International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 11s, 2025
https://theaspd.com/index.php

Isolation And Identification Of Fatty Acids In Serum Of People With Obesity

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Abstract
The study focused on obesity as a risk factor associated with all common and prevalent diseases. The isolation and identification of fatty acids in human serum is crucial for diagnosing essential fatty acid deficiencies, fatty acid metabolism disorders, and understanding lipid risk at the population level. In most cases, total fatty acyl analysis is performed using various methods such as gas chromatography (GC). The basic principle of this technique involves the use of acid- or base-catalyzed reactions to convert fatty acyl chains from intact lipids and non-esterified fatty acids into volatile FA methyl ester (FAME) species that can be detected using gas-coupled flame ionization detection (GC-FID) or mass spectrometry (GC-MS). Using various chromatographic parameters (including column materials) and detection methods, fatty acid analyzers can be monitored with multiple resolutions. Consequently, analysis times are increased according to these principles. In our study, five obesity-related fatty acids were isolated: alpha-linolenic acid, linoleic acid, oleic acid, palmitic acid, and stearic acid. Which has proven its direct relationship between obesity
Keywords; *High resolution mass spectrometers, Fatty acids, Obesity, Metabolic disturbances.*

INTRODUCTION

Excess weight, leading to obesity, is an increasingly prevalent health problem worldwide [1,2]. Dietary fat content has been noted as a key factor in the rise in global obesity rates [3,4,5]. According to studies by the World Health Organization, the number of obese people worldwide has nearly tripled since 1975 [6]. In the past, obesity was considered a problem of wealthy, high income, and developing countries, but now it is believed to affect people of low and middle socioeconomic status [7,8]. In obese individuals, energy consumption is much higher than the body's needs, leading to excessive fat accumulation due to the hypertrophy of fat cells [9,10].Energy imbalance results from consuming more calories than the body needs for its functions and health. Furthermore, people with limited physical activity tend to gain weight [11]. The size of lipid droplets in skeletal muscle increases [12]. A decrease in oxidative enzymes, a decrease in triglyceride turnover activity, and a decrease in lipid activity and oxidation are also observed. These mechanisms lead to adipose tissue retention. [13]. The energy in dietary fat comes mainly *from* saturated (SFA), monounsaturated (MUFA), and polyunsaturated (PUFA) fatty acids [14,15].The most common saturated fatty acids (SA) are stearic acid and palmitic acid (PA) (C16:0), which are typically consumed in large quantities in the diet [16]. The maximum recommended intake of these acids has not been determined, nor has a safe dietary level been established. Fatty acids are stored in lipid droplets within fat cells as triglycerides. Excess fatty acid accumulation leads to an increase in the size of mature fat cells. [17]. Hypertrophic adipocytes tend to be insulin resistant. This leads to increased lipolysis with decreased lipogenesis. Therefore, increased efflux of fatty acids away from adipose tissue represents a key pathophysiological feature of metabolic complications in obese individuals, such as inflammation, dyslipidemia, and impaired glycemic control [18].Fatty acids play a vital role in biological systems [19]. Fatty acid imbalance is associated with a variety of diseases, making the measurement of fatty acids in biological samples extremely important. Many analytical strategies have been developed to study fatty acids in many different biological samples [20].The structural diversity of fatty acids requires consideration of various factors when developing analytical methods, including extraction techniques, column selection, derivatization methods, and the selection of internal standards.Fatty acids (FA), especially polyunsaturated fatty acids (PUFA), play a role in lipid metabolism, blood sugar regulation, and inflammation [21]. This study focused on evaluating and identifying the level of fatty acids in patients and their relationship to obesity, lipid metabolism disorders, and weight loss.

MATERIALS AND METHODS

Study Group and Study Design

Morbidly obese patients (BMI= 36.5-50.0 kg/m^2) over the age of 20 were recruited. Patients were contacted prior to undergoing bariatric surgery, and their obesity history was determined in addition to a complete clinical examination. The World Health Organization classification of obesity was used.

EXTRACTION AND DERIVATION OF FATTY ACID IN SERUM.

