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ASSOCIATION OF SERUM 25-HYDROXYVITAMIN D WITH C-REACTIVE PROTEIN ACROSS BODY MASS INDEX CATEGORIES IN ADULTS

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ABSTRACT

Background: Vitamin D insufficiency and systemic inflammation frequently coexist with excess adiposity, but their relationship across the full body mass index (BMI) spectrum remains incompletely characterized.

Aim: To evaluate serum 25-hydroxyvitamin D [25(OH)D] and C-reactive protein (CRP) across BMI categories and determine whether CRP is independently associated with 25(OH)D.

Methods: This cross-sectional analysis included 200 adults allocated to five BMI groups (normal weight, overweight, obesity class I, obesity class II, and obesity class III; n = 40 per group). Group differences were tested with Kruskal–Wallis analysis and post hoc multiple comparisons. Associations between CRP and 25(OH)D were assessed using Spearman correlation in the total sample and BMI strata. A multivariable linear regression model adjusted for age, sex, smoking, BMI, triglycerides, high- and low-density lipoprotein cholesterol, glycated hemoglobin, and insulin resistance.

Results: Serum 25(OH)D decreased markedly across BMI categories ($H = 139.74$, $p < 0.001$), whereas CRP increased ($H = 122.68$, $p < 0.001$). The pooled association between CRP and 25(OH)D was strong and inverse ($\rho = -0.68$, $p < 0.001$). The association remained significant in normal-weight participants ($\rho = -0.78$, $p < 0.001$), participants with overweight ($\rho = -0.50$, $p < 0.001$), and the combined obesity group ($\rho = -0.24$, $p = 0.008$). The multivariable model was significant ($R^2 = 0.528$; adjusted $R^2 = 0.502$; $p < 0.001$), and CRP remained independently associated with lower 25(OH)D ($B = -0.483$, standardized $\rho = -0.211$, $p < 0.001$).

Conclusions: Higher BMI was accompanied by lower 25(OH)D and higher CRP. The inverse CRP–25(OH)D relationship persisted after adjustment for demographic and metabolic covariates, although the cross-sectional design does not establish causality.

Keywords: obesity; inflammation; adiposity; cardiometabolic risk; hypovitaminosis D

INTRODUCTION

Obesity is a major public-health challenge and is closely associated with metabolic dysfunction, insulin resistance, dyslipidemia, type 2 diabetes, and cardiovascular disease. A central biological feature of excess adiposity is systemic inflammation driven by adipocyte dysfunction, immune-cell infiltration, and altered cytokine signaling (Ellulu, Patimah, Khaza'ai, Rahmat, & Abed, 2017; World Health Organization, 2000). C-reactive protein (CRP), an acute-phase protein produced predominantly by the liver, is widely used as an accessible marker of inflammatory activity (Pepys & Hirschfield, 2003).

Vitamin D is classically involved in calcium and bone homeostasis but also participates in immune and

metabolic regulation. Serum 25-hydroxyvitamin D [25(OH)D] is the accepted indicator of vitamin D status because it reflects endogenous synthesis and dietary exposure (Holick, 2007). Low 25(OH)D concentrations are common in people with overweight and obesity. Proposed explanations include volumetric dilution, sequestration within adipose tissue, lower sun exposure, reduced physical activity, and obesity-related metabolic changes (Vanlint, 2013; Wortsman, Matsuoka, Chen, Lu, & Holick, 2000). Meta-analytic and genetic evidence supports a directional effect of greater adiposity on lower circulating 25(OH)D (Pereira-Santos, Costa, Assis, Santos, & Santos, 2015; Vimaleswaran et al., 2013).

Observational studies have also reported inverse associations between 25(OH)D and CRP (Amer & Qayyum, 2012; Yang et al., 2020). Nevertheless, this relationship is complex. Adiposity may confound or mediate part of the association, and randomized trials have not consistently shown that vitamin D supplementation reduces inflammatory biomarkers in adults with overweight or obesity (Gouveia et al., 2024; Rafiq et al., 2020). Evaluation across clearly defined BMI categories, together with multivariable adjustment, may help distinguish the contribution of inflammatory activity from the broader metabolic phenotype.

The aim of this study was to compare serum 25(OH)D and CRP concentrations across five BMI categories and to assess the overall, stratum-specific, and independently adjusted association between CRP and 25(OH)D in adults. We hypothesized that increasing BMI would be accompanied by lower 25(OH)D and higher CRP and that CRP would remain inversely associated with 25(OH)D after adjustment for relevant demographic and metabolic variables.

METHODS

Study design and participants

A cross-sectional observational analysis was performed using the doctoral research database. The dataset contained 200 adults aged 23–65 years, with 40 participants in each of five BMI categories: normal weight (Group 1), overweight (Group 2), obesity class I (Group 3), obesity class II (Group 4), and obesity class III (Group 5). Each group included 20 women and 20 men. The equal group sizes reflect the study design and should not be interpreted as population prevalence estimates.

