

Evaluation of Vitamin D3 serum as biomarker of metabolic activity and body composition in a study sample

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ABSTRACT

Background: Vitamin D has an important function on regulating calcium-phosphorus metabolism. It is involved in multiple extra-skeletal processes and supports physiological and metabolic functions.

Aim: to investigate the association between vitamin D levels and the lipid profile.

Material and Methods: Through a cross-sectional retrospective analysis, the participants underwent different assessments, in order to identify the possible correlations between vitamin D levels and changes in clinical, metabolic, and body parameters. Given the cross-sectional and retrospective nature of the study, observed associations should not be interpreted as causal, but rather as potential correlational findings warranting further prospective investigation.

Results: A correlation emerged between vitamin D levels and serum triglyceride concentration in women, with lower triglycerides observed in women with average ($p=0.028$) and optimal ($p=0.036$) vitamin D levels.

Conclusions: These exploratory findings suggest a potential correlational role for vitamin D in lipid assessment, warranting further study.

Key words: Vitamin D, Triglyceride, Women, Lipids, Metabolism, Biomarker



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Introduction

Vitamin D has a fundamental role as an essential micronutrient for our organism to function properly. Aside from having an important function on regulating calcium and phosphorus metabolism to maintain homeostasis, it is involved in a vast number of extra-skeletal physiological processes. This vitamin contributes to overall health, supporting immune system function, regulating neuromuscular activity, and playing an important role in chronic inflammatory conditions, autoimmune diseases such as multiple sclerosis, and neoplastic disorders.

Different studies have revealed that lacking vitamin D can be associated to some dermatological conditions like psoriasis², atopic dermatitis², vitiligo³ and melanoma⁴.

Furthermore, it has been observed that patients with a Body Mass Index (BMI) over 25 present a greater probability of lacking vitamin D⁵.

In aesthetic medicine, normalization of serum vitamin D levels has been shown to improve several skin parameters, particularly skin reactivity and hydration⁶.

Ongoing studies are currently revealing a complex interaction between vitamin D and adipocytes, with potential implications for the regulation of metabolism, inflammation, and overall health. However, further research is needed to fully understand the underlying mechanisms and clinical implications of this relationship.

This study aims to investigate the potential association between vitamin D levels and the lipid profile to better comprehend the role of this vitamin in metabolic and cardiovascular pathologies.

Materials and Methods

Participants

The study included adult patient in good health], compliant with the planned morpho-anthropometric measurements, and required blood tests. Patients with Primary Hyperparathyroidism, Intestinal Malabsorption Syndromes, Liver Diseases, Renal Diseases, ongoing or previous Neoplastic Diseases, past bariatric

surgery, chronic pharmacological therapy with anti-convulsants, antipsychotics, glucocorticoids, immunosuppressive corticosteroids, antiretrovirals, weight-loss drugs, hypolipidemic agents, and prolonged use of laxatives were excluded.

Inclusion/exclusion criteria were designed to minimize potential biases. Moreover, Vitamin D intake was not directly assessed, as this retrospective study relied only on serum levels and existing data.

The evaluation was based solely on the available variables, aiming to estimate the presumed contribution of vitamin D levels within those limits. The primary objective of the study was to assess a potential correlation between vitamin D and the lipid profile.

The study was conducted from January 2022 to January 2023. Each participant provided consent for the treatment of personal data and data privacy (Article 13, Legislative Decree 196/2003), signing a specific form. Once consent was obtained, participants in the study underwent medical, clinical, laboratory, and bioimpedance assessments.

Analyses performed

Analysed patients underwent clinical, laboratory, and bioimpedance assessments to obtain a comprehensive view of their health conditions. Clinical examinations included detailed assessments of their medical histories, pre-existing conditions, and risk factors in order to understand the overall context of their health. Laboratory analyses included blood tests to evaluate lipid profiles, inflammatory markers, and other key parameters relevant to metabolic function. Additionally, bioelectrical impedance analysis, a non-invasive method for assessing body composition, was performed to provide detailed information on the distribution of adipose and lean tissue in participants. The integration of these diverse assessment methodologies allowed for a complete and detailed overview of the patients' conditions, enabling the identification of potential correlations between vitamin D intake and variations in clinical, metabolic, and body parameters.

