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# Fracture risk, bone density and vitamin D in diabetic women

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## ABSTRACT

**BACKGROUND.** Aging is associated with the increasing prevalence of both type 2 diabetes mellitus and osteoporosis, especially in the elderly and female population.

**SUBJECTS AND METHODS.** We conducted a study to evaluate the correlation between vitamin D deficiency, type 2 diabetes (DMT2), and osteoporosis in a group of Italian women aged 40 to 75 years, comparing biochemical-clinical parameters, anthropometric parameters, and results of instrumental investigations, such as the MOC-DEXA examination.

**RESULTS.** The results show a lower bone mineral density and, consequently, an increased risk of osteoporosis fractures in patients with type 2 diabetes (DMT2). The responsible mechanism is particularly complex and not yet fully clarified. In addition, there is a negative correlation between plasma levels of vitamin D (25(OH)D) and an increase in insulin resistance with an increase in blood glycated hemoglobin (HbA1c); in other words, there is a higher mean vitamin D value in non-diabetic patients than in the population of women with diabetes.

**CONCLUSIONS.** Vitamin D deficiency plays a key role in the development of insulin resistance as well as in the development of osteoporosis and, in patients with type 2 diabetes mellitus, there is a greater loss of bone matrix integrity and consequently there is a significant increase in the risk of bone fracture. This figure is significant for future preventive and therapeutic clinical choices.

## KEYWORDS

TYPE 2 DIABETES MELLITUS
VITAMIN D
OSTEOPOROSIS
MOC-DEXA
WOMEN

## BACKGROUND

Aging is associated with the increasing prevalence of both type 2 diabetes and osteoporosis, and these chronic diseases are frequently associated with the elderly, especially in women. Although osteoporosis and type 2 diabetes (DMT2) seem to be not pathophysiologically related, numerous epidemiological studies have shown an increased risk of fractures among DMT2 patients. This higher risk is probably due to a combination of a greater risk of falling, regional osteopenia, reduced bone quality, and treatment effects. The pathophysiological processes underlying osteoporosis are related to estrogen deficiency but also depend on an increase in PTH[1] due to reduced levels of vitamin D and subsequent reduced intestinal calcium absorption. Vitamin D deficiency in postmenopausal women is certainly a risk factor for the development of osteoporotic disease.

