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Serum choline, leptin and interleukin-6 levels in fibromyalgia syndrome-induced pain: a case–control study

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Abstract

Background Fibromyalgia Syndrome (FMS) predominantly affects middle-aged women, characterized by musculoskeletal pain, fatigue, and cognitive issues. Choline, an endogenous molecule, may influence FMS due to its analgesic and anti-inflammatory properties. This study compared choline, leptin, and interleukin-6 (IL-6) levels in FMS patients and controls and examining their association with pain severity.

Methods Volunteers with FMS were clinically diagnosed at a Physical Medicine and Rehabilitation Department. The control group included pain-free volunteers. Pain severity was gauged using a numeric scale, dietary choline intake through a questionnaire. Serum choline, leptin and (interleukin)IL-6 levels were measured from fasting blood samples of volunteers with enzyme-linked immunosorbent assays (ELISA).

Results All FMS patients ($n=38$) and healthy volunteers ($n=38$) were female. Pain score in patients with FMS was 7.6 ± 0.2. Dietary choline intake was lower in patients with FMS than the controls ($p=0.036$). Serum choline and leptin levels were lower in the FMS group compared to control ($p=0.03$). Serum IL-6 levels were higher in the FMS group than in the control ($p<0.001$). There was weak positive correlation between IL-6 levels and pain scores and there were no correlation between leptin levels and pain scores in FMS.

Conclusions This research highlights FMS's complex nature, involving neurochemical, immunological, and nutritional factors. It suggests the significance of choline's anti-inflammatory effect, leptin's metabolic function, and IL-6's role in FMS pathology. The results suggest that reduced dietary choline might influence serum choline, leptin, and IL-6 levels, potentially impacting FMS-related pain. This points to the potential of supplementary choline intake in FMS management.

Trial registration Not applicable (Non-interventional study).

Keywords Choline, Interleukin-6, Leptin, Pain, Fibromyalgia, Nutrition, Inflammation

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Introduction

Fibromyalgia Syndrome (FMS) is characterized by widespread musculoskeletal pain along with fatigue, sleep disturbances, and cognitive issues. A defining feature of FMS is nociplastic pain, which arises from altered nociceptive processing in the central nervous system without clear evidence of tissue damage or inflammation. Understanding nociplastic pain is crucial for comprehending FMS's complex pain mechanisms, as it is believed to result from a combination of peripheral and central sensitization processes. Patients with FMS often experience allodynia, where non-painful stimuli are perceived as painful, due to changes such as increased excitability of pain pathways and altered levels of neurotransmitters like serotonin and norepinephrine, both essential for pain modulation. Recent research suggests that inflammatory markers, such as interleukin-6 (IL-6), may play a role in central sensitization mechanisms contributing to nociplastic pain [1].

FMS negatively affects quality of life, with prevalence varying between 2 and 8% depending on the diagnostic criteria used. It is commonly seen in 40–60-year-old individuals and predominantly in females. [2]. Effective management of FMS requires a personalized approach that integrates both pharmacological and non-pharmacological treatments. Pharmacotherapy for FMS includes duloxetine, milnacipran, and pregabalin, which modulate central pain pathways. Other commonly used off-label medications include amitriptyline, cyclobenzaprine, gabapentin, and naltrexone. While these drugs can provide symptom relief, their efficacy and side effects vary, highlighting the need for diverse treatment strategies [3]. Recent advancements in conservative treatments have shown that manual therapy, particularly myofascial techniques, also exercise therapy and CBT (cognitive behavioural therapy), can be effectively integrated into clinical practice, offering a valuable non-pharmacological option for managing fibromyalgia [4–6]. However, it is essential to develop novel drugs or supportive treatments to more effectively relieve FM-related pain, as current treatments vary in their effectiveness.

The factors involved in the pathophysiology of FMS are not clearly defined. It is known that patients with FMS have hypersensitivity to pain with the various painful stimuli such as heat, cold, mechanical and ischemic pressure compared to healthy controls. In the development of hypersensitivity autonomic nervous system dysfunction, hypothalamus–pituitary–adrenal axis abnormalities, immunological activation and the release of proinflammatory mediators contribute [7, 8]. Additionally, it is suggested that in FMS, the balance between cytokines is disrupted and an increase in proinflammatory cytokines is detected. Hyperalgesia has also been shown to be associated with the accumulation of proinflammatory substances and activation of microglia and correlated with pain severity evaluated with numerical rating scale. [9, 10]. It has been reported that the levels of proinflammatory cytokines interleukin (IL)−6 and IL-8 levels in cerebral spinal fluid and serum were increased in patients with FMS and are significantly associated with the severity of symptoms [11].

In addition, it has been shown that the proinflammatory mediator IL-6, which has been found to increase in the pathophysiology of FMS, increases leptin production. Leptin or 'satiety hormone' is an adipokine secreted from white adipose tissue in the body is involved in regulating appetite and energy and associated with the inflammatory response and neuropathic pain [9, 12]. Serum leptin levels of patients with FMS were found to be lower in some studies and higher in others compared to the control group, and there are conflicting results between leptin levels and clinical findings in different studies indicates that fibromyalgia patients who smoke tend to have reduced serum leptin levels and report higher pain experiences, suggesting that smoking may exacerbate pain perception and influence leptin regulation. Additionally, variability in serum leptin levels has been observed among fibromyalgia patients, with some cases showing higher leptin levels that correlate with increased disease severity, while others exhibit lower levels. Studies focusing on women with fibromyalgia who are overweight or obese have reported higher serum leptin levels compared to controls, with significant associations between leptin levels and clinical parameters. This implies that body composition may play a crucial role in the relationship between leptin and fibromyalgia. Collectively, these findings underscore the complex and inconsistent role of leptin in fibromyalgia, contributing to the conflicting results reported in the literature. [9, 13, 14].

