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Effect of plasma free fatty acids on lung function in male COPD patients

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Inflammation and oxidative stress play a pivotal role in COPD pathogenesis. Free fatty acids (FFA) as signaling molecules through a series of G-proteins coupled receptors, play an important role in regulation of the immune system and oxidative stress. For this reason, we decided to investigate the profile of FFA in the plasma in the COPD patients. This is a case-control study comparing 40 male patients with COPD and 40 healthy controls. Biochemical plasma parameters were measured by Autoanalyzer, Malondialdehyde by TBA, total antioxidant capacity via FRAP method and the concentration of free fatty acids were measured by gas chromatography. Then the relationship between the data and the spirometric findings of the patients was determined. In male COPD patients, fasting glucose, myristic acid, palmitic acid, stearic acid, oleic acid, elaidic acid, linoleic acid, linolenic acid and total FFA showed a significant difference with the control group. Also, a positive correlation between the medium chain FFA and lung function was observed. The results of the present study showed that the concentration of different free fatty acids is different in healthy people and male COPD patients, and these differences, especially in the case of medium and long chain fatty acids, can be related to the lung function.

Keywords COPD, Free fatty acids, Inflammation, Oxidative stress, Spirometriy

Chronic obstructive pulmonary disease (COPD) is a disorder lung function which reletad to chronic obstruction of the air flow in the lungs[1] with common symptoms of chronic cough, shortness of breath and abundant phlegm production[2]. Currently, According to WHO reports, COPD is the fourth cause of death in the world[3]. Also, based on performed studies, the prevalence of COPD in Iran is reported as 1.68 to 10%[4]. The molecular basis of COPD is affected by various factors, including genetic background, cellular aging, chronic inhalation of toxic agents, oxidative stress, innate and adaptive immune system function, autophagy and etc[5]. Systemic inflammation associated with COPD can establish the relationship between this diseade and other comorbidities such as cardiovascular diseases, diabetes, osteoporosis, and skeletal muscle dysfunction[1]. There is also considerable evidence that oxidative stress is one of the most important factors involved in the pathogenesis of COPD and taking antioxidants may be usefull in managing COPD[6,7].

Free Fatty acids are classified according to their number of carbon into short chain fatty acids (SCFAs), medium chain fatty acids (MCFAs) and long chain fatty acids (LCFAs)[8]. Beside to energy producing role of Free fatty acids (FFAs), they are very important mediator for many cellular functions and physiology such as production of oxidative stress status, inflammation and hormones and production of hormones and cytokines. Actions are mediated through free fatty acid receptors (FFARs). Four types of these receptors including FFAR1, FFAR2, FFAR3, and FFAR4 have been identified[8].

Blood concentarion FFA are associated with cardiovascular disease[9], increased risk of arthritis in obese people[10], Sleep-related breathing disorders[11], ROS production and COX-2 expression[12] and digestive system function as well as endocrine balance[13]. Long-chain FFAs can increase intracellular Ca^{2+} concentration through induction of Gq-coupled FFAR1 and FFAR4, leading to increased airway muscle contractions[14].

The level and profile of fatty acids affect inflammatory reactions and any changes in the type of free fatty acids in plasma and cell membranes of immune cells, can induce or inhibit inflammation by altration the profle of

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cytokines and other mediators[15]. Inflammation plays a central role in the occurrence of COPD, and the release of inflammatory mediators and destructive enzymes by inflammatory cells, especially in immune cells that have migrated to the lung tissue, cause the gradual destruction of lung tissue in COPD[16].

On the other hands, elevation of plasma FFA concentration in leads to an increase in activation NFκB and production intracellular free radicals, which indicates the role of fatty acids in increasing inflammation and oxidative stress, which creates a potential link between inflammation and disease[17]. Oxidative stress has been implicated in various diseases, including age-related diseases such as diabetes, chronic kidney disease, COPD, cognitive impairment, dementia, and cancer[18].

The relationship between fatty acid metabolism and the progression of COPD is of great clinical importance and has been the subject of numerous studies. The results of these studies show the relationship between the reduction of lung function and inflammation with the consumption of a diet containing ω6 type PUFA fatty acids and many studies have focused on evaluating the effectiveness of receiving ω3 fatty acids in COPD patients. However, these data cannot confirm with high certainty the existence of a positive correlation between fatty acid intake and lung function, as well as COPD progression and disease prognosis[19].

Despite conducting numerous studies on the role of fatty acids in COPD, all these studies have focused on the effects of fatty acids in the diet, and in fact, the conclusions rely on examining the composition of dietary fatty acids consumed or the experimental consumption of specific fatty acids in animal or human studies, however, because the body's fatty acid profile changes as a result of the body's natural metabolism, the effects of intestinal bacteria and the synthesis of fatty acids, especially in the liver, plasma free fatty acids profile is not only dependent on dietary fatty acids. For this reason we decided to investigate the profile of free fatty acids in the plasma of male COPD patients. The results of this study can help in identifying useful and harmful fatty acids affecting lung function so that by changing the diet and consuming sources of more useful fatty acids or by making synthetic agonists of these fatty acids, COPD patients can improve their breathing.