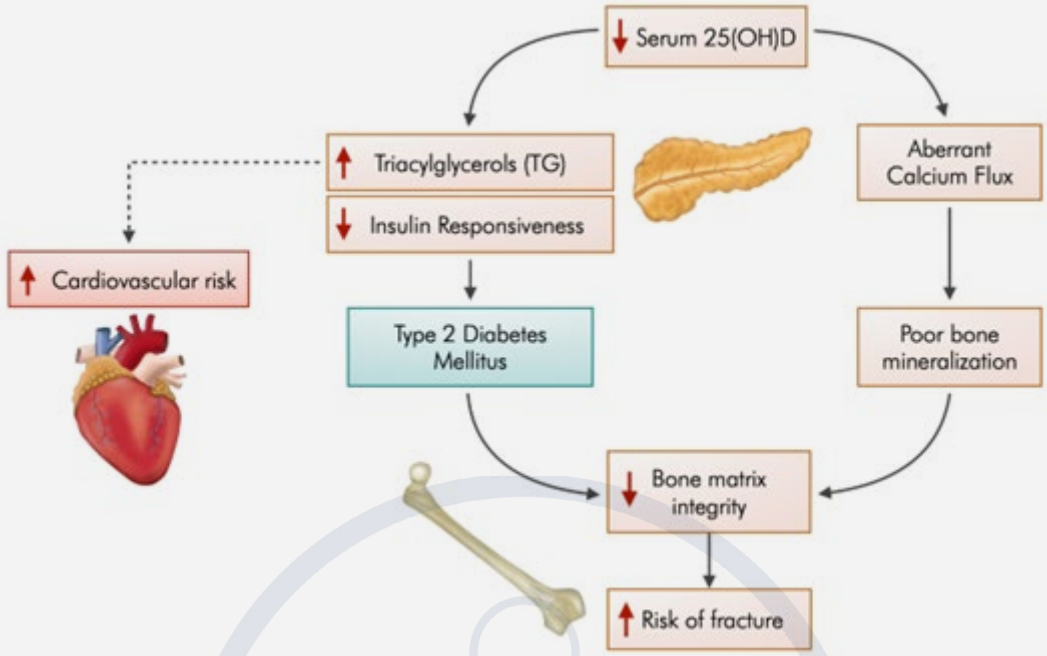


**Figure 1.** Representative diagram of the effect of vitamin D deficiency on insulin resistance, type 2 diabetes and osteoporosis.

## EFFECT OF VITAMIN D ON INSULIN RESISTANCE

The main and best-known function of vitamin D is to maintain the homeostasis of calcium and phosphorus and promote bone mineralization. However, recent evidence[1,2] suggests that vitamin D and calcium homeostasis may also be important for a variety of non-skeletal outcomes, including neuromuscular functions, psoriasis, multiple sclerosis, and colorectal and prostate cancer. Based on baseline and animal studies[3], vitamin D and calcium have also been suspected as risk modifiers for diabetes. Vitamin D deficiency has long been considered a risk factor for type 1 diabetes based on observational studies in animals and humans. More recently, accumulated evidence suggests that the altered homeostasis of vitamin D and calcium may play a role in the development of type 2 DM. Vitamin D can have a beneficial effect on improving pancreatic beta cell function, reducing insulin resistance, and improving systemic inflammation[4]. The main management of vitamin D deficiency is vitamin D supplementation to prevent or improve the disease. Several studies support that vitamin D supplementation can affect glucose homeostasis and improve insulin resistance. Among the mechanisms linking bone metabolism to DMT2, vitamin D deficiency has been extensively studied[5]. The relationship between metabolic control in DMT2 and bone metabolism has been the focus of numerous experimental and epidemiological studies. The results are often controversial, being influenced by the number of patients included in the survey, the study design, or blood glucose control measures. A significant increase in serum calcium levels and a reduction in serum fatty acid levels were found after taking vitamin D supplements[6]. Recently, a study from New Zealand[7] found that South Asian women with insulin resistance improved significantly after taking vitamin D supplements. Optimal vitamin D concentrations to reduce insulin resistance were found to be between 80 and 119 nmol/l, providing additional evidence for an increase in recommended adequate levels.

## INFLAMMATION, VITAMIN D, AND INSULIN RESISTANCE

Chronic inflammation is involved in the development of insulin resistance, which increases the risk of type 2 DM. It is known that VDR is expressed by macrophages and dendritic cells, which suggests that vitamin D plays an important role in the modulation of inflammatory responses[8-11]. Both cell types express the enzymes vitamin D-25-hydroxylase and 1α-hydroxylase and can produce 1,25-dihydroxyvitamin D. Numerous studies have supported the role of vitamin D, and

1,25-macrophages are cells with a large cytokine production capacity, in particular TNFα, which is one of the most important products released by these cells[12]. Transcriptional activation of the TNFα gene in macrophages largely depends on transcriptional activation dependent on nuclear factor NF-κB. In mouse macrophages stimulated by lipopolysaccharide (LPS)[13], 1,25-dihydroxyvitamin D overregulates IκB-α (the NF-κB inhibitor) by increasing the stability of mRNA and decreasing the phosphorylation of IκB-α. In addition, increasing levels of IκB-α may reduce the nuclear translocation of NF-κB. In addition, 1,25-dihydroxyvitamin D suppresses the expressions of the TLR2 and TLR4 proteins and mRNA in human monocytes in a time and dose-dependent manner[14,15]. Recently, it has also been suggested that inflammation and activation of the innate immune system could be adjusted downwards by hydroxyvitamin D from increased levels of inflammatory markers (TNFα, in summary, 1,25-dihydroxyvitamin D inhibits the release of the proinflammatory cytokine TNFα, regulates the activity of NF-κB, and suppresses the expressions of TLR2 and TLR4 proteins and mRNA in human monocytes, reducing the release of cytokines)[16]. Therefore, vitamin D can also work to reduce insulin resistance and the risk of diabetes by decreasing inflammatory responses.

