Is vitamin D status a determining factor for metabolic syndrome? A case-control study

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Abstract: This study was undertaken to assess vitamin D status in nonmenopausal women with metabolic syndrome (MeS) and to evaluate its possible role in inflammation and other components of MeS. A case-control study was conducted during late fall and winter 2009–10. A total of 375 women with waist circumference (WC) ≥88 cm were examined to find 100 who met MeS criteria according to the National Cholesterol Education Program (NCEP)/Adult Treatment Panel (ATP) III criteria (NCEP/ATP III). Of those without MeS, 100 age- and residence area-matched women were selected as a control group. Anthropometric and laboratory evaluations were performed. Waist-to-hip ratio (WHR), body mass index (BMI), homeostatic model of insulin resistance (HOMA-IR) and body fat mass (FM) were also evaluated. Women with MeS had significantly higher BMI, waist circumference (WC) and FM but lower serum osteocalcin than controls. There was no significant difference in serum 25 hydroxyvitamin D (25[OH]D), intact parathyroid hormone (iPTH) or vitamin D status between the two groups. Serum highly sensitive C-reactive protein (hsCRP) concentration was significantly higher in the MeS group, compared to the controls (3.4 ± 3.3 vs 2.0 ± 1.9 mg/L, P = 0.001). The difference remained significant even after controlling for BMI (P = 0.011), WC (P = 0.014) and FM (P = 0.005). When comparison was made only in those subjects with insulin resistance (HOMA-IR > 2.4), hsCRP was still higher in the MeS group (n = 61) than in the control group (n = 79) (P < 0.001). When data were categorized according to vitamin D status, in the MeS group significantly higher plasma glucose concentrations were observed in subjects with vitamin D deficiency compared to those with insufficiency or sufficiency (104.0 ± 11.7, 83.0 ± 11.3 and 83.2 ± 9.9 mg/dL, respectively, P = 0.001). Interestingly, their WC or WHR did not show any significant difference. In stepwise regression analysis, 25(OH)D was the main predictor of both hsCRP and plasma glucose. Vitamin D status may, at least in part, be a determining factor of systemic inflammation and the related metabolic derangements of MeS.

Keywords: metabolic syndrome, vitamin D, inflammation

Introduction

Metabolic syndrome (MeS) is characterized by several abnormalities including impaired glucose tolerance, blood lipid derangements, and prothrombotic and pro-inflammatory states. A number of studies have suggested abdominal obesity as the major culprit in MeS. It is believed that visceral fat, by secreting some inflammatory adipokines, brings about both insulin resistance and an inflammatory state. Elevated blood inflammatory biomarkers such as C-reactive protein (CRP), interleukin (IL)-6, IL-18 and tumor necrosis factor (TNF)-α have been found in individuals with MeS. Body fat mass (FM) and waist circumference (WC) have been found to be predictors of circulating highly sensitive C-reactive protein (hsCRP) in Iranian middle-aged women. Fat distribution seems...
to have a crucial role in the development of further metabolic disturbances. It has been shown that, unlike abdominal adiposity, gluteofemoral fat can be protective against metabolic derangements.

Extra fat deposition in the body, depending on its location, can adversely affect vitamin D status. Adipose tissue has been shown to act as a “metabolic well” for vitamin D, hence reducing its bioavailability. New studies support an inverse association between vitamin D status and insulin resistance, MeS and type 2 diabetes (T2D). A possible role for vitamin D deficiency in the pathogenesis of MeS has been recently proposed. Circulating 25 hydroxyvitamin D (25(OH)D) has been inversely associated with abdominal adiposity, hypertriglyceridemia, and hyperglycemia. Vitamin D status may indirectly affect MeS development. Low serum osteocalcin, a vitamin D-dependent Gla-protein, has recently been reported in subjects with MeS.

The immune-modulatory effect of vitamin D and its inverse link with inflammation draws more attention to its possible role in MeS. Obesity and MeS are usually accompanied by “low-grade systemic inflammation” and augmented oxidative stress, both of which are believed to have pivotal roles in development of MeS itself and in its further morbidities. It has been recently reported that even with the same body mass index (BMI), subjects with higher circulating inflammatory markers such as interleukin (IL)-6 are more likely to have insulin resistance. Low vitamin D status has been linked to inflammatory endothelial dysfunction in both diabetic and non-diabetic subjects. The oxidative-stress attenuating effect of vitamin D is a new finding that has been related to its anticancer effect. However, some other studies indicated that 1,25(OH)2D enhances inflammatory reaction in both human and murine adipocyte culture medium.