Samples were prepared according to the published method. A 200-µl serum sample was mixed with 50 µl of 0.05% H2SO4 solution, vortexed for at least 30 seconds, extracted with 2 ml of ethyl acetate using a vortex mixer for 60 seconds, and then centrifuged at 4,000 rpm for 10 minutes at 4°C. The ethyl acetate phase was evaporated to dryness under a nitrogen stream, and in the next step, 2 ml of a H2SO4-CH3OH-toluene mixture (5:90:5, v/v) was added to the residue and incubated at 75°C for 1 h with shaking every 20 min. Then the temperature of the samples was lowered to room temperature and 1 ml of saturated sodium chloride solution and 2 ml of hexane were added sequentially and mixed for 60 seconds by shaking with a vortex to obtain the targets. The organic phase was evaporated to dryness under nitrogen stream gas and the residue was re-dissolved in 200 µL of n-hexane, filtered using filters with 0.25µm pores, and stored at -20°C prior to the analysis.

WORKING CONDITIONS

The Shimadzu GCMS-QP2010 Plus is a multi-purpose instrument, including fatty acid analysis in human serum. Although operating conditions may vary depending on sample preparation and analytical requirements, the following general parameters are typically used: a flame ionization detector (FID) and a 30 m × 0.25 mm capillary column (DB-5), under the following conditions.

Paragraph name	Temperature
Injection area temperature	280c
Detector temperature	310c
Separation column temperature	80 (hold 2 min) –160 (25 C/MIN)-250c(10 c/min)
Gas flow rate	100Kpa

Statistical analysis

The statistical findings were statistically significant when the P value was less than 0.05 following data analysis using SPSS (version 27).

International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 11s, 2025
https://theaspd.com/index.php