## INTRODUCTION

The aim of the study was to evaluate the correlation between vitamin D deficiency, type 2 diabetes, and osteoporosis in a group of Italian women aged 40 to 75 years, comparing biochemical-clinical parameters, anthropometric parameters, and results of instrumental investigations, such as the MOC DEXA (Dual-Energy X-ray absorptiometry) test.

## SUBJECTS AND METHODS

The target population of this study consisted of 130 female adults aged 40 to 75 years (mean age 57.9 ± 9.8 years). Anthropometric measurements (body weight and height) were taken to assess the nutritional status. Anthropometric variables were measured according to WHO recommendations. To define overweight and obesity, we refer to the threshold values of the body mass index (kg/m$^2$). For the evaluation of biochemical-clinical parameters useful for the purposes of this study, analyses were carried out on biological samples (blood, urine).

**Clinical and biochemical assessment**

- For each subject, anthropometric and hematochemical parameters were detected:
- Body weight
- Body Mass Index (BMI)
- Hematochemical parameters: Vitamin D, Glycated Hemoglobin, Blood Glucose, Insulin, Total Cholesterol, Cholesterol-LDL, Cholesterol-HDL, Triglycerides.
- Bone density

**Anthropometric parameters**

Anthropometric measurements were taken in the morning and on an empty stomach in conjunction with blood sampling. Body weight was detected using a scale. Height was measured with the stadiometer (make and model). From the ratio of weight to height, the Body Mass Index (BMI), expressed in kg/m$^2$, was calculated.

**Haematochemical parameters**

Blood samples were taken between 08:00 and 09:00 after an overnight fast and analyzed. Serum concentrations of 25(OH)D were quantified by a chemiluminescence method (Diasorin Inc, Stillwater, USA). Serum insulin concentrations were measured by radioimmunology (Behring, Scoppito, Italy). Plasma glucose was determined using the glucose oxidase method (Sclavus, Siena, Italy). Plasma lipid concentrations (triglycerides, total cholesterol, HDL cholesterol) were quantified by the automatic colorimetric method (Hitachi; Boehringer Mannheim, Mannheim, Germany). LDL cholesterol was calculated using the Friedewald equation[17].

**Computerized bone mineralometry**

Computerized Bone Mineralometry (MOC) is a diagnostic technique used to evaluate bone mineralization. Measuring the density of bone mass can detect possible bone degeneration. Currently, the methods most used to perform mineralometry are the MOC DEXA, which requires the use of an X-ray apparatus, and the MOC QUS, which relies on ultrasound-based equipment.

## RESULTS

The survey, conducted in a population of 130 ethnic white women, assessing the relationship between the levels of 25(OH)D and the different anthropometric, biohumoral and anamnestic parameters, gave the results reported in the following paragraphs.

**Anthropometric parameters**

From the survey of anthropometric parameters and BMI in the studied sample (Table 1; Figure 2), it is clear that only 24% of the population was normal weight, while more than half of the subjects were overweight (61%), and the remaining 15% are in a condition of obesity.

**Table 1.** Anthropometric parameters detected in 130 subjects.

| Parameter | Average ± SD |
|---|---|
| Age (years) | 57.9 ± 9.8 |
| Weight (kg) | 69 ± 9.7 |
| Height (m) | 1.60 ± 0.1 |
| BMI (kg/m2) | 26.4 ± 3.3 |

Analysis of the correlation between BMI and age of the sample examined showed a substantially homogeneous distribution and the absence of a correlation between the two parameters ($R^2$=0). In other words, the conditions of overweight and obesity were homogeneously distributed in the sample examined.

