



## Association of Fatty Acid Synthase with Level of Fatty Acids in Osteoporosis Patients

Abeer Hassan Alwan<sup>1</sup> and Ekhlass M. Taha<sup>2\*</sup>

<sup>1,2</sup>Department of Chemistry, College of Sciences for Women, University of Baghdad, Baghdad, Iraq.

\*Corresponding Author.

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### Abstract

Osteoporosis (OP) is a bone disease that makes bones more brittle and increases the risk of fractures. It is the most common type in postmenopausal women and is characterized by decreased bone mineral density, skeletal microstructure disintegration, and increased bone fragility. This study aims to assess the connection between fatty acid (FA) and body mass index (BMI), T-score, lipid profile including triglyceride (TG), total cholesterol (TC), high-density lipoprotein (HDL), low-density lipoprotein (LDL), very low-density lipoprotein (VLDL), total antioxidant status (TAS), total oxidant status (TOS), FA synthase (FAS). The current study included 120 participants. Serum samples from postmenopausal women were collected and divided into three groups: 40 patients with OP (G2), 40 patients with osteopenia (G1), and 40 women as the control group. Gas chromatography (GC) to determine the levels of FAs and ELISA was used to determine the levels of FAS, whereas TAS, TOS, and lipid were assayed using a spectrophotometer. The study showed that OP had a significantly lower serum FAS level than osteopenia and control. The levels of palmitic, stearic, oleic, linolenic, and linoleic (TC, LDL) in the G2 group were significantly higher than those in the G1 group and the control group. The HDL, VLDL, TG, TAS, and TOS levels in G2 were considerably lower than in G1 and the control group. According to the receiver operating characteristic analysis, FAS showed high sensitivity and specificity. In conclusion, FAS, HDL, VLDL, TG, TAS, and TOS effectively monitored the progression of OP and FA levels.

**Keywords:** Osteoporosis, fatty acid, fatty acid synthase, lipid profile, total antioxidant status, total oxidant status.

### 1. Introduction

Osteoporosis (OP) is a systemic skeletal condition characterized by a limited decrease in



microstructural bone tissue and bone mass, increasing bone brittleness and fracture susceptibility (1). The WHO defines a fragility fracture as an insufficient injury to fracture normal bone; less dense and solid bone masses cause osteoporotic bones to become more porous (2); OP has a significant impact on public health and raises the risk of fragile fractures. Consequences for an individual might include discomfort, loss of function, low quality of life, institutionalization, and even death. There was also a significant increase in the social burdens. It is a serious medical condition that has affected 200 million people worldwide (3). As people get older, osteopenia and OP, the silent disease, become more common. The condition of having less bone mass, known as osteopenia, is typically asymptomatic and not yet thought to be pathologic (4). Osteoporosis is a clinical indication for treatment with anabolic or anti-resorptive drugs and may serve as an early sign of the disease (5).

Dual-energy X-ray absorptiometry (DEXA), the most accurate X-ray technique, is the most widely used method for determining bone mineral density (BMD) (6). In this paper, we report on several biochemical parameters involved in bone remodeling, which can lead to diseases like OP. These parameters include fatty acid, lipid profile, total antioxidant status (TAS), total oxidant status (TOS), and fatty acid synthase (FAS). Fatty acids are carbon chains with methyl and carboxyl groups at the ends (7). Synthesis of FAs in the hepatocytes and fat tissue is caused by excess of energy, FA synthesis takes place in the hepatocytes and fat tissue. The hepatocytes and fat tissue use FAS to store excess carbohydrate calories as triglycerides (TG) (8). Fatty acids function as a primary metabolic fuel, storing and transporting energy, and are essential building blocks for complex lipids (9). In addition, dietary lipids contain polyunsaturated FAs (PUFAs) (10). The PUFA overconsumption, which results in a high n-6 to n-3 PUFA ratio, may contribute to the pathogenesis of obesity and OP (11). GC has become widely accepted as a highly applicable tool in some FA research areas in micro-scale analytical work (12). The only mammalian enzyme that can synthesize FA is FAS (13), which is also crucial for embryonic development. Two multifunctional polypeptide chains with seven different functional domains compose the animal FAS.