In this study it was hypothesized that in the presence of high WC, vitamin D status is a contributing factor in MeS. To examine this hypothesis, a case-control study was conducted on nonmenopausal women residing in Tehran to: a) compare vitamin D status between subjects with and without MeS; b) determine the relationship between circulating 25(OH)D and the components of MeS; and c) examine the relationship between 25(OH)D and certain oxidative stress and inflammatory biomarkers.

**Subjects and methods**

**Study design**

This was a case-control study performed during the fall and winter, 2009–2010. Considering the higher prevalence of obesity and MeS in women than in men in Iran and the possible effects of menopause on the metabolism of bone and calcitropic hormones, only nonmenopausal women were studied in this research project. On the first visit, full information on the study design and objectives were given to all subjects in a face-to-face interview before they signed a written informed consent. A general questionnaire on demographic data and duration of direct sun exposure, based on recalled usual number of minutes/hours spent in daylight, was also completed. Duration of daily sun exposure was categorized as less than 10 minutes, 10 minutes to 1 hour, 1–2 hours and more than 2 hours. Then, blood pressure was measured and subjects were requested to return after an overnight (12–14 hours) fast for blood sampling. In this study, MeS was defined according to the National Cholesterol Education Program (NCEP)/Adult Treatment Panel (ATP) III criteria. The scientific and ethical issues of this study were approved by the Research Council and the Ethical Committee of the National Nutrition and Food Technology Research Institute (NNFTRI), respectively.

**Subjects**

To find 100 women with MeS, 375 women with abdominal obesity from health centers and school staffs from Tehran were examined. The inclusion criteria were: 1) willingness to participate; 2) aged 30–50 years; 3) being nonmenopausal; 4) waist circumference ≥ 88 cm; 5) absence of any clinical disease; 6) not being pregnant or lactating; and 7) not receiving any nutritional supplement for at least 3 months preceding the study. This latter criterion was actually found necessary because irregular supplement use was common and impossible to quantify accurately. Women who met three out of five criteria of NCEP/ATP III (including high WC), were identified as having MeS and allocated to the cases group. Of the remaining subjects, 100 age- and residence region-matched women were selected as a control group.

**Anthropometry and blood pressure**

Weight was measured with light clothes and without shoes to the nearest 0.1 kg using a digital scale (Seca 840; Seca GmbH, Hamburg, Germany). Height was measured without shoes to the nearest 0.1 cm with a measuring tape mounted onto the wall. A triangular ruler was put on the top of the subject’s head and then the number on the measuring tape at the intercept of the ruler and the tape was considered as the subject’s height. BMI was calculated using the equation BMI = weight (kg)/height(m)². Hip circumference (HC) and WC were both measured by a measuring tape to the nearest 0.1 cm. WC was determined at the midpoint between the, and subjects were requested to return after an overnight (12–14 hours) fast for blood sampling. In this study, MeS was defined according to the National Cholesterol Education Program (NCEP)/Adult Treatment Panel (ATP) III criteria. The scientific and ethical issues of this study were approved by the Research Council and the Ethical Committee of the National Nutrition and Food Technology Research Institute (NNFTRI), respectively.
lowest rib and iliac crest while the subject was in a standing position and after expiration. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured in a seated position, after a 5-minute rest, using a digital sphygmomanometer (BC08; Beurer GmbH, Ulm, Germany).

**Estimation of body fat mass (FM)**
The percentage of body FM was evaluated using a bioelectrical impedance analysis (BIA) system (Quadscan 4000; BodyStat Ltd, Onchan, Isle of Man, UK).

**Laboratory investigations**

**Blood sample handling**
Venous blood samples collected after 12–14 hours fasting were transferred into two tubes, either with or without the anticoagulant sodium fluoride. Plasma recovered from the anticoagulated blood sample was used to measure glucose and lipids within 2 hours of sample collection. Sera obtained after centrifugation of clotted samples at 1000 × g at room temperature were stored in aliquots at −80°C until the day of analysis.