Materials and methodes

Collection of samples

This case-control study was conducted with 40 male patients with COPD and 40 healthy men as controls (control group) from October 2021 until April 2022.

Inclusion criteria: All male patients who referred to the pulmonologist office and were under 65 years of age, and their FEV1/FVC ratio was less than 70%.

Exclusion criteria: all patient with reversibility after administration of bronchodilator (≥ 12% increase in the FEV1), patients with underlying disorders such as infectious, liver or kidney disease, patients with autoimmune disease or malignancy.

The diagnosis of COPD patients was performed by polmunologist using spirometer (Pony FX) with an FEV1/FVC ratio less than %70 The non-reversibility of the condition was checked by taking β2-agonist and re-doing spirometry. Increase in the FEV1 ≥ 12% and an absolute increase of ≥ 200 mL at least 15 min after administration was excluded.

The control group was selected from those who visited the clinical laboratory for health check-up tests.

Inclusion criteria: All men who referd to Javad Al-A'meh laboratoary for health check-up tests in Kerman city. Exclusion criteria: All subjects who had abnormal results in their routine tests including glucose, lipid profile, liver function tests, kidney function tests, blood cell count or urinalysis. Also, people who have declared in interview that they have a known disease or are taking drugs. People who had no symptoms of a specific disease in the initial interview and abnormal test results were referred to the clinic to confirm lung health.

We confirm that our study was conducted in accordance with the Declaration of Helsinki (2013). Also, the study protocol was reviewed and approved by the Research Ethics Committee of Kerman University of Medical Sciences (IR.KMU.AH.REC.1399.107). All participants in this study were aware about the study protocols. A written informed consent were obtained from all participants in this study. Then after, the patients' information was recorded in the questionnaire, which included questions such as age, gender, weight, height, history of smoking and other drugs, and history of disease. The amount of 10 ml of venous blood was taken and poured into tubes containing the anticoagulant EDTA. Then the samples were centrifuged at room temperature for 10 min at a speed of 2500 rpm. Plasma samples were stored in 1.5 ml microtubes in the freezer at -75 °C until the experiments.

Blood biochemical parameters

Blood biochemical factors including fasting blood sugar (FBS), triglyceride (TG) and total cholesterol, HDL cholesterol (HDL-c) by by an autoanalyzer (Selectra-XL). LDL cholesterol calculated with Fried-Wald formula.

Oxidative stress parameters

In order to measure total antioxidant capacity (TAC), the method of ferric reducing ability of plasma (FRAP) was used. In this methode, the changes in the absorbance of the solvent at the wavelength of 593 nm is the basis of measurement. 10 µl of plasma or standard sample was mixed with 140 µl of TAC measurement reagent and incubated for 5 min at 37 °C. Then the absorbance of the sample was read at a wavelength of 593 nm against the blank. Then, the optical absorbance (OD) of the sample was placed in the standard graph to obtain the TAC concentration in terms of Fe2 + µmol/ml or Fe2 + µM[20].

Malondialdehyde (MDA) reacts with thiobarbituric acid (TBA) at high temperature and produces a pink colored product which is measured by colorimetric method at 540 nm wavelength. 100 µl of plasma or standard sample was mixed with 200 µl of TBA reagent incubated for one hour in a boiling Bain-Marie. After the incubation time, the samples were placed in ice for 10 min. After cooling, the samples were centrifuged at 10,000 g for 10 min, and then the absorbance of the supernatant was read at 540 nm against the blank (reagent)[20].

Measurement of plasma free fatty acids

The measurement of fatty acids was done according to the previous our study except, that in this study, n-hexane instead of n-heptane was used[21].

Extraction of lipids from plasma

A solution of pentadecanoic acid in normal hexane (1 mg/ml) was used as an internal standard. To extract lipids from plasma, a reagent isopropanol-hexane-hydrochloric acid [1 M] (40-10-1 volume ratio, respectively) was used. butylated hydroxytoluene (BHT, 0.05 mg/ml) was added reagents to prevent lipid oxidation during extraction[22,23].

50 µl of pentadecanoic acid (1 mg/ml) and 4 ml of extraction reagent was added to 450 µl of plasma. The solution was vigorously vortexed for 20 min, then 2 ml of distillated water and 4 ml of normal hexane were added to the tube, and the tube was again vortexed for 5 min. After entrifugation at 4000 rpm for 10 min, the upper phase (hexane) which contains all the plasma lipid components was separated and placed inside the oven to fully evaporate[22,23].

Separation of plasma free fatty acids

To separate free fatty acids from other lipids, thin layer chromatography (TLC) was performed. For this purpose: 200–300 µL of chloroform was added to dissolve the lipids extracted in previous step. After spotting, the TLC plate was placed in the chromatography tank containing the solvent including hexane-diethyl ether-acetic acid in a volume ratio of 70-30-1. At the end of developing, Iodine was used to lipids band detection. According to the position of the standard bands of fatty acids, the fatty acid bands were identified and scraped from the TLC plate. Free fatty acids was dissoved in combination of chloroform and extracted[22,23].