Nutritional anamnesis

Each patient underwent a detailed analysis of his or her nutritional history, with the aim of obtaining quantitative and qualitative information regarding the caloric intake and distribution of various macronutrients. These included both simple and complex carbohydrates; lipids, subdivided into saturated, monounsaturated, and polyunsaturated fats; proteins of both animal and plant origin present in the diet.

The acquisition of this data was done through a series of specific questions which allowed for the collection of information on daily eating habits, number of meals taken during the day (such as breakfast, lunch, dinner, and snacks), specific choices related to foods consumed by each participant, and additional details on health conditions. During the interview, general data such as gender and date of birth were recorded, as well as medical and family history, including any medical conditions, medication and food supplement intake, presence of food allergies and intolerances. In addition, information regarding lifestyle was collected, including the level of physical activity (intense, moderate or sedentary) as well as any smoking habits.

Anthropometric and bioimpedance evaluations

Anthropometric data followed a validated protocol⁷. Weight and height used a SECA 711 scale/stadiometer (220 kg capacity, 100 g sensitivity; 200 cm max, 1 mm accuracy). Circumferences were measured with ergonomic tape (1–205 cm range, 1 mm accuracy). BMI was calculated as weight (kg)/height² (m).

Body circumferences were measured using a validated ergonomic tape measure for measuring circumferences, with a measurement range of 1 to 205 cm and an accuracy of 1 mm. BMI (body mass index) was calculated using weight and height.

To measure an individual's height accurately, the following were applied:

1. The subject should be barefoot and wear light clothing.

2. Feet should be placed on the scale plane with the heels together and the toes spread apart at an angle of about 60 degrees.
3. Ensure that the head is arranged so that the horizontal Frankfurter plane, which passes through the lower edge of the orbit and the acoustic meatus, is parallel to the ground.
4. Arms should be relaxed at the sides of the trunk, and the palms of the hands should face the thighs.
5. The back of the skull, shoulders and buttocks should be in contact with the altimeter or measuring instrument.
6. While the subject performs a deep inhalation, place the stadiometer bar on the highest point of the head, known as the vertex. Exert enough light pressure to slightly compress the hair.

Regarding the correct measurement of the patient's weight, these steps were followed:

1. Ensure that the scale is placed on a flat and stable surface.
2. Ask the subject to remove his or her shoes and wear light clothing.
3. Have the subject step onto the scale calmly and evenly, placing both feet in the center of the platform.
4. Make sure the subject is in an upright position, with weight evenly distributed on both feet and arms along the sides.
5. During the measurement, the subject should maintain a natural, relaxed posture, without grasping anything or leaning against walls.
6. Wait a few moments for the scale to stabilize the measurement and show the weight clearly and legibly.
7. Accurately record the weight value shown on the scale.

In the overall assessment of the patient, the waist circumference was also measured, which met the following criteria:

- The operator should stand in front of the subject in an upright position, with the abdomen relaxed, arms at the sides of the trunk and feet together.
- The measurement should not be taken above clothing.
- The inelastic tape measure should be placed at the midpoint between the upper end of the iliac crest and the lower edge of the costal arch in the lateral part of the abdomen.
- The tape measure should be in contact with the skin but should not produce compression, and its zero end should be positioned below the value to be recorded.
- The measurement should be taken at the end of normal exhalation and approximated to the nearest 0.1 cm.

After the waist circumference measurement, the measurement of buttocks or hip circumference was moved. The presence of 2 operators is required to ensure the correct positioning of the tape measure.

This measurement should also not be taken above clothing and should consider the following points:

- The first operator should kneel to the side of the subject so as to survey the maximum circumference of the buttocks.
- The inelastic tape measure should be placed at the level of the maximum circumference of the buttocks adherent to the skin but without compressing it.
- The second operator should stand on the opposite side of the patient so that the tape measure can be as parallel to the ground as possible.
- The measurement should be taken at the end of normal exhalation and approximated to the nearest 0.1 cm.