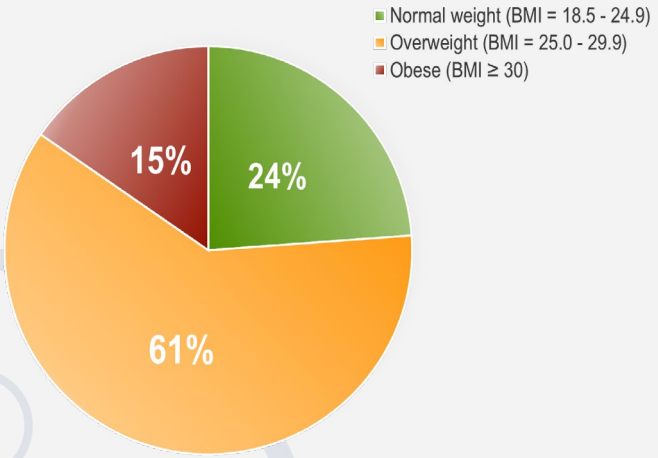


**Figure 2.** Distribution of BMI in the sample of 130 women studied.

**Correlations between hematochemical parameters and BMI**

For the diagnosis of DMT2, the correlation between the condition of being overweight and the alteration of the hematochemical parameters is of particular importance. Serum glucose levels are positively correlated by reaching for BMI values corresponding to the condition of overweight and obesity levels above the upper limits of normal. The relationship of the condition of overweight and obesity with the lipidemic picture, although not evidenced by the condition of hypertriglyceridemia (Figure 4), is detectable by the relationship between BMI and blood levels of HDL. As shown in the graph in (Figure 5), although the coefficient of determination is not high, the two parameters show an inverse correlation. More relevant is the ratio of BMI to glycated hemoglobin HbA1C, which, as shown in Figure 6, has a clear positive correlation.