Preparation of methyl ester of fatty acids

1 ml of BF3 solution in 14% methanol was added to the fatty acids tube of the previous step and closed in the tube. The solution was vigorously stirred and placed at 60 °C for 1.5 h. Then, 1.5 ml of saturated sodium bicarbonate solution and 2 ml of normal hexane were added to the tube. The tube was vortexed for 2 min and then centrifuged at 4000 rpm for 2 min and the supernatant phase containing fatty acid methyl esters (FAME) was separated[22,23].

Measurement of fatty acids

The inlet temperature was set to 300 °C and splitless mode. Nitrogen carrier gas with a gradient of 30 psi for 8 min and then increasing the pressure at a rate of 20 psi/min up to 90 psi and this pressure was maintained until the end of the work. The oven temperature was set at 45 °C for 1 min, then increased at a rate of 25 °C/min to 160∘C and at a rate of 30∘C/min to 195∘C. At this temperature, it was stopped for half a minute and then at a rate of 15 °C/min to 220 °C increased and this temperature was maintained until the end of separation[22,23].

The FID detector also had a temperature of 350 °C and the flow of hydrogen gas and air was 30 ml/min and 300 ml/min, respectively. The duration of each run was approximately 22 min and the injection volume was 1 µl[22,23].

After the end of chromatography, the type of fatty acids was determined based on the comparison of the retention time of each peak and with the standard and confirmed by spiking. Then, the area under the curve of each peak was normalized by dividing it by the peak area of the internal standard (pentadecanoic acid) and the concentration of each fatty acid was determined using the drawn standard graph[22].

Statistical analysis

All data were reported as mean ± standard error (Mean ± SE). Based on the results of data normality test, The differences of the variables between the case and control groups were compared using independent T-tests or Mann-Whitney tests. Spearman's or pearson test was also used to check the correlation between variables. The significance level in the tests was considered as P<0.05. Statistical analysis of data was done using SPSS version 26 software, and graphs were drawn using Graphpad prism software.

Results

Demographic information

The weight of the control group (72.6 ± 1.8 kg) was significantly ($P<0.0001$) higher than that of male patients with COPD (64.6 ± 2.96). Body mass index (BMI) in the COPD group (23.48 ± 1.08 kg/m2) was significantly lower than the control group (24.9 ± 0.36 kg/m2) ($P=0.012$). Current smoker and opium user in the COPD group was higher than control Group. Howerver, FVC, FEV1, FEV1/FVC and O_2 Sturation in COPD patients was significantly lower than control group. The severity of COPD in patints was determined based on GOLD criteria. Also risk of death of patiens based on BODE (Body mass index, airflow Obstruction, Dyspnea, and Exercise capacity) index was calculated. All results were summarized in Table 1.

Biochemical parameters

The results of this study showed that the level of FBS in COPD group (116.3 ± 4.2 mg/dl) has a significant difference with control people (95.5 ± 2.6 mg/dl) ($P<0.0001$). Total cholesterol level in COPD group (177.6 ± 7.5 mg/dl) is significantly higher than the control group (158.4 ± 3.9 mg/dl) ($P=0.026$). Also, HDL-c concentration in the male COPD patient group (2.9 ± 57.6 mg/dl) was significantly different from the Control group (2.6 ± 47 mg/dl) ($P=0.003$). The concentrations of triglyceride and LDL-c showed no difference in the two groups ($P>0.05$) (Fig. 1).

	Group		
	Control $n=40$	COPD $n=40$	*P* value
Height(m)	1.7 ± 0.1	1.66 ± 0.08	0.68
Weight (Kg)	72.6 ± 1.18	64.6 ± 2.96	**0.0001>**
BMI (Kg/m^2)	24.9 ± 0.36	23.48 ± 1.08	**0.012**
Age (year)	56.78 ± 1.2	61.77 ± 1.08	0.66
Smoking (pack years)	5.48 ± 1.7	20.86 ± 3.1	**0.0001>**
Opium usage (%)	22	65.9	**0.0001>**
WBC (cell/µl)	5945 ± 347	6487 ± 668	0.079
RBC (10^6 cell/µl)	5.14 ± 0.21	5.92 ± 0.18	**0.0042**
Hb (g/dl)	15.47 ± 0.48	16.9 ± 0.32	**0.017**
Hct (%)	43.72 ± 1.4	49.6 ± 2.1	**0.036**
Plt (10^3 cell/µl)*	246 ± 7	237 ± 11	0.387
SpO2(%)	95.4 ± 0.23	88.9 ± 0.8	0.0001>
FVC (%)	97.85 ± 1.4*	78.6 ± 2.7	0.0001>
FEV1(%)	74.18 ± 1.15*	48.7 ± 2.1	0.0001>
FEV1/FVC (%)	75.84 ± 0.478*	61.2 ± 2.3	0.0001>
COPD severity (*GOLD criteria*)			
Mild (FEV-1 ≥ 80%)	0(0%)	1(2.5%)	
Moderate (FEV-1: 50–79%)	0(0%)	19(47.5%)	
Severe (FEV-1: 30–49%)	0(0%)	15(37.5%)	
Very severe (FEV-1 ≤ 30%)	0(0%)	5(12.5%)	
BODE score			
0–2	40(100%)	19(47.5%)	
3–4	0(0%)	13(32.5%)	
5–6	0(0%)	5(12.5%)	
7–10	0(0%)	3(7.5%)	
Drug utsage			
Diabetes (*Metformin*)	0(0%)	4(10%)	
Hypercholesterolemia (*Statins*)	0(0%)	7(17.5%)	
Hypertension	0(0%)	5(12.5%)	
CNS (*Benzodiazepines*)	0(0%)	4(10%)	
Inhaler spray (*Bronchodilators*)	0(0%)	19(47.5%)	