Positioning these two chains head to tail creates two distinct centers for FA synthesis; FAS is the only human enzyme that converts dietary carbohydrates to fat (14). Additionally, it is the only eukaryotic enzyme that can produce palmitate, the precursor to most non-essential FAs substances under normal circumstances. Jiang et al. (15) aimed to initiate apoptosis and counteract the osteoporotic phenotype. The main goal of the group study (16) was to increase the expression of FAS by osteoblasts, which acted on the osteoclast precursor FAS to trigger apoptosis and reverse the osteoporotic phenotype. The association of FAs with oxidative stress biomarkers may contribute to the pathogenesis of several diseases. The term "oxidative stress" refers to a pro-oxidant/ antioxidant imbalance that favors antioxidants (17,18). Excess free radical damage to biomolecules can cause chronic inflammation, aging, and OP. It physiologically alters other parameters, such as the lipid profile, which some individuals experience as a risk factor for OP. This study aims to determine FAS, TAS, TOS, and lipid profile as suitable parameters to follow up on the progress in OP.

## 2. Materials and Methods

### 2.1. Study subjects

The researchers collaborated with Medical City (a consulting clinic department in the Republic of Iraq) with of 120 patients. These samples are divided into patients with newly diagnosed OP (n = 40) and patients with osteopenia (n = 40). The study involved 40 healthy individuals aged 40-60 years.

### 2.2. Blood sample collection and analysis

Blood samples (5 mL) were collected from volunteer subjects ten to twelve hours after fasting. Blood samples were collected from OP, osteopenia patients, and the control group under aseptic conditions and centrifuged for 15 minutes at 1000 g. Next, serum samples were divided into small aliquots for storage at  $-20\text{ }^{\circ}\text{C}$  until a portion of the acquired serum was used for lipid profile measurement. The body mass index (BMI) was calculated by dividing the weight (in kg) by the square of the height ( $\text{m}^2$ ) (19). Biochemistry (TG, TC, HDL, TOS, and TAS) was measured using a Biolabo kit. This study used a commercially available ELISA kit, following the manufacturer's instructions (MyBioSource, America), to measure the level of FAS; FA levels were assayed using the GC technique.

### 2.3. Statistical analysis

The statistical analysis tool (SPSS 25) was used to analyze the data. Mann-Whitney and Superman tests were used to analyze non-parametric distributed data at the 0.05 significance level. For normal parametric distribution data, ONEWAY-ANOVA with a 0.05 alpha level was used.

## 3. Results and Discussion

**Table 1** lists the mean $\pm$ SD for the age, BMI, and T.score of patients with OP, osteopenia, and healthy subjects.

The best way to distinguish between patients with OP, osteopenia, and control is by using the T-score. **Table 1** displays the T-score outcomes. The results showed a significant difference between OP, osteopenia, and control [(-3.15) (-1.88) (-0.63), respectively, with a considerable difference ( $p=0.0001$ ).

**Table 1.** Distribution of mean age, BMI, and T-score for patients groups and control.

Parameter	Mean $\pm$ SD			p-value
	Control Group	Group 1 Osteopenia	Group 2 OP	
Age	51.90 $\pm$ 8.60	53.30 $\pm$ 11	58.90 $\pm$ 10.30	0.017
BMI	32.60 $\pm$ 4.30	30.0 $\pm$ 3.70	28.90 $\pm$ 4.70	0.04
T-Score	-0.63	-1.88	- 3.15	0.0001

The findings revealed a significant difference in the age factor between the three groups for mean  $\pm$ SD ( $p= 0.017$ ). The results of the BMI are also shown in Table 1. The results show the Mean $\pm$ SD of OP, osteopenia, and control of BMI as (28.90  $\pm$  4.70), (30.0  $\pm$  3.70), and (32.60  $\pm$  4.30), respectively. This shows a discernible difference between the three groups ( $p=0.04$ ).

**Table 2** shows the serum levels of TC, TG, HDL, VLDL, and LDL, along with OP, osteopenia, and control. When the three groups were compared, there were highly significant differences in TC, TG, HDL, VLDL, and LDL levels.