Choline is synthesized in the body and it is an essential part of the diet. Choline is a precursor of acetylcholine (ACh) activates cholinergic anti-inflammatory pathway (CAP) by increasing cholinergic transmission via alpha-7 nicotinic acetylcholine (α7nAChR) receptor and inhibits its secretion of inflammatory mediators in central and periphery nervous system [15, 16]. Activation of cholinergic system produces analgesic effects in various acute or chronic pain models in both animals and humans [17–19]. Because of with its anti-inflammatory and analgesic effects; choline administration produced beneficial effects in reducing the dose of aspirin and morphine in acute and inflammatory pain models [20, 21]. Given the role of the cholinergic anti-inflammatory pathway (CAP) in modulating inflammation, it is plausible that low choline levels and insufficient dietary choline intake may contribute to the pathophysiology of fibromyalgia

syndrome (FMS) through inflammatory mechanisms; however, no prior information has been found on this relationship. This study aimed to compare blood levels of choline, leptin, and IL-6 between newly diagnosed, drug-naïve FMS patients who had not received any treatment and healthy controls. It also examined the associations between dietary choline intake, serum choline levels, and numeric pain scores.

Materials and methods

Characteristics of study and patients

This case–control study was approved by Clinical Research Ethics Committee of Dokuz Eylul University (No:469-SBKAEK) and carried out within the framework of the Declaration of Helsinki, Good Clinical Practices Guideline and relevant legislation provisions. The study conducted in Physical Medicine and Rehabilitation Department of Dokuz Eylul University Faculty of Medicine between March 2020 and July 2022.

Inclusion and exclusion criteria of the study

Patients over the age of 18, diagnosed with FMS by a Physical Medicine and Rehabilitation specialist, and who gave written consent to participate, were included in the study as the case group. Volunteers of similar age and gender, who did not have pain in the last 6 months and were not diagnosed with FMS, gave written consent to participate and were included in the study as the control group.

Exclusion criteria of the study for both groups were the presence of acute-chronic inflammatory disease, systemic autoimmune disease, severe cardiovascular system disease, serious respiratory problems, serious psychiatric illness, uncontrolled diabetes, and cancer. Patients who used nonsteroidal anti-inflammatory (NSAI) drugs in the last 48 h, those who received corticosteroid or anti-cytokine therapy within the last 3 months, and pregnant or breastfeeding volunteers were also excluded from the study [14]. Additionally, volunteers whose blood sample or questionnaire data were incomplete were also excluded from the study.

Diagnosis

Patients who have had widespread musculoskeletal pain (right and left halves of the body, lower and upper halves, and axial skeleton) for at least 3 months, characteristic symptoms, and tenderness in 11 of 18 defined tender points on physical examination [22] were clinically diagnosed with FMS clinically according to American College of Rheumatology (ACR) 2010 classification criteria. Pain assessment was evaluated using a 0–10 Numeric Pain Rating Scale (NPRS).

Data collection

Demographical data of the patients and controls, including age, height, weight, Body Mass Index (BMI, kg/m^2), and smoking habits were recorded.

Dietary intake of choline

Since there is a noticeable absence of standard procedures or dietary forms for determining dietary choline intake, adapted FFQ according to national dietary habits was used to determine participants' dietary choline intake. Total amount of dietary choline was obtained using a 28-item food frequency questionnaire (FFQ) consisting of commonly consumed foods in Turkish population with high choline contents include meat, poultry, fish, grains, legumes, nuts, dairy products, vegetables and eggs. This FFQ include the frequency (e.g. daily, weekly, and monthly) and portion size (e.g. glasses, cups and spoons) of each consumed food item during the last 1 month were asked by trained interviewers. All food items containing choline were converted to grams to calculate each participant's average daily total choline intake at baseline. Choline content of foods was calculated using the United States Department of Agriculture (USDA) Database for Choline Content of Common Foods. Total amount of choline intake were analyzed by a Dietitian with Nutrition Data Base Software (version 7.2, Mavi Elma Group, Turkey) [23].

Laboratory analysis

For biochemical measurements, 5 mL of fasting blood sample was taken from the cephalic vein from the volunteers between 9:30–11:30 in the morning. The samples were centrifuged at 3.000–3.500 rpm for 10 min, and obtained serum samples were seperated and kept in −20 °C until analysis. Choline (Biovision, #K615, USA), IL-6 (Invitrogen, KHC0061, USA) and Leptin (BOSTER-bio, EK0437, USA) levels were analysed by spectrophotometric methods with commercially available ELISA kits according to manufacturer's instructions.

Statistical analyses

Descriptive data were presented as numbers (n) and percentage (%) for categorical variables and mean and standard deviations (Mean ± SD) for continuous variables. Biochemical parameters (Choline, Leptin, IL-6) and other measurements of the patient and control groups were evaluated by using Student's t test in parametric conditions and Mann–Whitney U tests in nonparametric conditions. The correlation between different variables was investigated using Spearman correlation analysis. The data were analysed with Statistical Package for the Social Sciences (SPSS-24, SPSS INC. Chicago,IL,USA).

Recognizing the importance of maintaining statistical rigor, we recalculated the power of our study using OpenEpi, a widely recognized statistical tool for epidemiologic studies [24]. $p < 0.05$ was considered significant.