Body composition analysis was done through a bioimpedance analysis (BIA) using a 50 kHz single frequency instrument (BIA 101 Anniversary Sport Edition, Akern /RJL Systems, Florence, Italy). This analysis provided data such as resistance, reactance, phase angle (PhA), total body water (TBW), body cell mass (BCM), free fat mass (FFM) and fat mass (FM). Participants were placed in the supine position for BIA analysis, without shoes and socks. Electrodes

(BIATRODES Akern Srl Florence, Italy) were placed on the back of the right hand and the corresponding instep, following a specific protocol⁸. The data obtained was analysed using the Bodygram Plus software (software) version 1.1 (Akern Srl; Florence, Italy).

Biochemical assays

As part of the biochemical analysis, all participants underwent a series of measurements to assess hematological and biochemical parameters. All tests were conducted at the same accredited testing laboratory, using a latest generation chemical analyser to ensure the consistency and reliability of the results.

Prior to a venous blood sample, patients were required to follow an overnight fasting of 8-10 hours.

The analysed parameters included glucose, total cholesterol, LDL, HDL, triglycerides, PCR and vitamin D.

Regarding vitamin D3 measurement, detailed information on the assay's analytical sensitivity and coefficients of variation were not available from the original laboratory documentation, which represents a limitation in assessing the reproducibility of these measurements.

Statistical analysis

The data is reported as mean \pm SD. Student's t-test was used for comparisons between genders (appropriate for normally distributed continuous variables). Chi-square test and linear trend evaluated vitamin D-lipid associations ($p < 0.05$). All analyses used Graph-Pad v8.0.2. No multivariate models were applied due to the exploratory nature of the study and limitations in sample size; however, potential confounders (age, BMI, sex, and lifestyle factors) were acknowledged as possible influences. No corrections for multiple testing were performed.

Table 1. Anthropometric characteristics, body composition and hematochemical parameters of participants. Data are presented as mean \pm SD (Standard Deviation).

Anthropometric and biochemical characteristics of the sample (n 106)	Mean \pm SD
Height (cm)	162,28 \pm 9,65
Age (years)	54 \pm 13
Weight (kg)	88 \pm 24,40
Body mass index (BMI) (kg/m ²)	33,25 \pm 7,67
Waist circumference (cm)	103,7 \pm 19,3
Hip circumference (cm)	114,67 \pm 14,86
Waist to hip ratio (cm)	0,90 \pm 0,10
Resistance (Rz) (R)	499,33 \pm 92,36
Reactance (Xc) (R)	52,85 \pm 11,21
Phase angle (Pha) (°)	6,14 \pm 1,42
Triglycerides (mg/dL)	117,81 \pm 78,74
HDL cholesterol (mg/dL)	56,05 \pm 14,02
Blood glucose (mg/dL)	97,87 \pm 17,60
Vitamin D (ng/mL)	26,68 \pm 14,85
C-reactive protein (CRP) (mg/dL)	3,31 \pm 4,58

Results and discussions

Characteristics of participants

Table 1 shows the general characteristics and anthropometric and body composition parameters of the study population, divided by gender. The population sample includes a total of 106 participants, 71 females and 35 males. The mean age of the study population is 54 years old, with a standard deviation of 13. The mean weight is 88 kg, with a standard deviation of 24.40, while the mean height is 162.28 cm, with a standard deviation of 9.65. In the overall sample, the mean value of Body Mass Index (BMI) is 33.25 kg/m², with a standard deviation of 7.67.

Subdivision of sample by gender

When stratified by gender, men and women showed similar BMI values (33.88 \pm 7.04 vs 33.18 \pm 7.94 kg/m², NS). Statistically significant differences included: lower waist-to-hip ratio in women (0.86 \pm 0.08 vs 0.99 \pm 0.08, $p < 0.001$), lower triglycerides (107.01 \pm 54.06 vs 139.45 \pm 110.50 mg/dL, $p = 0.04$),

higher HDL cholesterol (60.48 \pm 13.56 vs 47.20 \pm 9.91 mg/dL, $p < 0.001$), and higher vitamin D (28.38 \pm 16.30 vs 20.86 \pm 9.14 ng/mL, $p = 0.04$). Both genders showed waist-to-hip ratios associated with moderate-to-high cardiovascular disease risk.

Statistically significant differences between the genders are shown in some parameters. In particular, the table shows that when the study sample is divided by gender, women show a lower waist-to-hip ratio and lower triglyceride blood levels than men. In addition, there are higher blood levels of HDL cholesterol and vitamin D among women than men. However, it is important to note that both men and women have a waist/hip ratio associated with a moderate-to-high risk for cardiovascular disease (CVD).