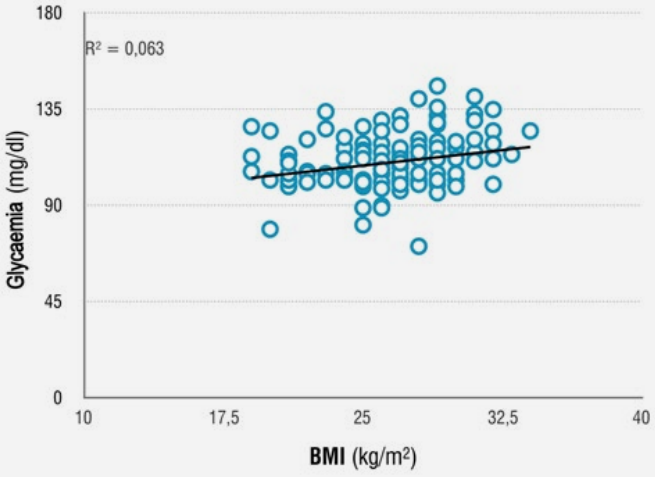


**Figure 3.** Relationship between BMI and blood glucose in the sample examined.

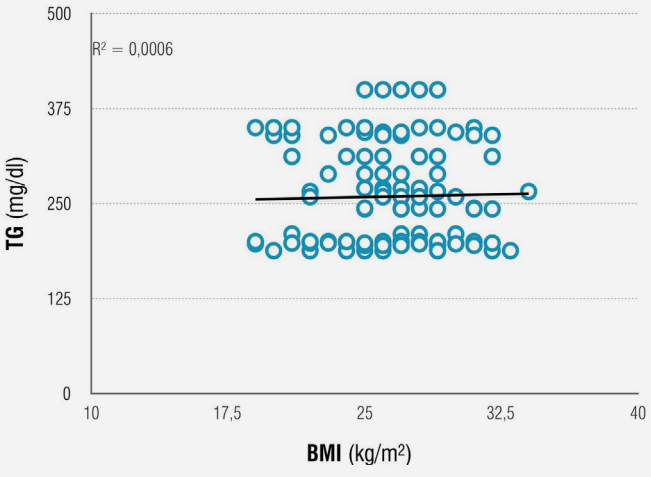


**Figure 4.** Relationship between BMI and triglyceridaemia in the sample examined.

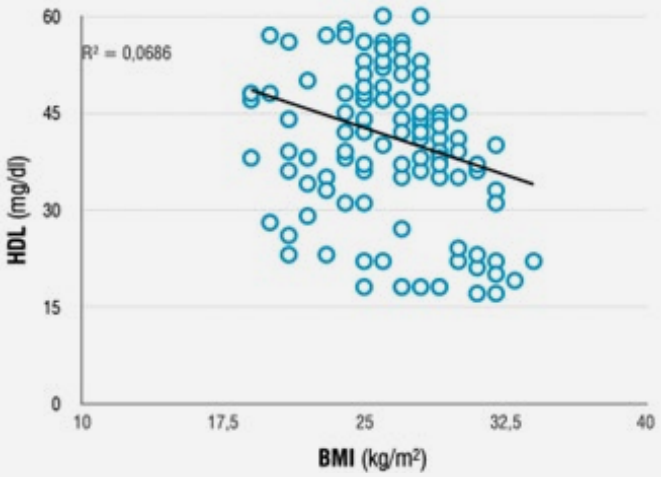


**Figure 5.** Relationship between BMI and HDL blood levels in the sample examined.

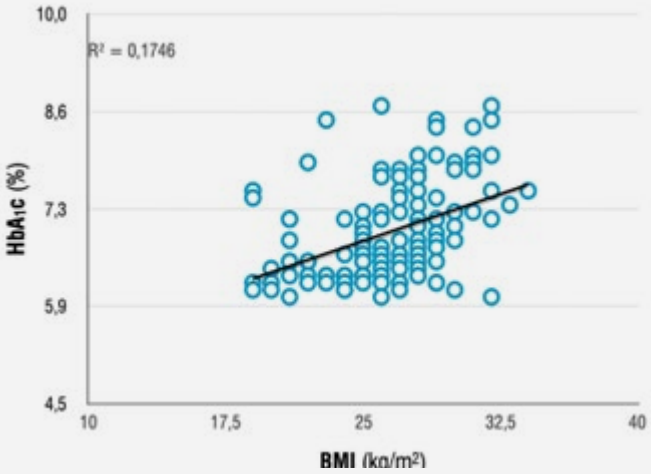


**Figure 6.** Relationship between BMI and HbA1C in the sample examined.

### Vitamin D

Regarding blood levels of vitamin D, reference ranges were considered to identify subjects with sufficient levels compared to those with insufficient or severe deficiency, and the results are given below.

It can be noted that:

- **95%** of the population (123 subjects) do not have a sufficient level of Vitamin D (≤ 30 ng/ml);
- In **50%** of the population (65 subjects), a severe deficiency of Vitamin D was found probably due to insufficient dietary intake (0 - 20 ng/ml);
- **45%** of the population (58 subjects) has Vitamin D insufficiency (21-30 ng/ml);
- Only **5%** of the population (7 subjects) have sufficient levels of Vitamin D (31-100 ng/ml).

This condition confirms the correlation between the presence of DMT2 and the state of inadequate availability of vitamin D.

### Correlations of parameters with Vitamin D levels

Analysis of the correlation between serum vitamin D levels and BMI in the population studied (Figure 7) does not show a significant correlation between the parameters. The data in the graph in Figure 8 show a significant inverse correlation between decreased plasma vitamin D levels and increased blood triglyceride concentrations in the study population. The data obtained suggest that decreased plasma vitamin D levels are associated with an increase in blood triglyceride concentration and an increased cardiovascular risk.

The examined population was divided into 3 groups, i.e., non-diabetic patients, patients who have been diagnosed with diabetes for less than 10 years, and patients who have been diagnosed with diabetes for more than 10 years, as shown in Table 3.