**Glycemic status and lipid profile**
Plasma glucose, triglycerides (TG), total cholesterol, low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) were measured using enzymatic colorimetric methods (all from Pars Azmoon, Tehran, Iran). Serum insulin was determined by immunoradiometric assay (IRMA) using a commercial kit (Biosource Diagnostics, Aartrijke, Belgium) and a gamma-counter system (Gamma I; Genesys, Grand Blanc, MI). Insulin resistance was evaluated by homeostasis model assessment of insulin resistance (HOMA-IR) index calculated using the following equation:27

\[
\text{HOMA-IR} = \frac{\text{fasting plasma glucose (mmol/L)} \times \text{serum insulin (mU/L)}}{22.5}
\]

**Inflammatory and oxidative stress biomarkers**
Serum concentrations of hsCRP were measured by immunoturbidometric method (Selectra E; Vital Scientific, Spankeren, the Netherlands). Malondialdehyde (MDA) was measured based on thiobarbituric acid reacting substances (TBARS) method as described originally28 with some minor modifications.29 For measuring total antioxidant capacity (TAC), a colorimetric method with Azino-bis(3-methylbenzothiazolin-6-sulfonic acid) diammonium salt (ABTS) reagent was used.30

Circulating 25(OH)D, intact parathyroid hormone (iPTH) and osteocalcin
Serum 25(OH)D was assayed using high-performance liquid chromatography (HPLC) as described earlier.31 In this study, vitamin D status was defined based on serum concentration of 25(OH)D as: deficiency ≤27.5 nmol/L; insufficiency 27.5 ≤25(OH)D < 50 nmol/L; and sufficiency ≥50 nmol/L.32 Serum iPTH concentrations were determined using enzyme-linked immunosorbant assay (ELISA) kits (DRG Instruments GmbH, Marburg, Germany and Biosource Diagnostics, Aartrijke, Belgium, respectively) and a plate reader (Stat Fax 3200; Awareness Technology Inc, Palm City, FL). The intra- and interassay variations for all ELISA tests were less than 5% and 6%, respectively, as stated by the manufacturers.

**Statistical analyses**
Data were expressed as mean ± standard deviation (SD). For quantitative data, comparison between groups was made using Student’s t-test. To control confounding factors in comparing data between groups, analysis of covariance was employed. Qualitative data was compared between groups using the Chi square test. Correlations between two sets of data were evaluated using Pearson’s equation. To predict the relationship between circulating 25(OH)D and MeS components, stepwise regression analysis was used. All statistical analyses were performed using Statistical Package for Social Sciences (SPSS) (v 16; SPSS Inc, Chicago, IL). In this study \( P < 0.05 \) was considered significant.

**Results**
Of the population examined for MeS, 28.3% met the NCEP/ATP III criteria. Subjects with MeS had significantly higher BMI, WC and FM. However, there was no significant difference in circulating 25(OH)D3 or intact parathyroid hormone (iPTH) between groups. Serum osteocalcin, a vitamin D-dependent bone protein, was significantly lower in women with MeS than in the control group (Table 1). The difference remained significant even after controlling for BMI \((P = 0.001), WC (P = 0.001)\) and FM \((P = 0.002)\). Neither duration of sun exposure \((\chi^2 = 2.478, P = 0.479)\) nor vitamin D status (Table 2) differed significantly between the two groups. Duration of daily direct sun exposure in 77.2% of the cases and 72.4% of the controls was between 10–60 minutes, and in respectively 25% and 31.6% was less than 10 minutes. Serum hsCRP concentration was significantly higher in the MeS group, compared to the controls \((P < 0.001)\). The difference remained significant even after controlling for BMI \((P = 0.011), WC (0.014)\)
with and without MeS ($r = -0.344, P = 0.016$; and $r = -0.357, P = 0.042$, respectively). However, after controlling for BMI or FM, only in the MeS group did the correlation remain significant ($r = -0.370, P = 0.012$ and $r = -0.305, P = 0.039$, respectively). Serum MDA and TAC also did not differ significantly between the two groups. Only the MeS group, showed a significant correlation between 25(OH)D and TAC ($r = -0.028, P = 0.048$), which remained significant even after controlling for BMI ($r = -0.335, P = 0.026$), FM ($r = -0.347, P = 0.021$) and WC ($r = -0.228, P = 0.031$). The association, however, disappeared after controlling for plasma glucose ($r = -0.162, P = 0.133$).