Hematochemical tests were conducted to evaluate biochemical parameters in the total sample, with further division by gender, as shown in Table 2. Parameters considered included blood triglycerides, HDL cholesterol, glucose, vitamin D and PCR.

Assessment of vitamin D in the female gender and correlation with lipid profile

Given the obvious significant difference in vitamin D blood levels between genders, the focus of the study was on female patients. In analysing the ranges of serum levels of female population, it was found that 35% of patients had a deficiency of the vitamin (≤ 20 ng/ml), 33% had an insufficiency and only 32% had an optimal concentration of the micro-nutrient (Figure 1).

Figure 2 shows that no significant correlation was found between increased vitamin D levels and HDL cholesterol levels.

In Figure 2, the relationship between HDL cholesterol and low, mean, and optimal serum levels of vitamin D is analyzed using a chi-square test and a linear trend, with a significance threshold set at $p < 0.05$.

More specifically, the data from our study does not indicate a linear or curvilinear trend between these two variables. This suggests that an increase in vitamin D levels does not necessarily lead to significant changes in HDL cholesterol, which is recognized for its protective effects on cardiovascular health.

Table 2. Subdivision of the sample into Males and Females, assessing anthropometric characteristics, body composition and hematological parameters of participants. Data are presented as mean \pm SD (Standard Deviation). Statistical differences between groups were evaluated using Student's t test for independent samples, according to variable distribution and variance homogeneity. Statistically significant values are reported in boldface. NS: not statistically significant.

Anthropometric and biochemical characteristics of the sample	Men (n 35) Mean \pm SD	Women (n 71) Mean \pm SD	p-value
Age (years)	50,74 \pm 15,15	56 \pm 11,09	
Height (cm)	173,34 \pm 5,60	156,87 \pm 5,68	
Weight (kg)	102,18 \pm 23,20	81,67 \pm 21,87	
Body mass index (BMI) (kg/m ²)	33,88 \pm 7,04	33,18 \pm 7,94	NS
Waist circumference (cm)	112,18 \pm 17,43	99,96 \pm 18,70	NS
Hip circumference (cm)	112,65 \pm 13,85	115,94 \pm 15,12	NS
Waist to hip ratio (cm)	0,99 \pm 0,08	0,86 \pm 0,08	<0,001
Resistance (Rz) (R)	434,00 \pm 73,45	526,51 \pm 86,91	NS
Reactance (Xc) (R)	48,81 \pm 11,05	54,95 \pm 11,03	NS
Phase angle (Pha) (°)	6,41 \pm 0,99	6,01 \pm 1,58	NS
Triglycerides (mg/dL)	139,45 \pm 110,50	107,01 \pm 54,06	0,04
HDL cholesterol (mg/dL)	47,20 \pm 9,91	60,48 \pm 13,56	< 0,001
Blood glucose (mg/dL)	100,34 \pm 21,24	96,92 \pm 15,52	NS
Vitamin D (ng/dL)	20,86 \pm 9,14	28,38 \pm 16,30	0,04
C-reactive protein (CRP) (mg/dL)	2,42	3,69 \pm 5,05	NS

Figures 1–3 are embedded as high-resolution images within this manuscript file for review purposes.

As serum vitamin D levels increased in women, blood triglycerides significantly decreased ($p=0.0131$). Reductions were observed at average ($p=0.028$) and optimal ($p=0.036$) vitamin D levels compared to deficient levels (Figure 3).

Discussion

In our study, a correlation emerged between vitamin D levels and serum triglyceride concentration in women, with lower triglycerides observed in women with average and optimal vitamin D levels. We do not observed a linear or curvilinear trend between HDL cholesterol and low, medium, and optimal serum levels of vitamin D. This suggests that an increase in vitamin D levels does not necessarily lead to significant changes in HDL cholesterol, which is recognized for its protective effects on cardiovascular health.

Recent literature further supports the growing interest in identifying efficient tools for the assessment of vitamin D status across different populations. Notably, several studies have focused on the development of practical screening instruments to detect hypovitaminosis D and related metabolic risks.