Results

Patient characteristics

All volunteers were female in both groups. Data collected from 76 female volunteers in 2 separate groups, comprising 38 volunteers with FMS and 38 controls. Two volunteers in the control group were excluded from the study analysis because their blood samples could not be taken and their biochemical analyzes could not be performed. The mean age of the volunteers was 43.6 ± 2.0 years (range 25–66) in the control group and 49.5 ± 2.2 years (range 20–69) in the FMS group.

All volunteers in the control group were pain-free. In the FMS group, the pain severity of the volunteers was 7.6 ± 0.2, which was significantly higher compared to the control group ($p < 0.0001$). The mean BMI was 24.2 ± 0.7 in the control group and 25.7 ± 0.8 in the FMS group, and there was no significant difference between the groups ($p = 0.153$) (Table 1). Eight participants in the FMS group had chronic conditions, including diabetes mellitus (2 patients), hypertension (5 patients), asthma (2 patients), hyperthyroidism (2 patients), and migraine (1 patient), all of which were under control. In the control group, four participants had chronic conditions, including diabetes mellitus (2 patients), hypertension (3 patients), and asthma (1 patient), which were also managed effectively. To provide additional context, the common medications used by these participants included ramipril for hypertension, metformin for diabetes, and salbutamol as a bronchodilator for asthma.

For the final sample size of 38 participants per group, we recalculated the power specifically for detecting differences in choline, IL-6 and leptin levels. The recalculated power for detecting a medium effect size (d = 0.50) was found to be 100% which indicates that, despite the reduction in sample size, the study retained sufficient power to detect statistically significant differences between the groups for these key variables.

Dietary intake of choline

Dietary choline intake within a month was significantly lower in the FMS group (263.3 ± 18.1 mg) compared to the control group (310.4 ± 19.8 mg; $p = 0.036$) (Fig. 1). A weak-moderate positive correlation was found between serum total choline levels and dietary choline intake in the control group ($R_s = 0.124$, $p = 0.47$) and the FMS group ($R_s = 0.201$, $p = 0,23$) (Supplementary Fig. 1).

Laboratory analyses

Serum choline and leptin levels were significantly lower in the FMS group (602.4 ± 99.3 µmol/mL and 50,852 ± 9309 pg/mL) compared to the control group (1793 ± 288.6 µmol/mL and 124,494 ± 30,562 pg/mL; $p = 0.03$ and $p = 0.03$) Serum IL-6 levels were significantly higher in the FMS group (3.3 ± 0.3 pg/mL) compared to the control group (2.1 ± 0.3 pg/mL; $p < 0.001$) (Fig. 2).

There was a weak positive correlation between total choline and leptin levels in patients with FMS ($R_s = 0.121$, $p = 0.47$). There was a weak negative correlation between total choline and IL-6 levels ($Rs = -0.058$, $p = 0.08$) and between total choline and pain scores ($Rs = -0.047$, $p = 0.211$) in patients with FMS. There was weak positive correlation between IL-6 levels and pain scores ($R_s = 0.025$; $p = 0.47$) and there were no or negligable correlation between leptin levels and pain scores in FMS ($R_s = 0.001$, $p = 0.689$) (Supplementary Fig. 2).

Table 1 Demographic characteristics of patients (FMS: Fibromyalgia Syndrome, BMI: Body Mass Index, NPRS: Numeric Pain Rating Scale)

	Control ($n = 38$)	FMS ($n = 38$)	p
Age (year, mean ± SD)	43.6 ± 2.0	49.5 ± 2.2	0.046
Age Range	25–66	20–69	
Smokers n (%)	13 (34.2%)	11 (28.9%)	0.510
BMI (kg/m^2)	24.2 ± 0.7	25.7 ± 0.8	0.153
Underweight (BMI lower 20 kg/m2; %)	10.6	13.2	
Normal (BMI between 20–25 kg/m2; %)	55.3	39.5	
Overweight (BMI between 25–30 kg/m2; %)	21.1	23.7	
Obese individuals (BMI over 30 kg/m2; %)	13.2	23.7	
Pain severity according to NPRS	0 ± 0	7.6 ± 0.2	< 0.001

Student's *t* test and Mann Whitney U test used for comparison of two groups. *, $p < 0.05$; ***, $p < 0.001$ vs. control