When data were categorized according to vitamin D status, only the MeS subjects with vitamin D deficiency showed significantly higher plasma glucose concentrations as compared to those with vitamin D insufficiency or sufficiency ($104.0 \pm 11.7, 83.0 \pm 11.3$ and $83.2 \pm 9.9$ mg/dL, respectively, $P < 0.001$). Interestingly, their WC ($100.9 \pm 9.7, 102.3 \pm 10.2$ and $108.6 \pm 12.2$ cm, respectively, $P = 0.240$) or waist-to-hip ratio (WHR) ($0.94 \pm 0.06, 0.97 \pm 0.03$ and $0.92 \pm 0.02$, respectively) did not differ significantly. In this group, none of the other MeS components had a significant difference among the categorized subgroups.

In stepwise regression analysis to establish a model to predict hsCRP with the components of MeS as independent variables, two models were obtained in which WC was the only predictor in both MeS and control groups, explaining 4.9% and 6.7% of hsCRP variability, respectively (Table 3). However, when 25(OH)D was added to the independent variables, in the control group, two models were obtained. In model 1, again WC was the only predictor explaining 29.9% of hsCRP variability. In model 2, both WC and 25(OH)D were the predictors explaining 45.7% of the variability. In the MeS group there was only one model in which 25(OH)D was the main predictor of hsCRP variability (Table 4).

In another analysis performed to predict plasma glucose in both groups with 25(OH)D, osteocalcin, hsCRP, BMI, WC and FM as independent variables, none of the variables entered the model in the control group. In the MeS group, however, only 25(OH)D entered the model, explaining 30.6% of plasma glucose variability ($P < 0.001$) (Table 5).

### Discussion

The occurrence rate of 28.3% of MeS in our subjects is noteworthy as only women with WC above 88 cm, who were all overweight or obese, were examined for the criteria of MeS. Although women with MeS had higher BMI, WC and FM, this finding indicates that abdominal obesity as defined by
NCEP/ATP III,1 despite its pivotal role, is not sufficient for development of MeS. Adipose tissue dysfunction may be more relevant to insulin resistance and other metabolic changes observed in MeS than the amount of fat mass itself.24 In support of this notion, it has been reported that in young South Asian men, insulin resistance could be present even in the absence of increased intra-peritoneal fat and this might be due to the large size and dysfunction of subcutaneous abdominal adipocytes.35 More work is needed to elucidate the role of adipose tissue cellularity and function in MeS development.

High occurrence of vitamin D deficiency and insufficiency in both groups of women with and without MeS (86% and 89%, respectively) is quite noticeable. Poor vitamin D status in obesity has already been reported.36 It is believed that extra body fat, by sequestering vitamin D, may reduce its bioavailability.7 The reasons for this high prevalence could be many: including blood sampling during the cold season; the high latitude of Tehran; air pollution; very inefficient direct sun exposure; and the fact that there is no vitamin D fortification program ongoing in Iran at this time. Moreover, according to the Islamic regulations, women must be veiled so that only face and hands (from wrist to the fingers) are allowed to be exposed. This may also contribute to the high occurrence of poor vitamin D status in our subjects.

The link between vitamin D status and metabolic syndrome has been, and still is, under debate.11,37 We found no difference in circulating 25(OH)D concentrations or vitamin D status between women with and without MeS. However, lower vitamin D status was accompanied by higher glucose concentrations and 25(OH)D was found to be the predictor of plasma glucose only in women with MeS. The relationship between vitamin D and glycemic status has been studied in both diabetic and nondiabetic subjects.38,39 In a recent clinical trial, vitamin D replenishment has led to the improvement of glycemic status in the subjects with T2D.40 Some evidence indicates that 1,25(OH)2D, the active form of vitamin D, can boost insulin secretion by enhancing calcium flux in pancreatic beta cells.41 An in vitro study showed that 1,25(OH)2D can induce the expression of insulin receptor and insulin responsiveness in U-937 human promonocytic cells.42

In the current study, serum 25(OH)D was not associated with lipid profile, WC, WHR, and BMI. Unlike this finding,

Table 3  Stepwise multivariate linear regression analysis with hsCRP as dependent variable in the whole study population