Results

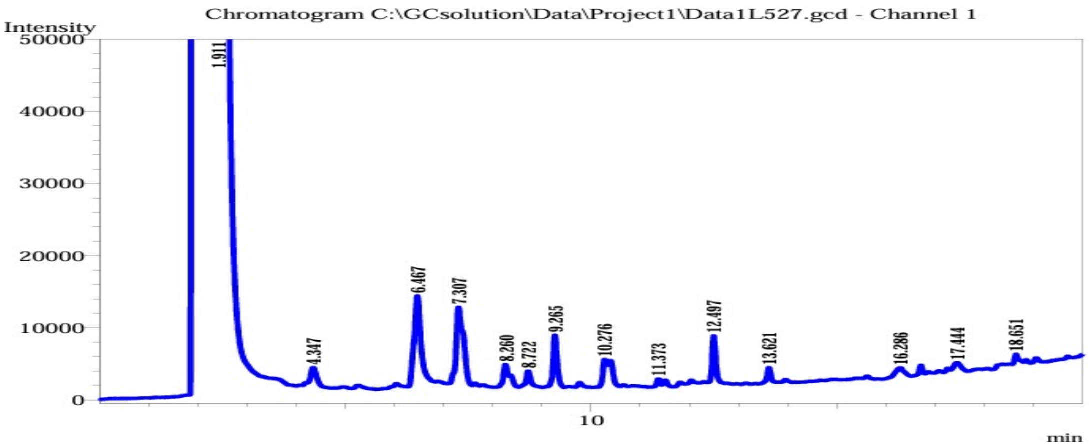

Figure 1: Male patient

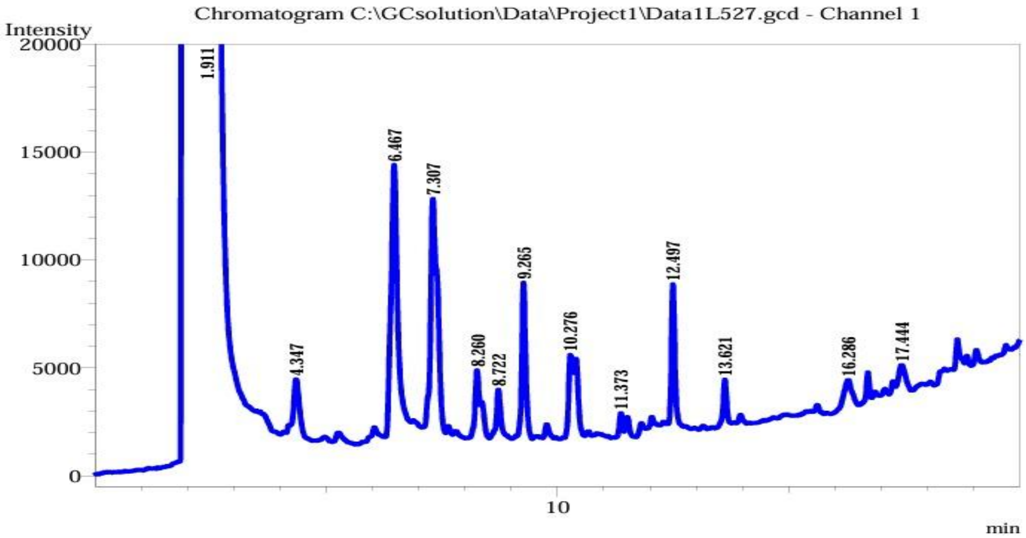

Figure 2: Female patient

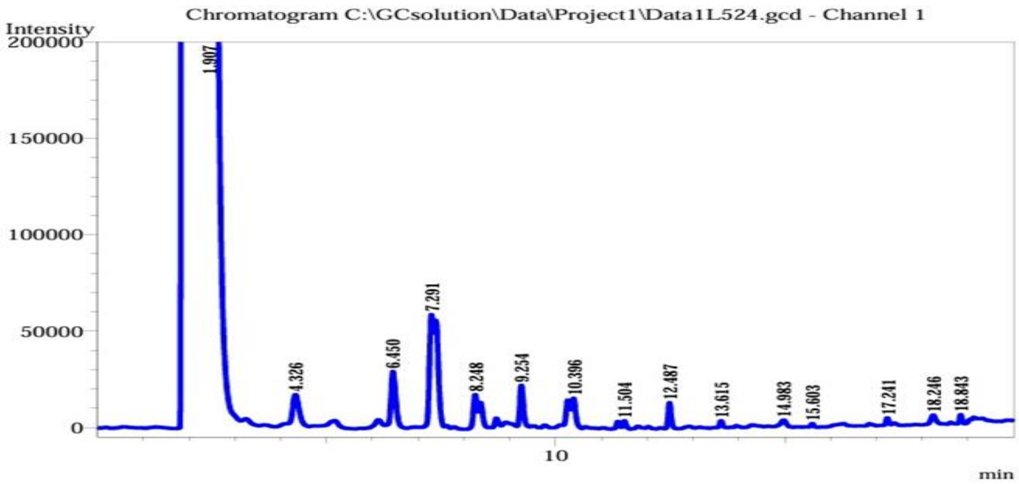

Figure 3: Male control

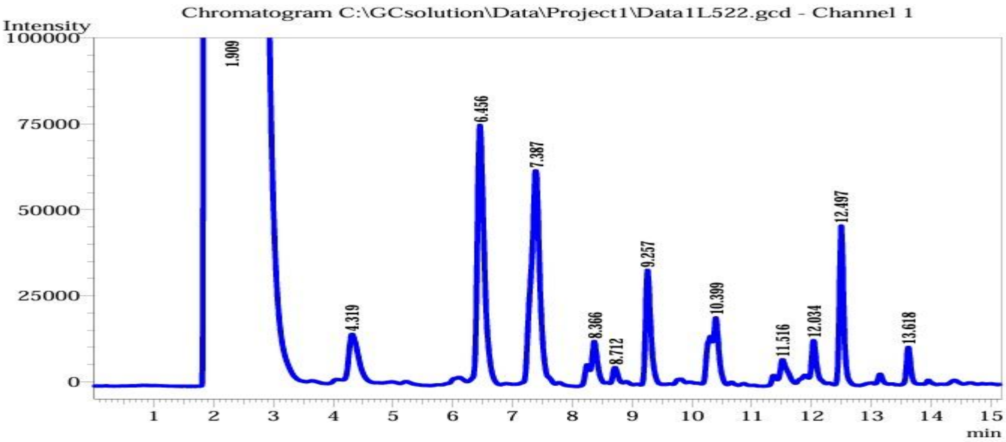

Figure 4: Female control

Table 1: Percentages of fatty acids in the serum of obese people and comparison with the percentages in normal people