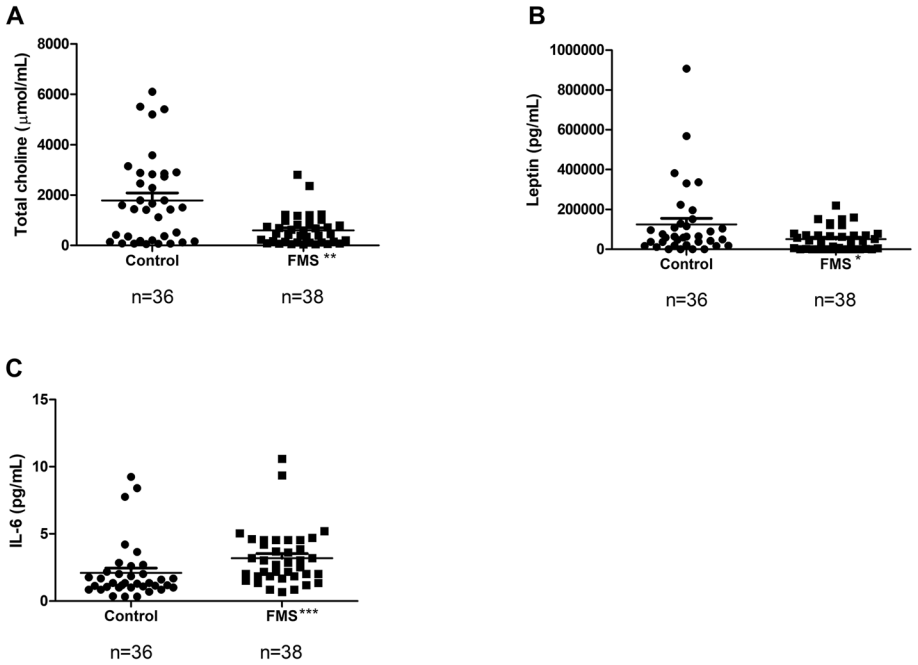

Fig. 1 Serum total choline, leptin and IL-6 levels of groups. Shown are serum total choline (A), leptin (B) and IL-6 (C) levels in control and FMS groups. The mean and standard deviation (SD) are shown in the figures. Mann Whitney U test used for comparison of two groups. *: $p = 0.002$, **: $p = 0.034$, ***: $p < 0.001$ vs control. FMS: Fibromyalgia Syndrome; IL-6: Interleukin 6

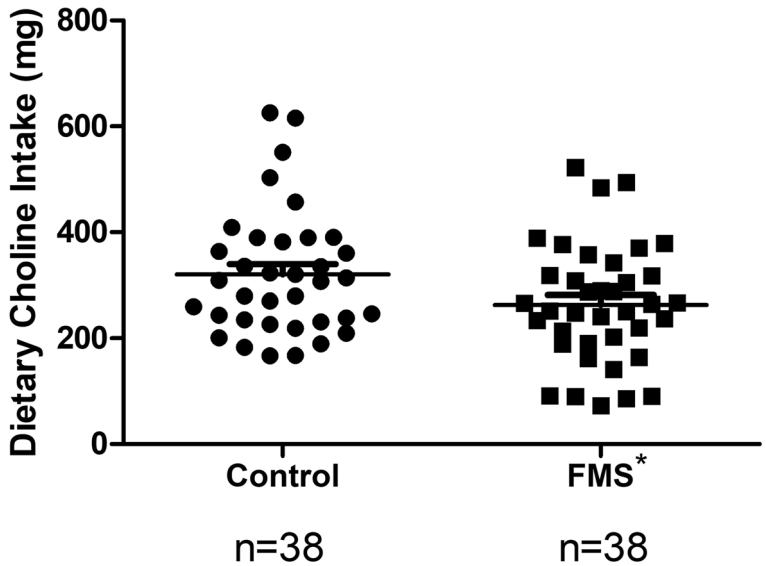

Fig. 2 Dietary choline intake of groups. Shown are dietary choline intake in control and FMS groups within a month. The mean and standard deviation (SD) are shown in the figures. Student's *t* test used for comparison of two groups. *: $p = 0.036$ vs control. FMS: Fibromyalgia Syndrome

Discussion

In our study, we investigated the role of choline, which suppresses inflammation by activating the cholinergic anti-inflammatory pathway (CAP) and possesses analgesic effects in the pathophysiology of Fibromyalgia syndrome (FMS). The results demonstrated that serum choline levels of newly diagnosed, drug-naive patients with FMS were significantly lower compared to the control group. Additionally, a weak-moderate positive correlation was found between serum choline levels and

choline consumption, while nutritional choline intake was lower in patients with FMS compared to controls.

All volunteers diagnosed in the FMS group were female in current study which is consistent with the majority of clinical studies since the FMS incidence is 8–9 times higher in women than in men [25]. A recent review on FMS patients indicate that the mean age of participants aligns with the middle-aged population, and there is a high percentage of women with fibromyalgia. Common limitations include small sample sizes for the intervention groups and the lack of physical tests specifically validated for FMS patients. Although our study focused on drug-naïve and newly diagnosed patients, the characteristics of our participants regarding age and gender were similar to those in other studies. These factors, along with the COVID-19 pandemic, contributed to the limited sample size and the lack of comprehensive physical testing [26]. The severity of pain, evaluated with the Numeric Pain Rating Scale (NPRS), was 7.6 ± 0.2 in the volunteers with FMS in this study. In line with our results, Napadow et al. showed that pain scores were found to be 4.8 ± 2.4 in 18 volunteers with FMS and significantly higher compared to matched healthy controls [27].

Choline is an essential nutrient, which is the precursor of the neurotransmitter acetylcholine, which has a role in many physiological events in the body. It contributes to the synthesis of acetylcholine, mediating the analgesic effect in various acute or chronic pain models in both animals and humans by stimulating nAChRs at high doses [17, 18, 28]. Choline also activates the CAP to control inflammation via cholinergic receptors through neuronal and humoral pathways [15]. Studies report that nAChR agonists and positive allosteric modulators may be potential drug targets for the treatment of neuroinflammatory and neuropathic pain [20, 29, 30].There is lack of comprehensive information about the change in blood choline levels in patients with FMS. Proton MR spectroscopy (1H-MR spectroscopy) analyzes showed that choline levels were low in the left hippocampus of patients with FMS, and the choline/creatine ratio measured from the right dorsolateral prefrontal cortices of patients with FMS has been associated with pain severity [31, 32]. On the contrary, the choline/creatinine ratio was found to be increased in the anterior cingulate cortex of patients with FMS and it has been suggested that it may be associated with improvement of the deterioration of parasympathetic dysfunction [33]. The finding that serum choline levels are lower than in the control group suggests that choline deficiency may have a role in the pathogenesis of FMS. However, contrasting findings in other brain regions suggest a complex, region-specific role of choline in FMS pathogenesis, necessitating further research to clarify the differences between the studies, as well as peripheral (i.e. blood) and central changes of choline.