Body weight and height were recorded using standardized anthropometric procedures, and BMI was calculated as weight in kilograms divided by height in metres squared. The categories followed conventional World Health Organization thresholds: normal weight, 18.5–24.9 kg/m^2; overweight, 25.0–29.9 kg/m^2; obesity class I, 30.0–34.9 kg/m^2; obesity class II, 35.0–39.9 kg/m^2; and obesity class III, ≥40.0 kg/m^2.

Laboratory variables

Fasting venous blood samples were collected after a 12-hour overnight fast and analyzed at the Private Health Institution Diagnostic Biochemical Laboratory, Molecular Diagnostics Laboratory and Diagnostic Microbiological Laboratory RAMUS, Skopje, Republic of North Macedonia, using validated laboratory procedures. Serum 25(OH)D was measured by chemiluminescent immunoassay (CLIA) as the principal indicator of vitamin D status, and CRP was analyzed as the marker of systemic inflammatory activity. Triglycerides, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), glycated hemoglobin (HbA1c), and the homeostatic model assessment of insulin resistance (HOMA-IR) were included as covariates in the multivariable analysis.

All clinical and laboratory information was analyzed in anonymized form. The study procedures were reported as compliant with the Declaration of Helsinki, and participants provided informed consent in the original doctoral protocol.

Statistical analysis

Continuous variables are presented as mean ± standard deviation (SD) or median (range), depending on distribution, and categorical variables as number and percentage. Between-group differences were assessed with Kruskal–Wallis analysis of variance by ranks; significant omnibus tests were followed by post hoc multiple comparisons. Smoking status was compared using the Pearson chi-square test.

Spearman rank correlation coefficients (ρ) were calculated for the association between CRP and 25(OH)D in the total sample, the normal-weight group, the overweight group, and the combined obesity classes I–III. A multivariable linear regression model was constructed with 25(OH)D as the dependent variable and age, sex, smoking status, BMI, triglycerides, HDL-C, LDL-C, CRP, HbA1c, and HOMA-IR as predictors. Results are reported as unstandardized coefficients (B), standard errors, standardized coefficients (β), 95% confidence intervals (CI), and p-values. All tests were two-sided, and $p < 0.05$ was considered statistically

significant. Values reported by the software as p = 0.000 are presented as p < 0.001.

RESULTS

Participant characteristics and between-group differences

The five groups differed significantly in age, smoking status, BMI, serum 25(OH)D, and CRP (Table 1). The normal-weight group was younger and contained fewer current smokers than several higher-BMI groups. Median 25(OH)D declined from 62.25 ng/mL in the normal-weight group to 8.35 ng/mL in obesity class III, whereas median CRP increased from 0.95 to 10.15 mg/L.

Table 1. Participant characteristics across BMI categories

Characteristic	Normal weight (n=40)	Overweight (n=40)	Obesity I (n=40)	Obesity II (n=40)	Obesity III (n=40)	Overall p
Women, n (%)	20 (50.0)	20 (50.0)	20 (50.0)	20 (50.0)	20 (50.0)	1.000
Age, years	38.63 ± 12.07	45.15 ± 12.06	51.98 ± 11.55	49.05 ± 11.01	46.13 ± 10.44	<0.001
Current smokers, n (%)	16 (40.0)	24 (60.0)	30 (75.0)	20 (50.0)	25 (62.5)	0.022
BMI, kg/m²	23.04 ± 1.51	27.55 ± 1.26	32.68 ± 1.37	37.49 ± 1.38	46.99 ± 7.61	<0.001
25(OH)D, ng/mL	62.25 (35.60–72.40)	27.65 (7.22–37.60)	12.40 (7.14–18.30)	10.65 (3.40–17.60)	8.35 (3.40–11.50)	<0.001
CRP, mg/L	0.95 (0.50–1.80)	2.10 (1.00–81.00)	4.25 (1.00–32.20)	8.30 (1.00–27.90)	10.15 (1.00–70.40)	<0.001

Values are mean ± SD, median (range), or n (%).

Serum 25(OH)D across BMI categories

The overall difference in serum 25(OH)D was significant (Kruskal–Wallis H[4, N = 200] = 139.74, p < 0.001). Mean ranks decreased stepwise from 180.44 in normal weight to 38.98 in obesity class III. Normal-weight participants had higher 25(OH)D than every other group (all p < 0.001). Participants with overweight had higher concentrations than those with obesity class I (p = 0.040), class II (p < 0.001), and class III (p < 0.001). Obesity class I also had higher concentrations than class III (p = 0.002); the remaining pairwise contrasts were not significant. Figure 1 shows the progressive decline in median values.