Table 1. Comparison of demographic information of COPD and healthy groups. * $n=23$. Significant values are in bold. Sampling was done by the Convenience method from men referring to medical centers ($n=40$). The results were presented as Mean ± SE and were compared with the non-parametric Mann-Whitney test for mass, BMI, smoking and opium use, and height and age with the independent sample T test. SpO_2, oxygen saturation; FVC, forced vital capacity; FEV1, forced expiratory volume second 1. COPD Severity Classification: mild: FEV_1 ≥80%, moderate: 50% ≤ FEV_1 <80%, severe: 30% ≤ FEV_1 <50% and very severe: FEV_1 <30%.

Oxidative stress indices

In order to evaluate the oxidative stress in the studied subjects, total antioxidant activity and malondialdehyde concentration were measured. The results showed that the total antioxidant capacity in the male COPD patients (196.29 ± 8.01 µmol/l Fe2+) has a significant difference with control people (265.7 ± 9.07 µmol/l Fe2+) ($P<0.0001$), however, As the Table 2 shows, the concentration of malondialdehyde in the plasma of patents with COPD (2.37 ± 0.14 µmol/l) is significantly higher than that of control people (1.53 ± 0.06 µmol/l) ($P<0.0001$).

Free fatty acids concentration

As Table 3 shows, Myristic acid in the COPD group (10.01 ± 0.48 µmol/l) was significantly lower than the control group (15.06 ± 0.88 µmol/l) ($P<0.0001$). Palmitic acid as the most abundant free fatty acid in the COPD group (147.4 ± 13.6 µmol/l) was significantly lower than the control group (182.8 ± 3.77 µmol/l) ($P<0.0001$). Stearic acid in the control group (68.2 ± 1.02 µmol/l) was significantly higher than the COPD group (48.6 ± 2.9 µmol/l) ($P<0.0001$) and oleic acid was also higher with $P<0.0001$ in the COPD group (92.4 ± 4.6 µmol/l) was significantly lower than the control group (119.8 ± 2.18 µmol/l). The only measured trans fatty acid was elaidic acid, which was significantly higher in the COPD group (5.2 ± 0.23 µmol/l) than the control group (4.38 ± 0.23 µmol/l) ($P=0.023$). Linoleic acid in the COPD group (57.87 ± 3.5 µmol/l) was significantly lower than the control group (81.36 ± 3.9 µmol/l) ($P<0.0001$). Also, alpha-linolenic acid and gamma-linolenic acid in the COPD group were (15.11 ± 0.64 µmol/l) and (4.7 ± 0.15 µmol/l), which were significantly different from the control group with values of (13.2 ± 0.91 µmol/l) and (3.24 ± 0.12µmol/l) ($P<0.0001$).