The TG levels were significantly higher in the OP and osteopenia groups ( $127.60 \pm 16.80$ ) ( $175.80 \pm 37.0$ ), respectively. Comparatively to the control group, ( $156.90 \pm 34.0$ ),  $p= 0.02$ , as shown in **Table 2** TC, LDL, VLDL, and HDL levels increased significantly in OP patients ( $166.19 \pm 20.0$ ), ( $92.10 \pm 23.25$ ), ( $25.50 \pm 3.30$ ), ( $47.60 \pm 6.0$ ), respectively and in osteopenia patients ( $149.0 \pm 19.0$ ), ( $76.10 \pm 27.0$ ), ( $33.90 \pm 9.50$ ), ( $50.70 \pm 0.36$ ), respectively. When compared to the control group ( $142.63 \pm 28.0$ ), ( $63.80 \pm 29.0$ ), ( $31.05 \pm 7.0$ ), ( $51.16 \pm 1.70$ ), respectively.

**Table 2.** Mean  $\pm$ SD values of TC, HDL, TG, LDL, and VLDL for all the studied groups.

Parameter	Mean $\pm$ SD			p-value
	Control Group	Group1 Osteopenia	Group2 OP	
TC (mg/dL)	142.63 $\pm$ 28.0	149.0 $\pm$ 19.0	166.19 $\pm$ 20.0	0.00
HDL (mg/dL)	51.16 $\pm$ 1.70	50.70 $\pm$ 0.36	47.60 $\pm$ 6.0	0.00
TG (mg/dL)	156.9 $\pm$ 34.0	175.80 $\pm$ 37.0	127.60 $\pm$ 16.8	0.02
LDL (mg/dL)	63.8 $\pm$ 29.0	76.10 $\pm$ 27.0	92.10 $\pm$ 23	0.00
VLDL (mg/dL)	31.05 $\pm$ 7.0	33.90 $\pm$ 9.50	25.5 $\pm$ 3.30	0.00

Dyslipidemia is a significant contributor to the development of cardiovascular disease (CVD), diabetes type II, stroke, and OP, but it can be treated with medication and lifestyle modifications (20). High serum TC, TG, or even both, or HDL-C levels were considered atherogenic variables in OP patients. It is characterized by abnormal lipid profile values (21). The study's findings in 2021 strongly imply that having higher levels of HDL and TC was associated with a higher risk of OP, which discovered a connection between high serum HDL levels and an elevated risk of OP (22). Estrogen deficiency, a steroid hormone that is an esterified form of cholesterol synthesis, is the leading cause of menopausal bone loss. In the human body (23). TG is the most prevalent lipid. The majority of organs can obtain energy from the byproducts of TG decomposition. The liver and other organs can also create TG, which is kept in fat tissue (24). A previous study showed no correlation between TG levels and the risk of OP in postmenopausal women. Increased blood levels of LDL can result in deposits on the arterial wall and other areas of the cardio-cerebral vascular system, which can block blood vessels by gradually forming atherosclerotic plaques (25). The literature, however, is still unclear regarding the connection between OP and LDL levels. This analysis revealed that the OP group had a higher LDL level than the control group.

As is shown in **Table 3**, the results of the FAS showed mean  $\pm$ SD of OP, osteopenia, and control [(5.10  $\pm$  0.70) (7.94  $\pm$  1.4) (5.74  $\pm$  0.90)], where the result indicates a significant change among three groups in the nitric oxide (NO) ( $p= 0.05$ ).

The TAS was determined using the. As shown in **Table 3**, the results showed OP, osteopenia, and control [(1.48  $\pm$  0.50) (1.37  $\pm$  0.60) (1.61  $\pm$  0.40)], where the result indicates a non-significant difference among the three groups in TAS ( $p > 0.05$ ). The TOS results showed

mean  $\pm$ SD of OP, osteopenia, and control with a non-significant change among three groups in total oxidant status ( $p > 0.05$ ), shown in **Table 3**. The only human enzyme complex that converts dietary carbohydrates to fat is called FAS (13). Previous studies concentrated on boosting osteoblastic FAS expression to interact with osteoclast precursor FAS and trigger apoptosis to reverse the osteoporotic phenotype (26).