Calcaterra et al. (2024) proposed an adapted questionnaire for assessing vitamin D deficiency in children, highlighting the importance of early identification of risk conditions in pediatric populations, especially considering the long-term implications for bone and metabolic health⁹. Similarly, in a 2022 study, Calcaterra et al. validated a screening tool for vitamin D insufficiency in children with obesity, underscoring how adiposity significantly impacts vitamin D bioavailability—even from early ages. These findings align with our observations regarding the inverse relationship between body fat distribution and serum vitamin D levels in adult women¹⁰.

Moreover, De Giuseppe et al. (2022) developed a short screening questionnaire for vitamin D deficiency

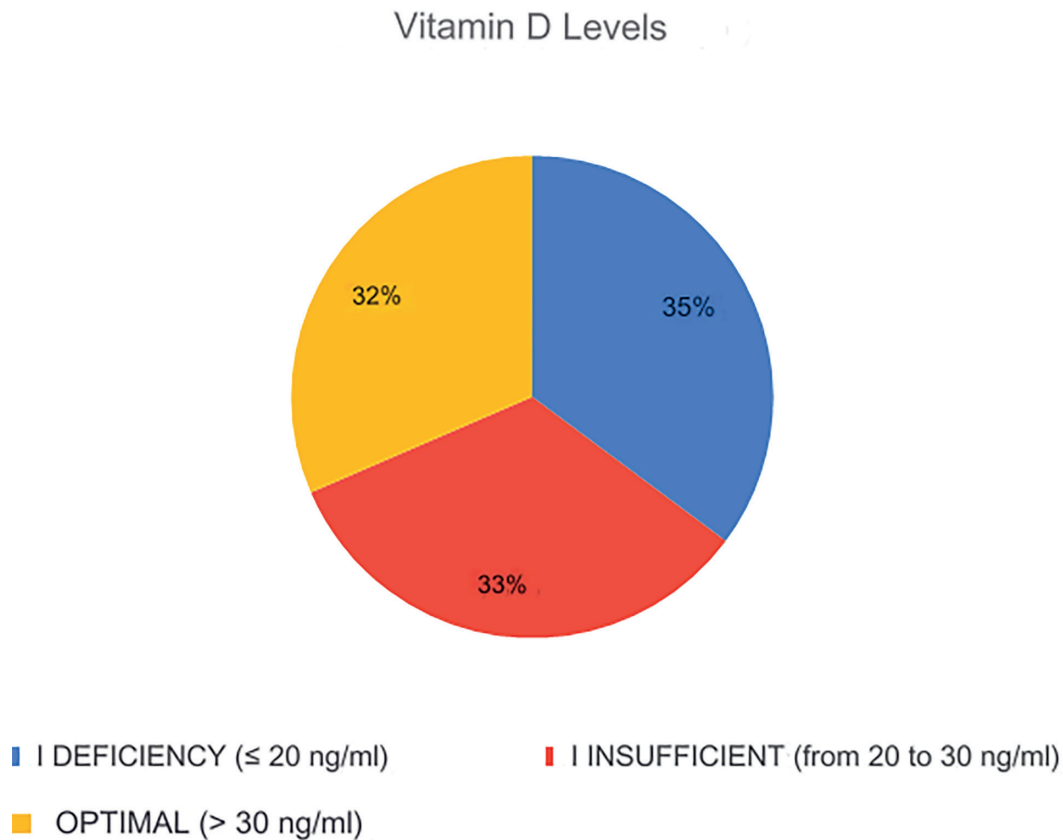


Figure 1. Average levels of Vitamin D present in the female sample analysed, highlighting the variability within the population studied. Vitamin D values in the chart are expressed in nanograms per millilitre.

in Italian adults, aiming to simplify the early identification of individuals at risk. This tool may have practical applications in clinical settings, particularly in aesthetic and preventive medicine, where vitamin D status is potentially associated with both metabolic markers and skin-related parameters, as partially observed in our study population¹¹.

Finally, Tagliaferri et al. (2019) investigated the controversial antioxidant role of vitamin D, emphasizing its potential involvement in modulating oxidative stress and inflammation. This supports the broader hypothesis of our research, where an improved vitamin D profile may positively influence metabolic and cardiovascular parameters through indirect anti-inflammatory and lipid-regulating mechanisms¹².