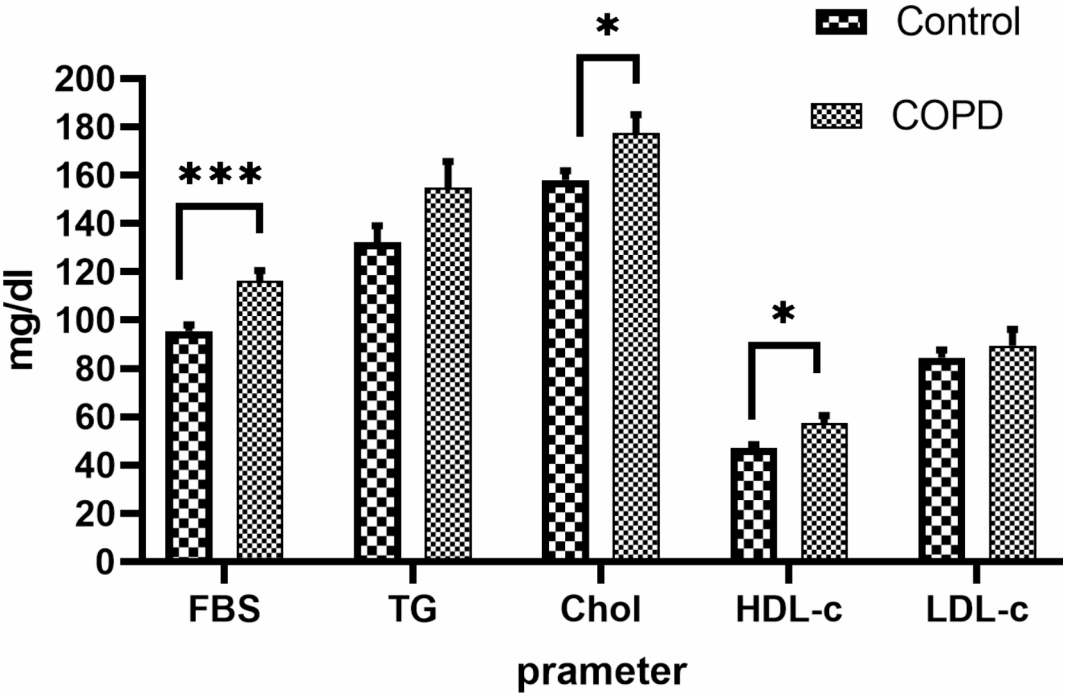

Fig. 1. Comparison of Biochemical parameters in COPD and control groups: Sampling was done by the Convenience method from men referring to medical centers ($n = 40$). (FBS) Fasting Blood Sugar, (Chol) total cholesterol, (TG) triglyceride, (LDL-c); LDL-cholesterol and (HDL-c) HDL-cholesterol were measured by enzymatic method and with an autoanalyzer. Results were presented as Mean ± SE and compared with the non-parametric Mann-Whitney test. $P < 0.0001$***, $P < 0.05$*.

	Group		
	Control	COPD	*P* value
TAC (μmol/l)	265.7 ± 9.07	196.29 ± 8.1	< 0.0001
MDA (μmol/l)	1.53 ± 0.06	2.37 ± 0.14	< 0.0001

Table 2. Comparison of oxidative stress status in COPD and control groups. Sampling was done by the Convenience method from men referring to medical centers ($n = 40$). Total antioxidant capacity (TAC) was measured by FRAP method and Malondialdehyde (MDA) concentration in plasma was measured by thiobarbituric acid (TBA) method in the plasma. The results are presented as Mean ± SE and compared with independent T-test. $P < 0.0001$***.

Concentration of long chain and medium chain fatty acids

The summation of concentration of medium chain fatty acids in male patients with COPD (10.86 ± 0.27 μmol/l) compared to the control group (10.39 ± 0.42 μmol/l) showed no difference ($P = 0.562$), while the total concentration of long chain fatty acids in COPD group (433.39 ± 18.2 μmol/l) was significantly lower than the control group (543.44 ± 6.94 μmol/l) ($P < 0.0001$). The results are shown in Table 4.

Concentration of saturated and unsaturated free fatty acids

The total concentration of saturated fatty acids in the COPD group (216.23 ± 15.2 μmol/l) compared to the control group (274.96 ± 4.5 μmol/l) showed a significant difference with $P < 0.0001$. Also The total concentration of unsaturated fatty acids in male patients with COPD (227.29 ± 7.8 μmol/l) was significantly lower than the control group (277.04 ± 4.44 μmol/l) ($P < 0.0001$). The results are shown in Table 4.

Concentration of Omega-3 and Omega-6 free fatty acids

The total concentration of omega-3 fatty acids in the COPD group (15.11 ± 0.64 μmol/l) was significantly higher ($P = 0.002$) compared to the control group (13.20 ± 0.9 μmol/l), and on the contrary, the amount of omega-6 fatty acids in patients with COPD (77.69 ± 3.4 μmol/l) was significantly lower than the control group (115.03 ± 2.7 μmol/l) ($P < 0.0001$). The results are shown in Table 4.

FFA(µmil/l)	Group		*P* value
	COPD	Control	
Capric acid(C10)	4.38 ± 0.2	4.61 ± 0.16	0.299
Lauric acid(C12)	6.48 ± 0.21	5.76 ± 0.35	0.73
Myristic acid(C14)	10.01 ± 0.48	15.06 ± 0.88	< 0.0001
Myristoleic acid (C14:1;9)	2.6 ± 0.14	2.56 ± 0.42	0.85
Palmitic acid (C16)	147.4 ± 13.6	182.8 ± 3.77	< 0.0001
Palmitoleic acid (C16:1;9)	24.7 ± 4.9	12.34 ± 1.11	0.718
Stearic acid (C18)	48.6 ± 2.9	68.2 ± 1.02	< 0.0001
Oleic Acid (C18:1;9)	92.4 ± 4.6	119.8 ± 2.18	< 0.0001
Elaidic acid (C18:1;9 trans)	5.2 ± 0.23	4.38 ± 0.23	0.023
Linoleic acid (C18:2;9,12)	57.87 ± 3.5	81.36 ± 3.9	< 0.0001
α-linolenic acid (C18:3;9,12,15)	15.11 ± 0.64	13.2 ± 0.91	< 0.0001
γ-linolenic acid (C18:3;6,9,12)	4.7 ± 0.15	3.24 ± 0.12	< 0.0001
Eicosadienoic acid (C20:2;11,14)	9.5 ± 0.29	9.6 ± 0.29	0.833
Arachidonic acid (C20:4;5,8,11,14)	15.02 ± 0.61	30.43 ± 3.9	0.809

Table 3. Comparison of free fatty acid profile in COPD and control groups. Sampling was done by the Convenience method from men referring to medical centers ($n = 40$). Free fatty acids in plasma samples were measured by gas chromatography, and then the results were presented as Mean ± SE and compared with non-parametric Mann-Whitney test.