The correlation between blood choline levels and dietary choline intake in both the FMS and control groups was also evaluated. Food frequency questionnaire (FFQ) results showed that the dietary intake of choline within a month was significantly higher in controls than in participants with FMS. A weak to moderate positive correlation was found between serum total choline levels and choline intake in the FMS group and healthy controls. Apart from pharmacological therapy, nutrition has recently become an important complementary therapeutic approach in the treatment of FMS recently. Studies reported that a vegetarian or mediterranean type diet may have positive effects on improving FMS symptoms in volunteers with FMS [34, 35]. In addition, deficiencies in certain essential nutrients may have a role in other FMS symptoms by inhibiting pain-preventing mechanisms [36]. It has been shown that there may be changes in serum choline levels due to dietary choline intake, and severe choline restriction in the diet can cause up to 50% reduction in circulating choline levels [37]. Therefore, the fact that fasting serum total choline levels in volunteers with FMS are lower than in controls in this study suggests that a diet low in choline may also contribute to FMS-related pain.

FMS is a chronic disease that affects the responses of the sympathetic nervous system and hypothalamus–pituitary–adrenal axis by causing changes in the systemic levels of proinflammatory cytokines. An increase in sympathetic activity is demonstrated in individuals with FMS and a positive correlation has been defined between inflammatory mediators and pain severity [10, 38]. Accordingly, muscle injury, hyperalgesia, activation of astrogliocytes, degenerative changes in hippocampus, increase in serum IL-1β, IL-6 and TNF-α levels were observed in rats with the FMS model [11]. It has been reported that IL-6 levels in cerebrospinal fluid (CSF) or serum are increased in patients with FMS and are significantly associated with the severity of symptoms like pain, fatigue, depression and stress [11, 39, 40]. A randomized controlled study showed that dietary choline intake reduces IL-6 levels in patients with metabolic syndrome accompanied by chronic inflammation, which is consistent with our results, suggesting that insufficient intake of choline in FMS contributes to the pathophysiology by impairing inflammation control [41].

In our study, choline and IL-6 levels, contributors of inflammation, were measured from fasting serum samples. Choline levels were found to be lower and IL-6 levels were higher in volunteers with FMS than in the control group. Our data corroborate recent findings in the field, demonstrating that individuals with Fibromyalgia Syndrome (FMS) have diminished levels of choline,

alongside elevated IL-6 levels, compared to the control group, signifying a pronounced inflammatory profile in FMS [42].

Leptin is a protein that found predominantly in adipose tissue or brain that contributes to the regulation of energy homeostasis and the transmission of a feeling of satiety. It has been reported that leptin levels in relation to painful conditions are either unchanged, increased, or decreased in studies compared with healthy controls. There are conflicting results presented by different study groups regarding its relationship with clinical symptoms [9, 13, 14, 43]. Studies have shown that adipokines, especially leptin and adiponectin, are associated with inflammatory response and neuropathic pain [9, 12, 44]. In case of chronic pain conditions, including FMS, leptin's role might extend beyond traditional understandings, influencing pain perception [43]. Female patients with painful multisomatoform disorder have been shown to have significantly higher leptin levels compared to controls [43]. In a study comparing 16 FMS volunteers with 21 healthy controls, it was found that there was no change in leptin levels in the FMS group compared to the control [45]. In another cross-sectional study, leptin levels in volunteers with FMS were found to be low, similar to our study [46]. Recent findings suggest that adipokines exhibit complex interactions within inflammatory cascades, impacting pain signaling in chronic pain conditions like Gulf War illness and osteoarthritis [47, 48].

Regarding the cholinergic system and leptin interaction, in different studies, it was shown that oral choline supplementation caused a significant decrease in serum leptin levels, while in another study, choline administration did not cause a significant change in serum leptin levels [49, 50]. Dietary patterns, specifically those rich in choline like the Mediterranean diet, not only influence adipokine profiles but also modulate broader inflammatory markers, including C-reactive protein (CRP), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α) [51–53].

Notably, our study found that leptin levels in FMS patients were decreased compared to controls, reflecting the variability observed in the literature regarding how leptin responds to painful conditions, which appears to be highly personalized and may depend on the type and severity of pain individuals experience.

Moreover, recent investigations, building upon the findings of previous studies point out an increase in leptin levels in FMS patients after three months of treatment with tricyclic antidepressants (eg, amitriptyline), serotonin reuptake inhibitors (SSRIs), and serotonin/noradrenaline reuptake inhibitors (SNRIs) [45], suggesting that leptin's role extends beyond a static biomarker to a potentially active participant in the pathophysiology of FMS influenced by therapeutic interventions [43]. Considering previous evidence coupled with the finding of the study, choline might be considered as a potential treatment option in managing FMS, targeting both metabolic and pain modulation pathways including leptin levels. In the present study, no significant correlation was observed between serum choline levels and dietary choline intake, leptin, or IL-6 levels in the FMS group. Similarly, no significant correlations were noted between these markers and pain scores.

