ramos contributed to conception, design, data acquisition, data analysis, and interpretation; drafted the manuscript; critically revised the manuscript; gave final approval; and agrees to be held accountable for all aspects of work, ensuring integrity and accuracy. M. J. Martínez-Ramírez contributed to conception, design, data acquisition, data analysis, and interpretation; drafted the manuscript; critically revised the manuscript; gave final approval; and agrees to be held accountable for all aspects of work, ensuring integrity and accuracy. M. L. Del Moral contributed to conception, design, data acquisition, data analysis, and interpretation; drafted the manuscript; critically revised the manuscript; gave final approval; and agrees to be held accountable for all aspects of work, ensuring integrity and accuracy.

### Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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