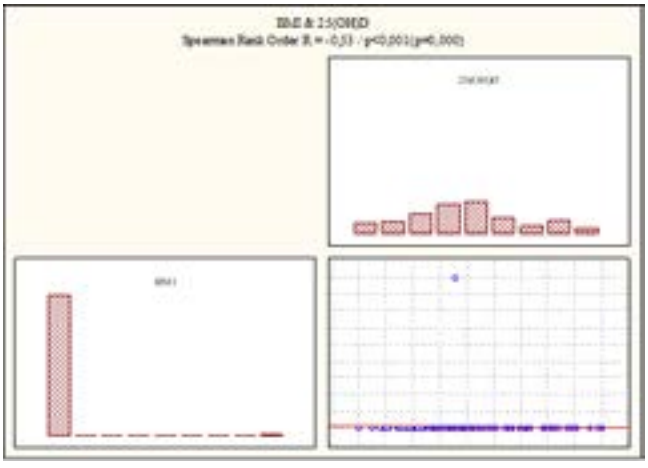


Figure 1. Median serum 25(OH)D concentrations across BMI categories. Ranges are reported in Table 1.

C-reactive protein across BMI categories

CRP differed significantly across groups (Kruskal–Wallis H[4, N = 200] = 122.68, p < 0.001), with mean ranks increasing from 26.35 in normal weight to 154.26 in obesity class III. CRP was lower in normal weight than in all higher-BMI groups (all p < 0.001). The overweight group had lower CRP than obesity class II and III (both p < 0.001), and obesity class I had lower CRP than obesity class III (p = 0.004). Other pairwise contrasts were not statistically significant. Median values are shown in Figure 2.

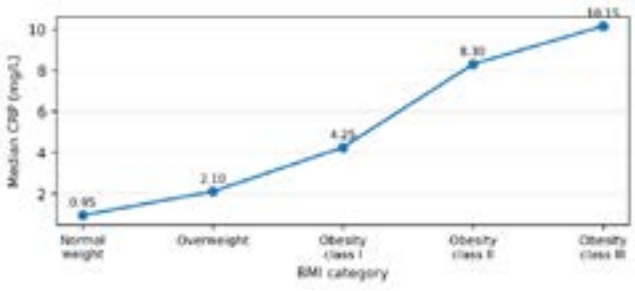


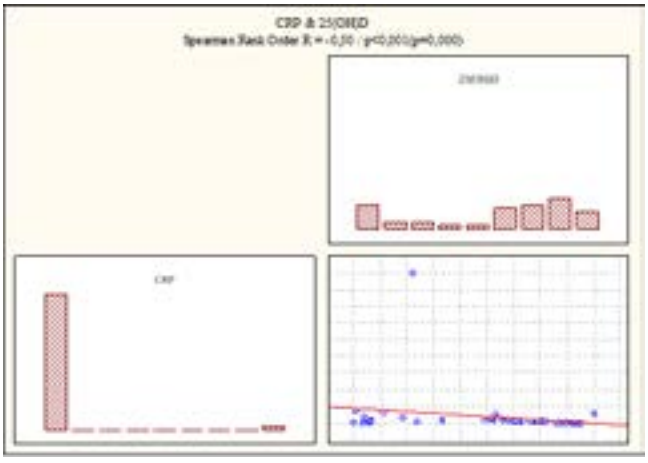
Figure 2. Median serum CRP concentrations across BMI categories. Ranges are reported in Table 1.

Association between CRP and 25(OH)D

In the total sample, CRP and 25(OH)D were strongly and inversely correlated (**ρ** = −0.68, p < 0.001). The association remained significant within the prespecified BMI strata, although its magnitude decreased with greater adiposity: strong in normal weight, moderate in overweight, and weak in the combined obesity group (Table 2).

Table 2. Spearman correlations between CRP and 25(OH)

Population	n	Spearman ρ	p
Total sample	200	−0.68	<0.001
Normal weight	40	−0.78	<0.001
Overweight	40	−0.50	<0.001
Obesity classes I–III combined	120	−0.24	0.008



Multivariable linear regression

The multivariable model was statistically significant ($R = 0.726$, $R^2 = 0.528$, adjusted $R^2 = 0.502$; $F = 20.654$, $p < 0.001$). After adjustment, higher CRP remained independently associated with lower 25(OH)D ($B = -0.483$, standardized $\beta = -0.211$, $p < 0.001$). Age, triglycerides, HDL-C, LDL-C, and HbA1c were also significant independent predictors, whereas sex, smoking status, BMI, and HOMA-IR were not significant in this model (Table 3).