Study Design Limitations

Major limitations: (1) cross-sectional/retrospective design precluding causal inference; (2) no data on sun exposure, dietary vitamin D intake, or supplementation; (3) no multivariate adjustment for confounders (age, sex, BMI, lifestyle); (4) small, unbalanced sample (n=106, female-predominant); (5) single-center recruitment limiting generalizability.

This study employs a cross-sectional, retrospective design, which inherently limits causal inference. The retrospective nature of the data collection, while providing valuable preliminary insights, does not permit the establishment of temporality or mechanistic causality. As a result, the observed associations between serum vitamin D3 levels, metabolic activity, and body composition parameters must be interpreted as correlational findings only. Prospective, longitudinal studies

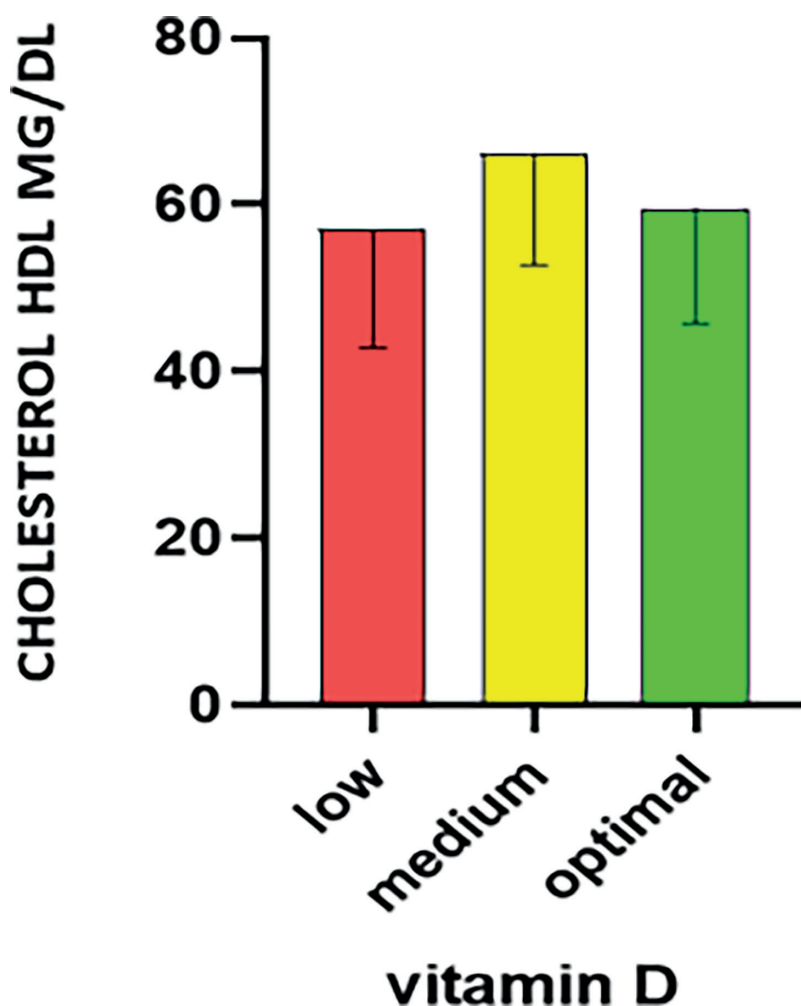


Figure 2. Correlation between HDL cholesterol and low, mean and optimal serum levels of vitamin D. Chi-square test and linear trend was used, with a significance level of $p < 0.05$.

with controlled intervention designs are necessary to establish definitive causal relationships.

This exploratory, unadjusted analysis identified statistically significant associations between vitamin D levels and triglyceride concentrations in women ($p=0.0131$), which should be interpreted cautiously by taking into consideration the study's design limitations.

Epidemiological investigations have also demonstrated a link between waist-to-hip ratio—an indicator of fat distribution—lipid metabolism, and vitamin D levels. Because vitamin D is fat-soluble, it tends to accumulate in adipose tissue, which can reduce its availability in circulation. Consequently, individuals

with a higher waist-to-hip ratio, often associated with visceral fat and elevated triglycerides, may display lower vitamin D levels. Additionally, visceral fat releases pro-inflammatory cytokines that may further impair vitamin D metabolism and contribute to cardiovascular risk.