**Table 3.** Mean  $\pm$ SD values of FAs and TOS and TAS for all the studied groups.

parameter	Mean			p-value
	Control group	Group 1 Osteopenia	Group 2 OP	
FAS (mg/dl)	5.74 $\pm$ 0.90	7.94 $\pm$ 1.40	5.10 $\pm$ 0.70	0.00
TOS (mM/L)	0.21 $\pm$ 0.10	0.29 $\pm$ 0.10	0.15 $\pm$ 0.10	0.8
TAS (mM/L)	1.61 $\pm$ 0.40	1.37 $\pm$ 0.60	1.48 $\pm$ 0.50	0.2

The results are shown in **Table 4**, along with the mean and SD of the Oleic. The studied group differed significantly in terms of results ( $p = 0.05$ ) compared with the control group. According to **Table 4** palmitic and stearic  $\alpha$ -linolenic and linoleic results, there was no significant difference ( $p > 0.05$ ) between the OP and mild OP groups and the control group in terms of palmitic and stearic  $\alpha$ -linolenic and linoleic. Various studies have been conducted during the past few years (27). The FAs produced by marrow adipocytes are significant, with an excessive amount of Palmitic acid (PA), a saturated FA (28). Postmenopausal OP causes PA to be the FA that is most prevalent in the human bone marrow environment, which suggests that PA may be involved in the pathogenesis of this condition. On the other hand, high levels of cell death and apoptosis were induced by PA alone (29).

**Table 4.** Mean  $\pm$ SD values of palmitic, oleic, stearic,  $\alpha$ -linolenic, and linolic for all the studied groups.

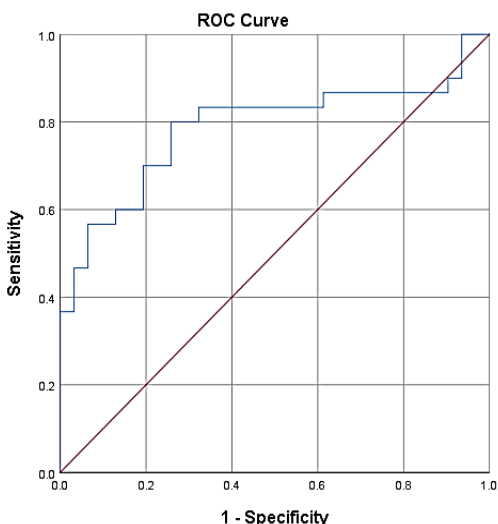
Parameter %	Mean $\pm$ SD			p-value
	Control Group	Group 1 Osteopenia	Group 2 OP	
Palmitic	3.20 $\pm$ 0.0	3.31 $\pm$ 0.20	3.45 $\pm$ 0.30	0.3
Stearic	1.59 $\pm$ 0.10	1.49 $\pm$ 0.10	1.63 $\pm$ 0.10	0.2
Oleic	16.50 $\pm$ 0.30	16.60 $\pm$ 0.20	16.80 $\pm$ 0.10	0.05
$\alpha$ -linolenic	25.0 $\pm$ 0.20	24.60 $\pm$ 0.30	24.80 $\pm$ 0.30	0.1
Linolic	1.98 $\pm$ 0.001	1.94 $\pm$ 0.008	1.98 $\pm$ 0.001	0.3

It has been demonstrated that linoleic acid has a favorable impact on bone metabolism. These results indicate that linoleic acid modulates markers of inflammation and osteoclastogenic factors, which may prevent the loss of bone and muscle mass (30). Consequently,  $\alpha$ -linolenic acid's effects on osteoclast function and bone loss were investigated (31). Also, oleic acid, minerals, and vitamins reduce CV risk factors and induce positive adjustments in a few markers of bone metabolism (32).

The receiver operating characteristic (ROC) curve for patients with osteopenia indicates a sensitivity of 80% for FAS and a specificity of 70.8%, with a cutoff value of 5.4, as presented in **Table 5** and **Figure 1**.

**Table 5.** The ROC result analysis of FAS in OP patients.

Parameters	Area	Sensitivity	Specificity	Cutoff	Asymptotic 95% Confidence Interval	
					Lower Bound	Upper Bound
FAS	78.6	80	70.80	5.40	0.66	0.90



**Figure 1.** The ROC curve for FAS in OP patients.

**4. Conclusion**

The FA results show significant differences between the three groups (OP, Osteopenia, and control). The level of FAs appeared high at the beginning of the disease and then decreased as the disease progressed. The levels of lipid profile (TC, TG, LDL, and VLDL) were significantly involved in the OP group. The FAS is a good prognosis factor in the development of OP.

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**Conflict of Interest**

The authors declare that they have no conflicts of interest.

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## Ethical Clearance

The study was conducted with ethical principles. The study protocol was reviewed and approved by Baghdad University, College of Science for Women, a local ethics committee, according to document number CSEC/044/0071, on 26 September 2021 to get this approval.

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