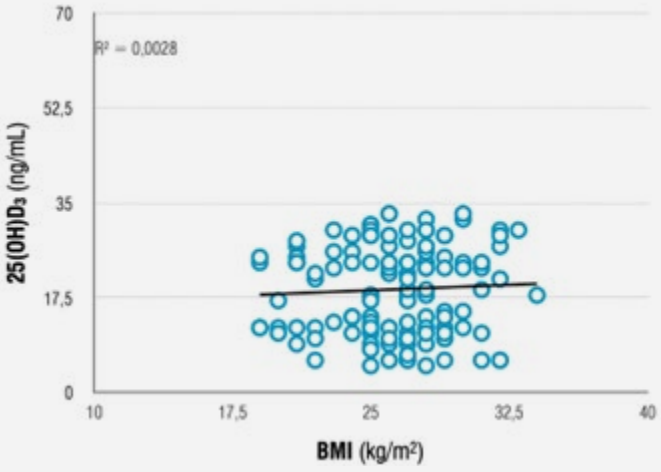


**Figure 7.** Relationship between BMI and vitamin D in the sample examined.

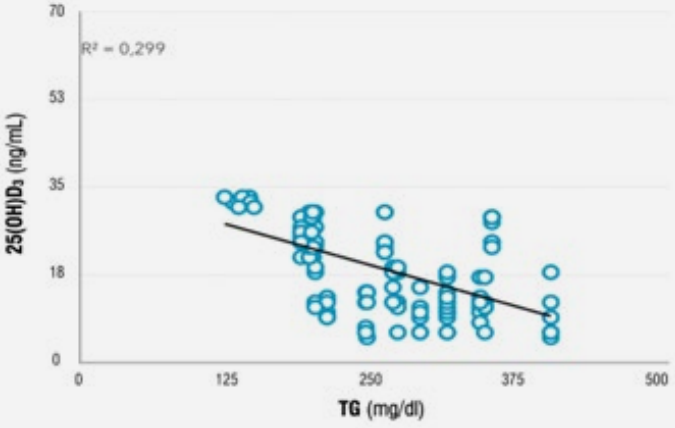


**Figure 8.** Relationship between blood levels of triglycerides and vitamin D in the sample examined.

**Table 2.** Biochemical parameters detected in 130 subjects.

| Biochemical parameter | Average ± (SD) | | Normal reference values | Number of patients out of range |
|---|---|---|---|---|
| Vitamin D (ng/mL) | 19.07 | ± (8.28) | (*) | See Table 3 |
| Glycated haemoglobin (HbA1c) % | 6.92 | ± (0.69) | < 7** | 54 |
| Glycaemia(mg/dL) | 92.46 | ± (7.40) | 65-110 | 62 |
| Insulin (µU/mL) | 12.25 | ± (8.24) | 4-24 | 35 |
| Total cholesterol (mg/dL) | 188.09 | ± (30.55) | < 200 | 49 |
| Cholesterol-LDL (mg/dL) | 99.78 | ± (21.64) | < 100 | 46 |
| Cholesterol-HDL (mg/dL) | 63.04 | ± (22.12) | > 45 | 82 |
| Triglycerides (mg/dL) | 146.03 | ± (11.7) | < 150 | 123 |

*0-20 deficiency, 21-30 insufficiency, 31-100 sufficiency, >100 Toxicity
**ADA, Diabetes Care 2006

**Table 3.** Plasma levels of vitamin D in the studied population.

| Subdivision of population into 3 subgroups | Vitamin D (ng/mL) (Average ± SD) |
|---|---|
| Non-diabetic patients | 21.06 ± 9.6 |
| Patients with diabetes for < 10 years | 19.44 ± 7.9 |
| Patients with diabetes for >10 years | 18.55 ± 8.5 |

It is noted that:

- In non-diabetic patients, there is a higher mean vitamin D value than in the population with full-blown diabetes.
- Patients who have been diagnosed with type 2 diabetes mellitus for less than 10 years have higher mean vitamin D levels than patients with diabetes for more than 10 years.
- There is a correlation between decreased mean blood vitamin D levels and increased insulin resistance and type 2 diabetes mellitus.