In conclusion, FMS is a chronic disease accompanied by inflammatory processes. As an indicator of inflammation, increased IL-6 levels in patients in the FMS group compared to the control confirm this. Additionally, it is possible that insufficient dietary choline intake and choline deficiency may contribute to the pathophysiology of FMS by negatively affecting inflammatory processes. Further studies are needed to investigate the role of choline in the pathophysiology of FMS, which activates CAP, provide communication between cholinergic nerves and the immune system, and plays an important role in the control of inflammation. This study was conducted during the COVID-19 pandemic and subsequent lockdowns significantly impacted patients with FMS. Reduced access to face-to-face healthcare, decreased physical activity, and heightened feelings of isolation, anxiety and depression exacerbated symptoms of FMS. This situation highlighted the importance of accessible, remote care options and supportive treatment strategies to manage chronic pain related to FMS [54]. Additionally randomized clinical trials can provide more evidence of the effects of choline and its possible therapeutic role in FMS-induced pain. Moreover, low levels of serum leptin were detected, which necessitates further research with a higher number of cases to clarify the relationship between choline and leptin due to the contradictory findings in the studies.

Limitations

The study has several limitations. Firstly, it focuses on newly diagnosed, drug-naïve FMS patients, limiting the applicability of results to those already receiving treatment. The sample size, initially estimated for 64 participants per group to achieve 80% power at a 95% confidence level (medium effect size, $d = 0.50$), was reduced due to the pandemic, concluding with 38 participants per group over 2.5 years. While blood choline levels were measured to improve accuracy alongside self-reported dietary intake, reliance on self-reports introduces potential inaccuracies. Lastly, the significant difference in the mean age between the FMS and control groups may introduce confounding effects, as age can influence various physiological and biochemical parameters. Additionally, because healthy controls

without chronic diseases were included in the study, this age difference might reflect the characteristics of the selected population rather than the condition itself. Future studies could enhance accuracy by focusing solely on blood choline levels and their correlation with dietary intake. It is also recommended to perform validated food consumption frequency to determine the dietary choline intake specific to the Turkish population. The lack of control over pre-testing physical activity could have affected cytokine levels during data collection, adding variability between groups. This is a noteworthy limitation, as differences in baseline cytokine levels could be influenced by varying physical activity, and it is also acknowledged in the limitations section. Further research is necessary to confirm and extend these findings.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12891-025-08337-0.

Supplementary Material 1.

Supplementary Material 2.

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Authors' contributions
EB, EH, EA, AG and MAA contribute conception and design of the study. EB, IT, EA contibuted the acquisition of data. RU conducted statistical analyses. EB, EH, SK, RU and MAA contributed the analysis and interpretation of data. EB, EH, RU and MAA drafted the article, and all authors revised it critically.

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Data availability
The data that support the findings of this study are available from the corresponding author upon request.

Declarations

Ethics approval and consent to participate
This case–control study was approved by Clinical Research Ethics Committee of Dokuz Eylul University (No:469-SBKAEK) and carried out within the framework of the Declaration of Helsinki, Good Clinical Practices Guideline and relevant legislation provisions. Patients over the age of 18, diagnosed with FMS by a Physical Medicine and Rehabilitation specialist, and who gave written consent to participate, were included in the study as the case group. Volunteers of similar age and gender, who did not have pain in the last 6 months and were not diagnosed with FMS, gave written consent to participate and were included in the study as the control group.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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References

1. Martínez-Lavín M. Centralized nociplastic pain causing fibromyalgia: an emperor with no cloths? Clin Rheumatol. 2022;41:3915–7.
2. Heidari F, Afshari M, Moosazadeh M. Prevalence of fibromyalgia in general population and patients, a systematic review and meta-analysis. Rheumatol Int . 2017;37(9):1527–39.
3. Martinez JE, Guimarães I. "Fibromyalgia - are there any new approaches?". Best Pract Res Clin Rheumatol. 2024;38(1):101933.
4. Audoux CR, Estrada-Barranco C, Martínez-Pozas O, Gozalo-Pascual R, Montaño-Ocaña J, García-Jiménez D, et al. What Concept of Manual Therapy Is More Effective to Improve Health Status in Women with Fibromyalgia Syndrome? A Study Protocol with Preliminary Results. Int J Environ Res Public Health. 2023;20(2):1061.
5. Clauw DJ. Fibromyalgia: a clinical review. JAMA. 2014;311:1547–55.
6. Macfarlane GJ, Kronisch C, Dean LE, Atzeni F, Häuser W, Fluß E, et al. EULAR revised recommendations for the management of fibromyalgia. Ann Rheum Dis. 2017;76:318–28.
7. Adler GK, Geenen R. Hypothalamic–Pituitary–Adrenal and Autonomic Nervous System Functioning in Fibromyalgia. Rheum Dis Clin. 2005;31:187–202.
8. Sluka KA, Clauw DJ. Neurobiology of fibromyalgia and chronic widespread pain. Neuroscience. 2016;338:114–29.
9. Bokarewa MI, Erlandsson MC, Bjersing J, Dehlin M, Mannerkorpi K. Smoking is associated with reduced leptin and neuropeptide y levels and higher pain experience in patients with fibromyalgia. Mediators Inflamm. 2014;2014:627041.
10. Ohgidani M, Kato T, Hosoi M, Tsuda M, Hayakawa K, Hayaki C, et al. Fibromyalgia and microglial TNF-α: Translational research using human blood induced microglia-like cells. Sci Rep. 2017;7(1):11882.
11. Ernberg M, Christidis N, Ghafouri B, Bileviciute-Ljungar I, Löfgren M, Bjersing J, et al. Plasma Cytokine Levels in Fibromyalgia and Their Response to 15 Weeks of Progressive Resistance Exercise or Relaxation Therapy. Mediators Inflamm. 2018;2018:3985154.
12. Aktas G, Sit M, Tekçe H. New adipokines: Leptin, adiponectin and omentin. Abant Med J. 2013;2(1):56–62.
13. Ataoğlu S. The Relations between Serum Leptin Level with Disease Activity and Inflammatory Markers in Fibromyalgia Patients. North Clin Istanbul. 2017;5:102–8.
14. Paiva ES, Andretta A, Batista ED, Lobo MMMT, de Miranda RC, Nisihara R, et al. Serum levels of leptin and adiponectin and clinical parameters in women with fibromyalgia and overweight/obesity. Arch Endocrinol Metab. 2017;61:249–56.
15. Pavlov VA, Wang H, Czura CJ, Friedman SG, Tracey KJ. The cholinergic anti-inflammatory pathway: a missing link in neuroimmunomodulation. Mol Med. 2003;9:125–34.
16. Cansev M, Ilcol Y, Yilmaz S, Hamurtekin E, H Ulus I. Peripheral administration of CDP-choline, phosphocholine or choline increases plasma adrenaline and noradrenaline concentrations. Auton Autacoid Pharmacol. 2008;28:41–58.