Fatty acid	Mean ± SD		P-Vale
	Male control	Male patient	
a_lenolenic_acid(µg/ml)	0.82 ± 0.008	0.50 ± 0.017	0.000
Lenolic_acid(µg/ml)	1.54 ± 0.010	1.10 ± 0.068	0.000
Oleic_acd(µg/ml)	0.98 ± 0.010	0.63 ± 0.064	0.000
Palmatic_acid(µg/ml)	0.40 ± 0.008	0.68 ± 0.029	0.000
Stearic_acid(µg/ml)	0.15 ± 0.005	0.31 ± 0.018	0.000
Fatty acid	Mean ± SD		P-Vale
	Female control	Female patient	
a_lenolenic_acid(µg/ml)	0.98 ± 0.008	0.59 ± 0.078	0.000
Lenolic_acid(µg/ml)	1.62 ± 0.008	1.28 ± 0.035	0.000
Oleic_acd(µg/ml)	1.12 ± 0.008	0.71 ± 0.035	0.000
Palmatic_acid(µg/ml)	0.50 ± 0.013	0.77 ± 0.041	0.000
Stearic_acid(µg/ml)	0.25 ± 0.008	0.46 ± 0.031	0.000
Fatty acid	Mean ± SD		P-Vale
	Female patient	Male patient	
a_lenolenic_acid(µg/ml)	0.59 ± 0.078	0.50 ± 0.017	0.085
Lenolic_acid(µg/ml)	1.28 ± 0.035	1.10 ± 0.068	0.004
Oleic_acd(µg/ml)	0.71 ± 0.035	0.63 ± 0.064	0.085
Palmatic_acid(µg/ml)	0.77 ± 0.041	0.68 ± 0.029	0.012
Stearic_acid(µg/ml)	0.46 ± 0.031	0.31 ± 0.018	0.000

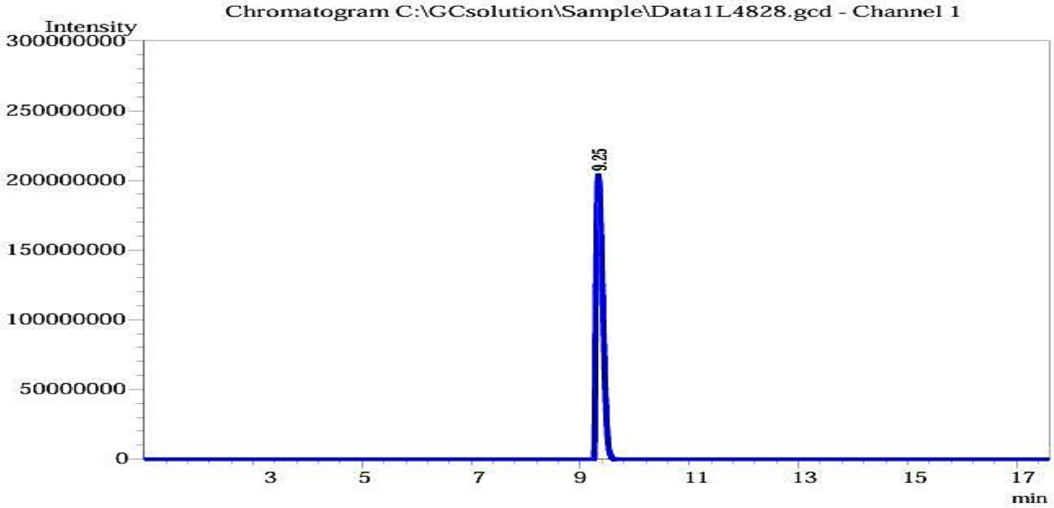

Figure 5: a-linolenic (ALA)