FFA (µmol/l)	Group		*P* value
	COPD	Control	
Saturated free fatty acids	216.23 ± 15.2	274.96 ± 4.52	< 0.0001
UnSaturated free fatty acids	227.04 ± 7.8	277.04 ± 4.44	< 0.0001
Long chain free fatty acids	433.39 ± 18.7	543.14 ± 6.9	< 0.0001
Medium chain free fatty acids	10.86 ± 0.27	10.39 ± 0.4	0.562
Omega-3 free fatty acids	15.1 ± 0.64	13.3 ± 0.9	0.002
Omega-6 free fatty acids	77.69 ± 3.4	115.03 ± 2.76	< 0.0001
Total free fatty acids	443.52 ± 6.97	552.01 ± 6.09	< 0.0001

Table 4. Comparison of different types of free fatty acids. Sampling was done by the Convenience method from men referring to medical centers ($n = 40$). Free fatty acids in plasma samples were measured by gas chromatography, and then the results were presented as Mean ± SE and compared with non-parametric Mann-Whitney test.

Concentration of total free fatty acids

The total amount of free fatty acids in male COPD patients (443.52 ± 18.6 µmol/l) was significantly lower than the control group (552.01 ± 6.97 µmol/l) ($P < 0.0001$). The results are shown in Table 4.

The relationship between spirometric indices and the concentration of free fatty acids in patients with COPD.

Examining the relationship between variables showed that capric acid has a positive relationship with FVC, FEV1 and FEV1/FVC ratio with a significant correlation coefficient of 0.749, 0.658 and 0.764 respectively. Also, there was a significant positive relationship between the concentration of arachidonic acid and FVC with a correlation coefficient of 0.360 and $P = 0.016$. Of course, a negative and significant correlation was also observed between palmitic acid concentration with FVC, FEV1 and FEV1/FVC ratio with correlation coefficient − 0.796, -0.598 and − 0.768, respectively. Another long-chain saturated fatty acid, namely stearic acid, also showed a negative and significant relationship with FVC and FEV1. Other fatty acids did not show a relationship with the values of spirometric indices (Table 5).

The relationship between spirometric indices and the differnt types of plasma free fatty acid in patients with COPD.

The results of the Spearman test showed that the concentration of saturated fatty acids has a negative and significant correlation with the correlation coefficient − 0.729, -0711 and − 0.695, respectively, with FVC, FEV1 and FEV1/FVC ratio.

Medium chain fatty acids with correlation coefficients of 0.567, 0.727 and 0.611 showed a positive and significant relationship with FVC, FEV1 and FEV1/FVC ratio, respectively. Long chain fatty acids also had an

	FVC	FEV1	FEV1/FVC
Capric acid(C10)			
r	0.749**	0.658**	0.764**
P value	< 0.0001	< 0.0001	< 0.0001
Lauric acid(C12)			
r	-0.016	-0.026	0.035
P value	0.920	0.869	0.824
Myristic acid(C14)			
r	0.129	0.072	-0.075
P value	0.403	0.645	0.630
Myristoleic acid (C14:1;9(			
r	-0.075	0.013	0.130
P value	0.626	0.934	0.401
Palmitic acid (C16)			
r	-0.796**	-0.598**	-0.768**
P value	< 0.0001	< 0.0001	< 0.0001
Palmitoleic acid (C16:1;9)			
r	0.071	0.063	0.026
P value	0.645	0.684	0.866
Stearic acid)C18(			
r	-0.353*	-0.330*	-0.157
P value	0.019	0.029	0.308
Oleic acid (C18:1;9)			
r	-0.185	-0.146	0.027
P value	0.228	0.346	0.862
Elaidic acid)C18:1;9 trans)			
r	-0.020	-0.022	-0.076
P value	0.8990	0.887	0.622
Linoleic acid (C18:2;9,12)			
r	-0.164	-0.187	-0.081
P value	0.287	0.224	0.601
α-linolenic acid (C18:3;9,12,15)			
r	-0.011	-0.033	-0.120
P value	0.941	0.833	0.436
γ-linolenic acid (C18:3;6,9,12)			
r	0.038	0.078	0.047
P value	0.808	0.615	0.762
Eicosadienoic acid (C20:2;11,14)			
r	-0.171	-0.079	0.003
P value	0.266	0.609	0.982
Arachidonic acid (C20:4;5,8,11,14)			
r	0.360*	0.299	0.086
P value	0.016	0.059	0.578

Table 5. The relationship between spirometric indices and the concentration of free fatty acids in patients with COPD. Spirometric indices were obtained by spirometric device and after taking bronchodilator. Considering the lack of normal distribution of the data, the relationship between the variables was calculated with the Spearman test and $P < 0.05$ was considered significant. FEV1, forced expiratory volume in the first second; FVC, forced vital capacity

inverse and significant relationship with FVC, FEV1 and FEV1/FVC ratio with a correlation coefficient of -0.572, -0.644 and − 0.431 respectively.