17. Bartolini A, Ghelardini C, Fantetti L, Malcangio M, Malmberg-Aiello P, Giotti A. Role of muscarinic receptor subtypes in central antinociception. Br J Pharmacol. 1992;105:77–82.
18. Gurun MS, Leinbach R, Moore L, Lee CS, Owen MD, Eisenach JC. Studies on the safety of glucose and paraben-containing neostigmine for administration. Anesth Analg. 1997;85:317–23.
19. Wang Y, Su D-M, Wang R-H, Liu Y, Wang H. Antinociceptive effects of choline against acute and inflammatory pain. Neuroscience. 2005;132:49–56.
20. Abbas M, Rahman S. Effects of alpha-7 nicotinic acetylcholine receptor positive allosteric modulator on lipopolysaccharide-induced neuroinflammatory pain in mice. Eur J Pharmacol. 2016;783:85–91.
21. Bagdas D, Sonat FA, Hamurtekin E, Sonal S, Gurun MS. The antihyperalgesic effect of cytidine-5′-diphosphate-choline in neuropathic and inflammatory pain models. Behav Pharmacol. 2011;22:589–98.
22. Wolfe F, Rasker JJ. The Evolution of Fibromyalgia, Its Concepts, and Criteria. Cureus. 2021;13(11):e20010.
23. Patterson KY, Bhagwat SA, Williams JR, Howe JC, Holden JM, Zeisel SH, et al. USDA Database for the Choline Content of Common Foods Relase 2. Nutrient Data Laboratory, Beltsville Human Nutrition Research Center, ARS, USDA. 2015;1–57
24. Sullivan KM, Dean A, Soe MM. OpenEpi: a web-based epidemiologic and statistical calculator for public health. Public Health Rep. 2009;124:471–4.
25. Colomer-Carbonell A, Sanabria-Mazo JP, Hernández-Negrín H, Borràs X, Suso-Ribera C, Garciá-Palacios A, et al. Study protocol for a randomised, double-blinded, placebo-controlled phase III trial examining the add-on efficacy, cost-utility and neurobiological effects of low-dose naltrexone (LDN) in patients with fibromyalgia (INNOVA study). BMJ Open. 2022;12:e055351.
26. Silva, Lais Carneiro; Dittgen, Michelle; Meroni, Roberto; Sanchez-Romero, Eleuterio A.; Rossettini, Giacomo; Villafañe, Jorge Hugo; Corbellini C. Exercise Training for Women With Fibromyalgia Syndrome. Top Geriatr Rehabil. 2024;40:147–57.
27. Napadow V, LaCount L, Park K, As-Sanie S, Clauw DJ, Harris RE. Intrinsic brain connectivity in fibromyalgia is associated with chronic pain intensity. Arthritis Rheum. 2010;62:2545–55.
28. Yaksh T, Grafe M, Malkmus S, L. Rathbun M, C. Eisenach J. Studies on the Safety of Chronically Administered Intrathecal Neostigmine Methylsulfate in Rats and Dogs. Anesthesiology. 1995;82(2):412–27.
29. Bagdas D, Wilkerson JL, Kulkarni A, Toma W, AlSharari S, Gul Z, et al. The α7 nicotinic receptor dual allosteric agonist and positive allosteric modulator GAT107 reverses nociception in mouse models of inflammatory and neuropathic pain. Br J Pharmacol. 2016;173:2506–20.
30. Hone AJ, McIntosh JM. Nicotinic acetylcholine receptors: Therapeutic targets for novel ligands to treat pain and inflammation. Pharmacol Res. 2023;190:106715.
31. Fayed N, Garcia-Campayo J, Magallón-Botaya R, Andrés-Bergareche H, Luciano J, Andres E, et al. Localized 1H-NMR spectroscopy in patients with fibromyalgia: A controlled study of changes in cerebral glutamate/glutamine, inositol, choline, and N-acetylaspartate. Arthritis Res Ther. 2010;12(4):R134.
32. Petrou M, Harris RE, Foerster BR, Mclean SA, Sen A, Clauw DJ, et al. Proton MR spectroscopy in the evaluation of cerebral metabolism in patients with fibromyalgia: Comparison with healthy controls and correlation with symptom severity. Am J Neuroradiol. 2008;29:913–8.
33. Jung YH, Kim H, Lee D, Lee JY, Lee WJ, Moon JY, et al. Abnormal neurometabolites in fibromyalgia patients: Magnetic resonance spectroscopy study. Mol Pain. 2021;17:1744806921990946.
34. Donaldson MS, Speight N, Loomis S. Fibromyalgia syndrome improved using a mostly raw vegetarian diet: an observational study. BMC Complement Altern Med. 2001;1:7.
35. Tomaino L, Serra-Majem L, Martini S, Ingenito MR, Rossi P, La Vecchia C, et al. Fibromyalgia and Nutrition: An Updated Review. J Am Coll Nutr. 2021;40:665–78.