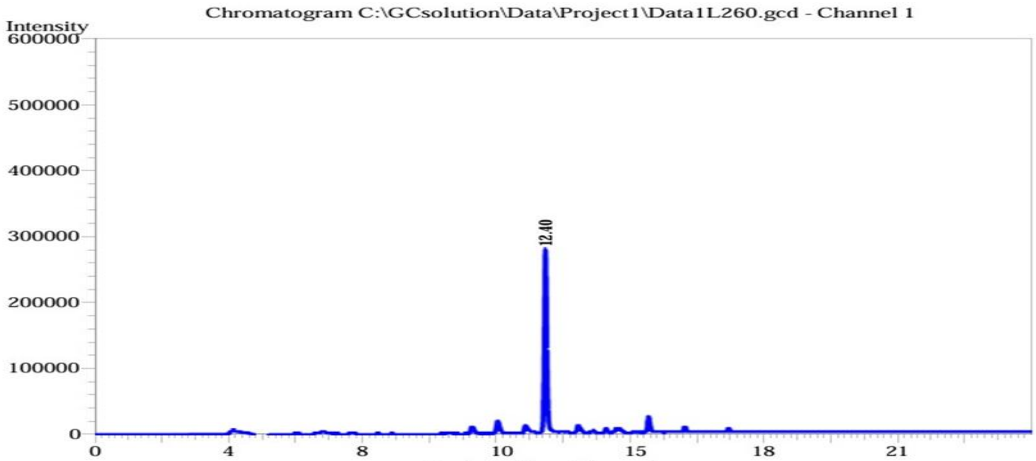

Figure 6: Lenolic acid **(LA)**

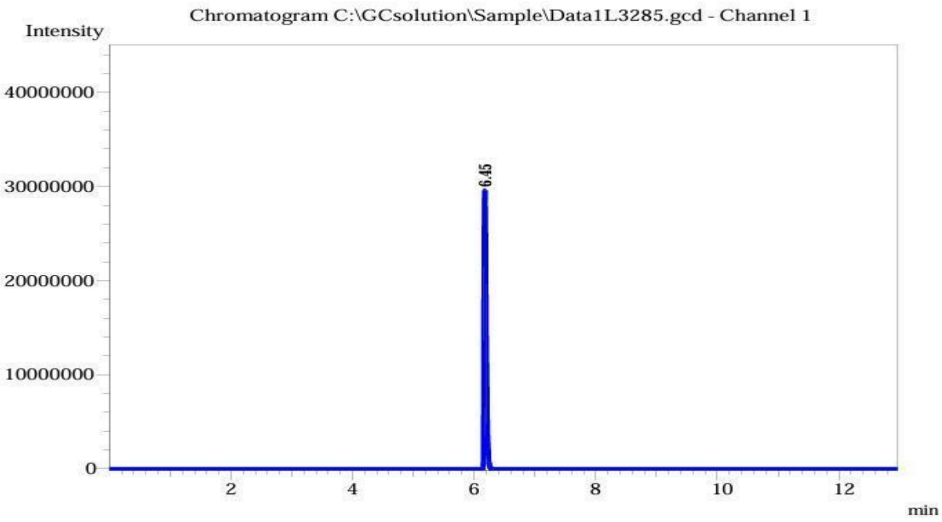

Figure 7: Olic acid (OL)

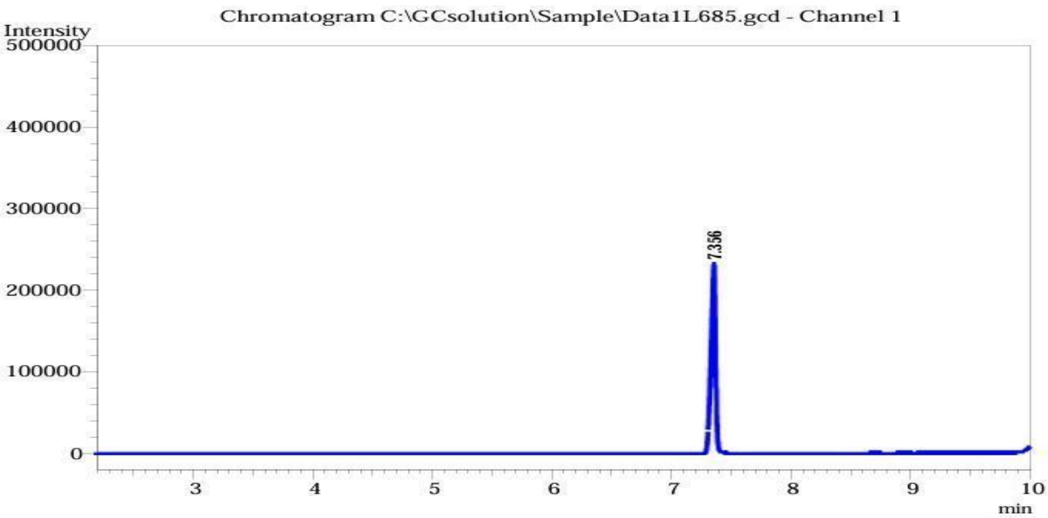

Figure 8: Palmitic acid (PA)