And finally, the concentration of total free fatty acids in plasma with a correlation coefficient of -0.577, -0.642 and − 0. 425 showed a positive and significant relationship with FVC, FEV1 and FEV1/FVC ratio, respectively. All results were summarized in Table 6.

	FVC	FEV1	FEV1/FVC
Saturated free fatty acids			
r	-0.729**	-0.711**	-0.695**
P value	< 0.0001	< 0.0001	< 0.0001
UnSaturated free fatty acids			
r	-0.153	-0.109	0.007
P value	0.320	0.479	0.963
Omega-3 free fatty acids			
r	-0.011	-0.033	-0.120
P value	0.941	0.833	0.436
Omega-6 free fatty acids			
r	-0.135	-0.170	-0.110
P value	0.381	0.2690	0.479
Medium chain free fatty acids			
r	0.567**	0.727**	0.611**
P value	< 0.0001	< 0.0001	< 0.0001
Long chain free fatty acids			
r	-0.572**	-0.644**	-0.431**
P value	< 0.0001	< 0.0001	0.004
Total free fatty acids			
r	-0.577**	-0.642**	-0.425**
P value	< 0.0001	< 0.0001	0.004

Table 6. The relationship between spirometric indices and the differnt types of plasma free fatty acid in patients with COPD. Spirometric indices were obtained by spirometric device and after taking bronchodilator. Considering the lack of normal distribution of the data, the relationship between the variables was calculated with the Spearman test and $P < 0.05$ was considered significant. FEV1, forced expiratory volume in the first second; FVC, forced vital capacity.

Discussion

Chronic obstructive pulmonary disease (COPD) is defined as a common disease caused by exposure to toxic particles. This disease is attributed to chronic inflammation that leads to airway obstruction[24]. On the other hand, recent studies show that FFAs are signaling molecules which according to their length of carbon chain, regulate various cellular processes. For this reason, some types of fatty acids have been suggested to play a role in the prevention and treatment of inflammatory and metabolic disorders. These effects of free fatty acids are mediated via FFA receptors (FFARs), which are expressed in various tissues[25]. Considering the importance of inflammation in the occurrence of COPD and the role of free fatty acids in inflammation, we decided to determine the profile of free fatty acids in male patients with COPD.

At the first, we sow a significant difference of opium consumption between control and COPD groups. To determine the possibility of the confounding effect of opium use, we examined the relationship between opium use and different markers, which did not show a significant relationship with the independent and dependent factors of the study. Therefore, the difference in opium consumption in the two groups of this study is not considered a confounding factor that needs to be adjusted[26].

The results of this study showed that fasting blood sugar in male COPD patients is significantly higher than healthy control group. In the study of Acharyya et al., it was found that the blood sugar of people with COPD is significantly higher than the control people and the prevalence of metabolic syndrome in people with COPD was reported as 46%[27]. COPD is associated with disturbances in the metabolism of lipids and glucose[28]. The increase in blood glucose in COPD patients can be related to the therapeutic use of corticosteroid[29]. Because for these results, both pulmonologists and diabetologists should consider the risks and implications associated with this potentially important pathophysiological interaction and actively screen for type 2 diabetes as a COPD comorbidity[30]. It is also known that although opium use reduces blood sugar in the short term, long-term use increases blood sugar and diabetes becomes more severe[31]. Therefore, the increase in blood sugar in COPD patients may be due to higher consumption of opium in them compared to the control group.

The concentration of total cholesterol and HDL-c in male COPD patients was significantly higher than the control group. In the study of Markelic it was found that COPD patients, have increased level of HDL cholesterol compared to healthy people, which is consistent with our study, but unlike this study, no difference was observed in the total cholesterol level of patients and healthy people[32]. Also, Ivanovska et al. showed that the level of total cholesterol, LDL and HDL cholesterol increases in patients with very severe forms of COPD[33]. Lipid metabolic pathways are clearly altered in COPD, and these changes may directly alter cell functions such as the production of specific mediators, immune regulators, or cell death that ultimately causing COPD[34]. However, studies on the metabolic pathways of different types of lipids in lung cell types in vitro, animal models, and also in COPD patients are needed[34] but taken together, these findings, physicians should screen COPD patients for elevated

triglyceride and cholesterol levels to reduce the risk of cardiovascular disease and mortality in these patients[35]. In the study Ivanovska et al.[36], the increase of HDL cholesterol is related to the use of steroids, and it is confirmed that the increase of HDL-c in these patients is not associated with a decrease in the risk of heart diseases.

In our study, lower antioxidant activity and higher MDA concentration were observed in male COPD patients compared to normal subjects, which indicates the presence of oxidative stress in COPD patients compared to healthy subjects. Oxidative stress is one of the basic mechanisms of COPD, and it is caused by a disturbance in the prooxidant-antioxidant balance[37]. Also this imbalance has the key role in exacerbation of COPD[38]. Oxidative stress in the lungs is the results of ROS released from activated inflammatory cells, such as neutrophils and macrophages, caused by exposure to environmental factors such as cigarette smoke, air pollutants[37]. Treatments antioxidant system appear to reduce COPD attacks by modulating oxidative stress[39] and Inhibitors of mitochondrial ROS production are new therapeutic options for the treatment of COPD caused by oxidative stress[40].