**MOC-DEXA**

About the results of the MOC-DEXA tests, it should be noted that mean normal T-Score (score of -1.01 ± 8.95) was found in non-diabetic patients, except for patients who had fractures not attributable to osteopenia and/or osteoporosis, but rather to trauma of different etiology. In diabetic patients, the mean T-score values are outside the normal range (T score ≤-1) (Table 4). The mean T-score values of the spine in the tract (L1-L4) and the left femur (both neck and whole) are classifiable as a condition of osteopenia (T-score between -1 and 2.5). It is clearly inferred that in patients with type 2 diabetes mellitus, there is a loss of bone matrix integrity, and consequently, there is a significant increase in the risk of bone fracture.

**Table 4.** Bone mineral densitometry examination computerized in patients with DMT2

| Region | Location | T-score Young adult (Average ± SD) | BMD (g/cm2) (Average ± SD) |
|---|---|---|---|
| Colonna | L1 - L4 | -1.57 ± (7.02) | 1.185 ± (22.01) |
| Left femur | Neck | -2.21 ± (11.24) | 0.987 ± (12.03) |
| Left femur | Entire | -2.21 ± (11.24) | 1.253 ± (8.25) |

The results show that there is a lower bone mineral density (and consequently an increased risk of osteoporosis fractures in patients with type 2 diabetes (DMT2). The responsible mechanism is particularly complex and not yet fully clarified. Certainly, insulin resistance negatively affects bone health. Several mechanisms have been proposed to explain the possible influences of diabetes on bone metabolism, including glycosuria, AGE, low levels of IGF-1 or altered plasma insulin levels, impaired renal function, and chronic inflammation. In addition, due to increased insulin resistance, the use of glucose in tissues is reduced, its levels increase, and consequently, the levels of HbA1C increase; this is a condition related to susceptibility to diabetes in subjects with increased resistance to insulin related to obesity. In addition, factors related to the complications of diabetes and/or its management, such as poor metabolic control or the use of certain hypoglycemic drugs, may affect osteoporosis and/or the risk of fractures in patients with T2DM. The risk of osteoporosis fractures is more evident (approximately 6 times higher) in both sexes in type 2 diabetics (doubled risk in postmenopausal women compared to the general population). The correlation between osteoporosis and type 2 diabetes, highlighted in these observations and discussed in the introductory part of this work, is a topic of considerable interest that numerous studies continue to deepen.

## CONCLUSIONS

There is a negative correlation between plasma levels of vitamin D (25(OH)D) and an increase in insulin resistance with increased blood concentrations of glycated haemoglobin ($HbA_{1c}$); in other words, there is a higher mean vitamin D value in non-diabetic patients than in the population of women with diabetes. Vitamin D deficiency is associated with an increased blood concentration of triglycerides and an increased cardiovascular risk. Vitamin D deficiency plays a key role in the development of insulin resistance as well as in the development of osteoporosis. In patients with type 2 diabetes mellitus, there is a greater loss of bone matrix integrity, and consequently, there is a significant increase in the risk of bone fracture. Type 2 diabetes mellitus is associated with an ever-increasing prevalence of osteoporosis in the female population. The present study conducted on a population of Italian women aged between 40 and 75 years clearly shows a correlation between decreased plasma vitamin D levels and increased insulin resistance and type 2 diabetes mellitus with reduced bone mineral density and consequent increased risk of osteoporosis fractures. This is probably due to the low bone quality in DMT2 subjects, as documented by the MOC-DEXA exam. Our observations show that vitamin D levels are not related to age, gender, serum insulin levels, metabolic parameters. These data reinforce the hypothesis that osteoporosis is an “unknown” complication of type 2 diabetes mellitus and that low plasma levels of 25(OH)D are a critical element for triggering this vicious circle.

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**Conflict of Interest**
The author declares that they have no conflicts of interest. The article is not under evaluation anywhere, and it is not submitted elsewhere.

**Policy on Ethics**
The author declares that informed consent was obtained from the subjects who adhered to it with complete free-

dom for the following observational study, which has been conducted in accordance with the Helsinki Declaration.

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