However, our database did not include parameters such as seasonal variation, dietary intake of vitamin D, sun exposure habits, supplementation practices, or outdoor physical activity levels, as these variables were not originally considered in the data collection process. These omissions represent significant limitations in interpreting serum vitamin D3 levels, as seasonality,

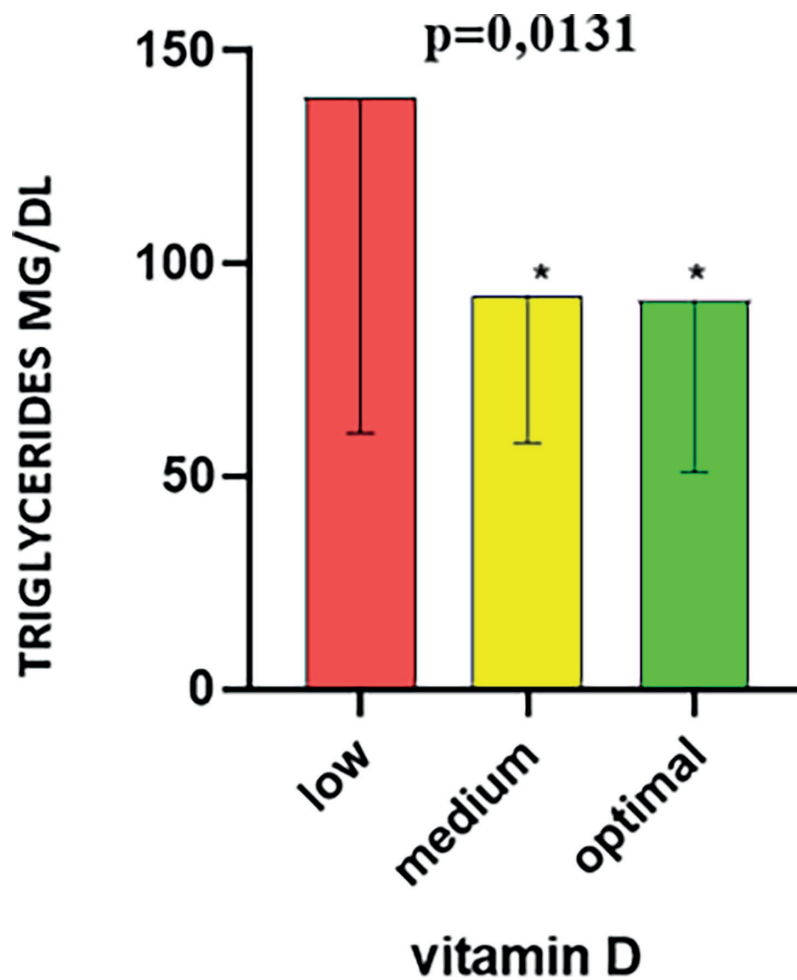


Figure 3. Correlation between triglycerides and serum amount of vitamin D. Chi-square test and linear trend was used, with a significance level of $p < 0.05$.

sun exposure, and dietary intake are known to substantially influence circulating 25(OH)D concentrations. Therefore, the interpretation of our findings must be cautious, and future studies should prospectively collect these variables to provide a more comprehensive understanding of vitamin D metabolism and its associations with metabolic parameters.

Furthermore, given the retrospective design and sample characteristics, multivariate adjustment for potential confounding variables (such as age, sex, BMI, and lifestyle factors) was not performed. This represents a significant limitation of the study, as it reduces the ability to account for confounding effects that may influence the observed associations between vitamin D

and metabolic parameters. The unadjusted nature of these analyses means that observed correlations may be partially influenced by unmeasured or uncontrolled confounders. Future prospective studies should employ multivariable regression models adjusted for demographic and lifestyle factors to better establish independent associations.