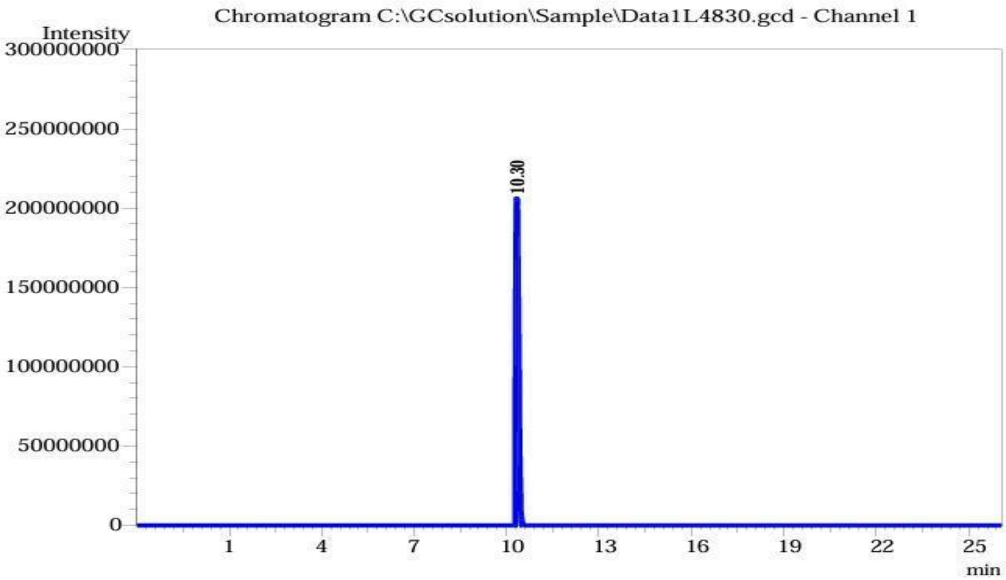

Figure 9: Stearic acid (SA)

DISCUSSION

Obesity and overweight are widespread worldwide, increasing the risk of cardiovascular diseases, including systemic inflammation [22], hypertension, and dyslipidemia. Accordingly, the results that we obtained, which indicate a lower level of the unsaturated fatty acids alpha-linolenic, linoleic fatty acid, and also oleic acid in obese people compared to normal people as Figure (1,2,3,4) and Table 1, are probably due to the following reasons:Isolation ALA as showed in Figure 5 which classified as an n-3 polyunsaturated fatty acid (PUFA), is an essential fatty acid. ALA is abundant in plant foods such as rapeseed, chia seeds, perilla, walnuts, and flaxseeds [23]. The average dietary intake of ALA is generally higher than the recommended intake, a plant-based essential polyunsaturated fatty acid associated with a reduced risk of cardiovascular disease [24]. The aim of this study and meta-analysis was to investigate the effects of alpha-linolenic acid in overweight or obese individuals because dietary alpha-linolenic acid intake is associated with a reduced risk of cardiovascular disease and death from any cause [25]. ALA downregulates the gene expression of nitric oxide synthase, cyclooxygenase-2, and TNF-α via the inhibition

International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 11s, 2025
https://theaspd.com/index.php