In our study, the concentration of myristic, palmitic, stearic, oleic and linolenic fatty acids in male COPD patients showed significant difference with control subjects. Also, a significant relationship was observed between capric, palmitic, stearic and arachidonic acids with spirometric indices. In addition, the relationship between total saturated, medium chain, long chain and total fatty acids with FEV1 and FEV1/FVC ratio was significant. In COPD patients, reduction of total free fatty acid concentration was reported previously[41]. Also In the present study, a higher concentration of ω3 fatty acids was observed in patients with COPD, while these factors did not show a relationship with lung function in COPD patients. It has been suggested that anti-inflammatory supplements may improve the prognosis of COPD patients, and it has been shown that the balance between omega-3 levels and omega-6 levels determines whether these eicosanoids have anti-inflammatory or pro-inflammatory effects in patients with COPD[42]. In a study of 250 COPD patients, it was shown that a high level of the omega-3 fatty acid alphalinolenic acid in the diet of subjects was associated with a lower level of pro-inflammatory cytokines such as TNF.α[42]. In vivo and in vitro data also support a mechanism by which activation of the PUFA-FFAR4 axis results in less deleterious effects on blood flow and limited macrophage migration[43] but we didn't see any correlation between omega-3 fatty acids and lung function prameters, which may be due to the increase in omega-6 fatty acids in the dose of these patients. In Kemper et al.[44] study, it was found that higher levels of omega-3 fatty acids such as eicosapentaenoic acid and docosahexaenoic acid are related to better lung function. But in our study, these fatty acids were not detected. The reason being that we specifically measured plasma non-sterified fatty acids that can act as ligands for fatty acid receptors. In the study of Cepeda et al., it was also found that the consumption of pentadecanoic acid as a long-chain saturated fatty acid is related to an increase in the FEV1/FVC ratio[45]. However, in our study, on the contrary, plasma long-chain fatty acids were associated with a decrease in FEV1/FVC. The reason for this difference is that in the mentioned study, the fatty acid concentration in the plasma was not measured and it was only consumed as a food supplement. Digestive bacteria play a critical role in the metabolism of fatty acids and their conversion into short chain types. In a meta-analysis study, it has been concluded that the daily consumption of higher amounts of omega-6 polyunsaturated fatty acids than omega-3 is associated with an increased risk of COPD[46]. In our study, this relationship was observed with long-chain saturated fatty acids. The reason for this discrepancy can be the different design of the two studies as well as the lack of measuring the blood concentration of fatty acids in the above study.

FFA have also recently been shown to increase the formation of reactive oxygen species (ROS), including hydroxyl radical (OH·), superoxide anion ($-O_2$), and hydrogen peroxide (H_2O_2)[47]. Therefore, considering the role of oxidative stress in the induction of COPD, the increase of free radicals can be considered as one of the mechanisms of the effect of some types fatty acids in the development of COPD.

In the present study, a negative relationship between long chain fatty acids and lung function was shown. Recent research has shown that obesity-associated free fatty acids (FFAs), for example, palmitic acid and stearic acid, are considered novel pro-inflammatory agents[48]. Palmitic acid also causes mitochondrial damage and mtDNA leakage into the cytosol, thereby activating the molecular pathways, which in turn activates inflammation[49]. Therefore, these fatty acids may play a role in reducing lung function through increasing inflammation.

Conclusion

Overall, the results of this study showed that the concentration of different types of fatty acids including myristic acid, palmitic acid, stearic acid, linoleic acid and linolenic acid in male COPD patients is lower than healthy people. Also, a positive correlation between the concentration of medium chain fatty acids and lung function was observed, while this correlation was negative for long chain, saturated and total fatty acids with spirometric indices. The positive relationship between medium chain fatty acids and lung function indicates that the consumption of food sources containing this type of fatty acids, such as coconut oil, milk, yogurt, butter, and cheese, can play a role in improving the respiratory conditions of patients. Although the existence of these relationships confirms the importance of free fatty acids in male COPD patients, but considering the role of saturated long-chain fatty acids in causing inflammation and the opposite effect of medium-chain and unsaturated acids in inhibiting inflammation through fatty acid receptors, the interpretation of the results requires conducting studies.

Data availability

All data analyzed during this study are included in this article. Further inquiries can be directed to the corresponding author.

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Author contributions
RY: Conception and design; Experiments; Data Analysis; Writing; Review and Editing.HF: Supervision; Experiments; Data Analysis; Writing; Review and Editing.SY: Experiments; Data Analysis; Writing; Review and Editing.BS: Experiments; Data Analysis; Writing; Review and Editing.BD: Supervision; Conception and design; Experiments; Data Analysis; Writing; Review and Editing.

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Declarations

Competing interests
The authors declare no competing interests.

Ethical approval
We confirm that our study was conducted in accordance with the Declaration of Helsinki (2013). Also, the study protocol was reviewed and approved by the Research Ethics Committee of Kerman University of Medical Sciences (IR.KMU.AH.REC.1399.107).

Additional information
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