In our sample, significant differences were noted between men and women in waist-to-hip ratio, with both genders showing values associated with a moderate-to-high risk of cardiovascular disease (CVD), consistent with findings by De Koning et al.¹³

Sample size and generalizability

The relatively small and unbalanced sample composition, with 71 women and 35 men (total n=106), significantly limits the generalizability of these findings to broader populations. The predominance of female participants, while reflecting the demographics of aesthetic medicine clinics, restricts the applicability of gender-specific conclusions. The modest sample size reduces statistical power and increases the likelihood of Type II errors, particularly for subgroup analyses. Furthermore, recruiting from a single outpatient clinic with specific inclusion and exclusion criteria may introduce selection bias, limiting the generalizability of the findings. Future research employing larger, more heterogeneous cohorts from multiple centers and diverse geographic regions would substantially improve the robustness and generalizability of conclusions drawn from such analyses.

Another relevant point relates to the widespread hypovitaminosis D observed in our sample, with a more pronounced deficiency in men than in women. Our data support those of Capuano et al.¹⁴, who found that only 13.3% of women and 11.1% of men had adequate serum 25(OH)D levels (≥ 30 ng/mL), while deficiency (< 20 ng/mL) affected 59.3% of women and 55.1% of men in their large Italian cohort.

Given the known association between serum vitamin D levels and bone mineral density (BMD), and the increased risk of fractures among older individuals with vitamin D deficiency¹⁵, the routine monitoring of this micronutrient becomes essential, especially in aging populations.

Our study also recorded gender-based differences in lipid profiles: women showed higher mean values of both HDL and vitamin D, and lower levels of triglycerides, compared to men. This is consistent with established reference values, where HDL > 50 mg/dL is considered optimal for women and > 40 mg/dL for men¹⁶. These physiological differences may help explain gender-based disparities in cardiovascular risk.

In addition to classical risk factors such as hypertension and hyperlipidemia, recent research has highlighted the emerging role of vitamin D in cardiovascular health. For example, the systematic review and meta-analysis by Jani et al. showed an inverse relationship between 25(OH)D levels and CVD risk in over

65,000 subjects. Similarly, a study on men with type 1 diabetes found that low vitamin D levels were associated with higher rates of coronary artery calcifications.

In our sample, an interesting trend emerged among women: as serum vitamin D levels increased, triglyceride levels significantly decreased. This finding is consistent with previous reports suggesting a potential association between vitamin D status and triglyceride metabolism, although causality cannot be inferred from the present design.

In the context of Aesthetic Medicine, recent findings suggest that optimizing serum vitamin D levels may also play a supportive role in improving skin health. A clinical study by Ferroni (2021) evaluated 27 adult female patients undergoing vitamin D supplementation over a three-month period, without the use of any concurrent aesthetic treatments. The study recorded a significant improvement in several objective skin parameters—including hydration, sebaceous regulation, and reduced reactivity—following the normalization of vitamin D levels. These results align with the known immunomodulatory and anti-inflammatory properties of vitamin D, which may help restore skin barrier function, reduce oxidative stress, and promote epidermal homeostasis. Therefore, maintaining adequate vitamin D status could represent a valuable adjunct in anti-aging protocols and non-invasive aesthetic interventions, especially in patients with suboptimal baseline levels.

Moreover, vitamin D's impact on systemic inflammation may contribute to improvements in cutaneous parameters. It plays a role in keratinocyte differentiation, modulates cutaneous immune responses, and enhances the function of the skin barrier². These mechanisms support the hypothesis that restoring adequate vitamin D levels may yield benefits for skin hydration, texture, and resilience.

Conclusions

This retrospective, cross-sectional analysis found a statistically significant association between higher serum vitamin D levels and lower triglyceride concentrations in women, warranting confirmation in prospective studies.

These preliminary findings may contribute to future research on vitamin D's potential role in metabolic assessments but do not support its current use as a clinical biomarker.

The effects of vitamin D on skin health, along with its roles in immunomodulation and inflammation, support the potential utility of supplementing this micronutrient as an anti-aging intervention, particularly in individuals with low serum levels.

Future prospective, adequately powered, multivariate-adjusted studies on larger, diverse populations are required to establish vitamin D's potential role in metabolic and cardiovascular health.

Conflict of Interest and Ethical Statement: This retrospective analysis utilized fully anonymized data collected during routine clinical practice, without any prospective intervention or additional data collection. Per institutional guidelines and Italian regulations for observational retrospective studies (Legislative Decree 196/2003, as amended), [formal IRB/ethics committee approval was not required]. All patients had previously provided written informed consent for anonymized data use in research and compliance with privacy legislation. The authors declare no conflicts of interest.

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