36. Bjørklund G, Dadar M, Chirumbolo S, Aaseth J. Fibromyalgia and nutrition: Therapeutic possibilities? Biomed Pharmacother. 2018;103:531–8.
37. Panel E, Products D. Dietary Reference Values for choline. EFSA J. 2016;14(8):1–76.
38. Kosek E, Altawil R, Kadetoff D, Finn A, Westman M, Le Maître E, et al. Evidence of different mediators of central inflammation in dysfunctional and inflammatory pain–interleukin-8 in fibromyalgia and interleukin-1 β in rheumatoid arthritis. J Neuroimmunol. 2015;280:49–55.
39. Geiss A, Rohleder N, Anton F. Evidence for an association between an enhanced reactivity of interleukin-6 levels and reduced glucocorticoid sensitivity in patients with fibromyalgia. Psychoneuroendocrinology. 2012;37(5):671–84.
40. Mendieta D, Luz De La Cruz-Aguilera D, Isabel Barrera-Villalpando M, Becerril Villanueva LE, Arreola R, Hernández-Ferreira E, et al. IL-8 and IL-6 primarily mediate the inflammatory response in fibromyalgia patients. J Neuroimmunol. 2016;15:290:22–5.
41. Dibella M, Thomas MS, Alyousef H, Millar C, Blesso C, Malysheva O, et al. Choline intake as supplement or as a component of eggs increases plasma choline and reduces interleukin-6 without modifying plasma cholesterol in participants with metabolic syndrome. Nutrients. 2020;12:1–12.
42. Giorgi V, Bazzichi L, Batticciotto A, Pellegrino G, Di Franco M, Sirotti S, et al. Fibromyalgia: one year in review 2023. Clin Exp Rheumatol. 2023;41:1205–13.
43. Achenbach J, Rhein M, Glahn A, Frieling H, Karst M. Leptin promoter methylation in female patients with painful multisomatoform disorder and chronic widespread pain. Clin Epigenetics. 2022;14:1–7.
44. Lim G, Wang S, Zhang Y, Tian Y, Mao J. Spinal leptin contributes to the pathogenesis of neuropathic pain in rodents. 2009.
45. Ablin JN, Aronov N, Shimon I, Kanety H, Pariente C, Aloush V, et al. Evaluation of leptin levels among fibromyalgia patients before and after three months of treatment, in comparison with healthy controls. Pain Res Manag. 2012;17:89–92.
46. Olama S, O Elsaid T, El-Arman M. Serum Leptin in Egyptian patients with fibromyalgia syndrome: Relation to disease severity. Int J Rheum Dis. 2013;16(5):583–9.
47. Gløersen M, Steen Pettersen P, Neogi T, Jafarzadeh SR, Vistnes M, Thudium CS, et al. Associations of Body Mass Index With Pain and the Mediating Role of Inflammatory Biomarkers in People With Hand Osteoarthritis. Arthritis Rheumatol (Hoboken, NJ). 2022;74:810–7.
48. Hodgin KS, Jones CL, Younger JW. Fatigue and Pain Severity in Gulf War Illness Is Associated With Changes in Inflammatory Cytokines and Positive Acute Phase Proteins. J Occup Environ Med. 2022;64:905–11.
49. Elsawy G, Abdelrahman O, Hamza A. Effect of Choline Supplementation on Rapid Weight Loss and Biochemical Variables Among Female Taekwondo and Judo Athletes. J Hum Kinet. 2014;40:77–82.
50. Kiyici S, Basaran NF, Cavun S, Savci V. Central injection of CDP-choline suppresses serum ghrelin levels while increasing serum leptin levels in rats. Eur J Pharmacol. 2015;764:264–70.
51. Stefanadis C, Pitsavos C, Detopoulou P, Antonopoulou S, Panagiotakos DB. Dietary choline and betaine intakes in relation to concentrations of inflammatory markers in healthy adults: the ATTICA study. Am J Clin Nutr. 2008;87:424–30.
52. Cobos-Palacios L, Muñoz-Úbeda M, Gallardo-Escribano C, Ruiz-Moreno MI, Vilches-Pérez A, Vargas-Candela A, et al. Adipokines Profile and Inflammation Biomarkers in Prepubertal Population with Obesity and Healthy Metabolic State. Child (Basel, Switzerland). 2022;9(1):42.
53. Sánchez-Rosales AI, Guadarrama-López AL, Gaona-Valle LS, Martínez-Carrillo BE, Valdés-Ramos R. The Effect of Dietary Patterns on Inflammatory Biomarkers in Adults with Type 2 Diabetes Mellitus: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. Nutrients. 2022;14(21):4577.
54. Romero EAS, Rolando LM, Villafañe JH. Impact of Lockdown on Patients with Fibromyalgia. Electron J Gen Med. 2022;19(3):em366.

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