of nuclear factor kappa B (NF-κB) and mitogen-activated protein kinase pathways [26]. In addition, ALA might improve the obese phenotype by reducing adipocyte hypertrophy, protein concentrations of inflammatory markers monocyte chemoattractant protein-1 and TNF-α, and T-cell infiltration in adipose tissue, ddietary alpha-lipoic acid improves blood lipid levels by reducing triglycerides, total cholesterol, LDL cholesterol, and LDL cholesterol [27]. The fatty acid LA was also isolated, as shown in Figure 6. High consumption of LA leads to elevated levels of inflammatory markers such as plasminogen activator inhibitor type 1, fibrinogen, soluble vascular adhesion molecules, cytokines, tumor necrosis factor alpha, C-reactive protein, and others Several epidemiological studies have assessed the association between diet and nutrition and the risk of MetS Specifically, intakes of omega-6 (n-6) [28]. Polyunsaturated fatty acids (PUFAs) are associated with risk factors of MetS. n-3 PUFAs have positive effect on health in suppressing the synthesis of thromboxane's, atherosclerotic plaque, and inflammation [29]. Additionally, in many clinical trials, it has been reported that sufficient intake of n-3 PUFAs has beneficial effects on the managements of triglycerides, fasting blood sugar, high-density lipoprotein (HDL) cholesterol, and insulin resistance [27]. The consumption of n-6 PUFAs instead of saturated fat can have positive effect on blood lipid management, leading to a decrease in total cholesterol and an increase in HDL-cholesterolprevious studies reported inconsistent evidence on the associations between n-6 PUFAs and MetS risk Furthermore, [30]. A high-fat or high-carbohydrate consumption leads to the accumulation of excess body fat, while a low incidence of atherosclerosis and improvement of impaired pancreatic β-cell secretory function have been associated with the intake of diets rich in monounsaturated fatty acids, especially oleic acid, 18:1(n-9)36Oleic acid is not found only in olive oil, but also in many vegetable oils (*e.g.* high-oleic varieties of soybean and canola), nuts, fruits, and animal products (*e.g.* beef, pork, and eggs)[32]. Diets supplemented with monounsaturated oleic acid as Figure 7 can be correlated with valuable outcomes on body structure, thereby adding to the controlling and inhibition of obesity. Also, a product of oleic acid, oleoylethanolamide, has been shown to decrease hunger and consequent food intake [33]. Therefore, diets high in oleic acid may play a role in regulating food intake, body weight, and liver function (EE) by stimulating the AMPK signaling pathway, which induces and increases SIRT1/PGC-1α activity to regulate fatty acid oxidation rates; prevent NLRP3/caspase-1 inflammasome-associated obesity; and stimulate OEA synthesis, leading to increased FOx and EE in the presence of PPAR-α; and decreased SCD1 activity. Together, these results support the advice not to restrict the intake of OA-rich diets to maintain a healthy body weight [29]. The beneficial effects of diets enriched in OA in regulating body weight lead to the conclusion that diets enriched in OA should be included in obesity-management programs [30]. In addition, our study found an increase in the levels of saturated fatty acids for both palmitic acid (Figure 8) and stearic acid (Figure 9) in obese people compared to their level in normal people for both sexes as Table 1, palmitic acid (PA) constitutes 27% of total free fatty acids in plasma. Studies have concluded that elevated PA levels cause glucose metabolism disturbances and an inflammatory response [31]. Importantly, it has been shown that the presence of high levels of PA that occurs as a result of obesity can significantly enhance the expression of KLF7, although the exact mechanism is unclear [32].

Stearic acid (SA) (C18:0) is a dietary long-chain saturated fatty acid that has been shown to reduce metastatic tumor burden. Dietary SA may reduce visceral fat, according to growing evidence and preliminary observations that visceral fat is associated with metastases and poor survival rates. we hypothesized that dietary SA may reduce visceral fat. Named after the Greek term meaning "solid fat," SA plays a regulatory role in various aspects of energy metabolism and signal transduction and has gradually entered the public eye as an important component of many physiological cellular functions, its applications range from serving as a source of somatic energy to participating in endogenous biosynthesis, similar to palmitate, SA serves as a substrate for the enzyme stearoyl-coenzyme A desaturase [33].It stimulates the conversion of states to oleates, and participates in the synthesis of complex lipids, including triglycerides. Also, SA acts as a bio-signaling molecule in pathological processes such as diabetes, cardiovascular diseases, growth, nervous system disorders, and liver injury [34] Our study showed that in patients with clinically severe obesity before bariatric surgery, PA (C16:0) and SA were predominant among the total saturated fatty acids in plasma, but we found lower values of linoleic acid, alpha-linolenic acid and oleic acid in patients with metabolic syndrome, which is in agreement with what others have found [35] PA induces pro-inflammatory mechanisms through Toll-like receptor 4 (TLR4)-mediated inflammatory signals and reactive oxygen species (ROS) in a TLR-independent manner [36] and impairs hepatic glucose and lipid metabolism and induces central leptin resistance [37].

CONCLUSION

In cases of morbid obesity, five fatty acids, alpha-linolenic, linoleic, oleic, palmitic, and stearic were isolated from the blood of patients. This was evident in elevated levels of the saturated fatty acids palmitic and stearic in obese individuals of both sexes, with correspondingly lower levels of the three isolated unsaturated fatty acid, alpha-linolenic, linoleic, and oleic. The proportion of isolated fats from patients was higher in women than in men of the same age group. Therefore, patients are advised to follow a healthy diet free of saturated fats, especially these two acids, to avoid the complications of metabolic syndrome and the diseases resulting from it.

Acknowledgment

The researchers are grateful to Mosul University for their help.

Ethical Consideration

The study was authorized by our organization (19545, 1/5/2023) and the board of review of authors. It was conducted in accordance with institutional policy, all applicable national laws, and the principles of the Helsinki Declaration.

Conflict 0f Interests

The author confirm that this article content has no conflicts of interest.

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