Table 3. Multivariable linear regression with serum 25(OH)D as the dependent variable (N = 200)

Predictor	B	SE	ρ	95% CI for B	p
Sex (1)	1.472	2.205	0.036	−2.878 to 5.821	0.505
Age	−0.334	0.091	−0.198	−0.514 to −0.154	<0.001
Smoking (1)	−2.333	2.231	−0.056	−6.734 to 2.068	0.297
BMI	−0.00004	0.000	−0.005	−0.001 to 0.001	0.918
Triglycerides	−7.226	1.314	−0.366	−9.819 to −4.633	<0.001
HDL-C	−5.744	2.719	−0.139	−11.108 to −0.380	0.036
LDL-C	−5.803	1.397	−0.266	−8.559 to −3.046	<0.001
CRP	−0.483	0.124	−0.211	−0.729 to −0.238	<0.001
HbA1c	−2.443	1.145	−0.144	−4.703 to −0.183	0.034
HOMA-IR	−0.046	0.058	−0.043	−0.160 to 0.067	0.424

B, unstandardized coefficient; β, standardized coefficient; CI, confidence interval. Binary-variable coding follows the original study database.

DISCUSSION

This study demonstrated a clear gradient across BMI categories: serum 25(OH)D declined as adiposity increased, whereas CRP increased. The central finding was a strong inverse association between CRP and 25(OH)D in the total sample. Importantly, the relationship was not limited to the pooled data; it remained significant in normal-weight, overweight, and obese participants, although the correlation weakened across the higher-BMI strata. CRP also remained an independent negative predictor of 25(OH)D after adjustment for demographic, lipid, glycemic, and insulin-resistance variables.

The progressive reduction in 25(OH)D is consistent with the established association between adiposity and lower vitamin D status. Reduced bioavailability due to distribution into a larger body compartment and retention in adipose tissue are plausible mechanisms (Vanlint, 2013; Wortsman et al., 2000). The systematic review by Pereira-Santos et al. (2015) showed a higher frequency of vitamin D deficiency in obesity, while Mendelian-randomization evidence suggested that higher BMI contributes causally to lower 25(OH)D (Vimaleswaran et al., 2013). The present stepwise pattern across five BMI categories supports a dose-related association, but the cross-sectional design cannot determine temporal direction.

The rise in CRP with BMI is biologically plausible because adipose-tissue expansion promotes macrophage recruitment and pro-inflammatory cytokine signaling (Ellulu et al., 2017). The inverse CRP–25(OH)D association agrees with population-based findings from Amer and Qayyum (2012) and Yang et al. (2020). The persistence of the association after adjustment suggests that CRP captures information not fully represented by BMI alone. Nevertheless, Rafiq et al. (2020) reported that adiposity explained a substantial part of the vitamin D–inflammation relationship, emphasizing that residual confounding and pathway overlap remain possible.

The lower stratum-specific correlation in obesity may reflect restricted ranges of 25(OH)D among participants with obesity, biological heterogeneity, or floor effects at very low concentrations. It may also indicate that additional determinants—such as season, sunlight exposure, dietary intake, supplement use, comorbidities, and medication—become increasingly important once severe adiposity is established. Therefore, the pooled coefficient should not be interpreted as uniform across all BMI levels.

The findings support clinical awareness that low 25(OH)D and elevated CRP can coexist in adults with excess adiposity, but they do not establish that vitamin D supplementation will reduce inflammation. A recent meta-

analysis of randomized trials in adults with overweight or obesity found no significant effect of isolated vitamin D supplementation on CRP, interleukin-6, or tumor necrosis factor concentrations (Gouveia et al., 2024). Accordingly, vitamin D and CRP should be interpreted within the broader clinical and metabolic context rather than used as evidence of a direct therapeutic pathway.

Strengths of the study include the balanced number of participants across five BMI categories, equal sex distribution within groups, analysis across a wide BMI range, stratum-specific correlations, and multivariable adjustment. Several limitations require consideration. The cross-sectional design precludes causal inference, and the stratified equal-size sampling prevents estimation of population prevalence. Age and smoking differed across groups, although both were included in the regression model. Seasonal exposure, diet, supplements, physical activity, and detailed comorbidity information were not available for the present analysis. CRP values reached 81.0 mg/L in the overweight group and 70.4 mg/L in obesity class III; without repeat measurements or clinical adjudication, acute inflammatory contributors cannot be excluded in all participants. Finally, the pooled correlation may partly reflect separation among the prespecified BMI groups.

CONCLUSIONS

Increasing BMI was associated with progressively lower serum 25(OH)D and higher CRP. CRP showed a significant inverse association with 25(OH)D in the total sample and within BMI strata and remained independently associated after multivariable adjustment. These findings identify a close observational relationship between vitamin D status, adiposity, and inflammatory activity, but prospective studies with repeated CRP measurements and detailed assessment of seasonal, lifestyle, and treatment factors are required to clarify causality and clinical implications.

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