<table>
<thead>
<tr>
<th>Group</th>
<th>Independent variables</th>
<th>B</th>
<th>SE</th>
<th>β</th>
<th>t</th>
<th>P-value</th>
</tr>
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<tbody>
<tr>
<td>MeS</td>
<td>WC, FPG, HDL, TG, SBP and DBP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Model 1: WC</td>
<td>0.074</td>
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<td>0.221</td>
<td>2.188</td>
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<td>0.064</td>
<td>0.025</td>
<td>0.259</td>
<td>2.581</td>
<td>0.011</td>
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<td>MeS</td>
<td>WC, FPG, HDL, TG, SBP, DBP and 25(OH)D</td>
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<td></td>
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<tr>
<td></td>
<td>Model 1: 25(OH)D</td>
<td>−0.059</td>
<td>0.029</td>
<td>−0.289</td>
<td>−2.048</td>
<td>0.046</td>
</tr>
<tr>
<td>Control</td>
<td>Model 1: WC</td>
<td>0.130</td>
<td>0.036</td>
<td>0.547</td>
<td>3.640</td>
<td>0.001</td>
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<tr>
<td></td>
<td>Model 2: 25(OH)D</td>
<td>−0.059</td>
<td>0.020</td>
<td>−0.400</td>
<td>−2.946</td>
<td>0.006</td>
</tr>
</tbody>
</table>

| WC | 0.142 | 0.032 | 0.601 | 4.426 | <0.001 |

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; DBP, diastolic blood pressure; FPG, fasting plasma glucose; HDL-C, high-density lipoprotein cholesterol; hsCRP, highly sensitive C-reactive protein; LDL-C, low-density lipoprotein cholesterol; TG, triglycerides; WC, waist circumference.

Table 4  Stepwise multivariate linear regression analysis with hsCRP as dependent variable

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<thead>
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<th>β</th>
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<td>0.601</td>
<td>4.426</td>
<td>&lt;0.001</td>
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Abbreviations: DBP, diastolic blood pressure; FPG, fasting plasma glucose; HDL-C, high-density lipoprotein cholesterol; hsCRP, highly sensitive C-reactive protein; LDL-C, low-density lipoprotein cholesterol; TG, triglycerides; WC, waist circumference; 25(OH)D, 25-hydroxyvitamin D.
the association of vitamin D status with some components of MeS such as serum HDL and triglycerides has been reported.\textsuperscript{43} Data from the Third National Health and Nutrition Examination Survey (NHANESIII) showed an inverse association between 25(OH)D and MeS.\textsuperscript{44} In a study performed on 1017 morbidly obese subjects (68% female), parathyroid hormone (PTH) was identified as the predictor of MeS, even though no relationship between serum 25(OH)D and MeS was observed.\textsuperscript{45}

Circulating 25(OH)D has been inversely associated with MeS in 1654 American adults independent of calcium intake and PTH, which were positively associated with MeS in older men.\textsuperscript{46} Other studies, however, have failed to show any contribution of vitamin D status in MeS.\textsuperscript{47–49} In a cross-sectional study on 542 Arab Americans, only men showed an association between vitamin D deficiency and components of MeS\textsuperscript{50} and there was no such association in Asian Indians at all.\textsuperscript{49} In the latter study, the prevalence of MeS in 441 Asian Indians, comprising 237 men and 204 women, was 27.9% and the occurrence of vitamin D insufficiency, defined as 25(OH)D < 50 nmol/L, was 65.5% with no gender difference.\textsuperscript{49} The discrepancies seen in the results of studies may be due to the different study populations, the seasons of the studies and the methods used for determination of 25(OH)D. The very high occurrence of vitamin D deficiency in our subjects could be due to blood sampling during cold seasons, when dermal synthesis of vitamin D is negligible. Moreover, it has been demonstrated that both radioimmunoassay (RIA) and competitive protein-binding assay (CPBA) may overestimate circulating 25(OH)D, compared to the HPLC method.\textsuperscript{31} That was used in this study.

The significantly lower serum concentrations of osteocalcin in women with MeS compared to the control group was of great importance. Lower serum osteocalcin concentrations in MeS have also been reported in other studies.\textsuperscript{14,15,51,52} Increased risk of MeS, independent of serum glucose, has been observed in Korean men and women who were in the lower quartiles (Q1–Q3) of serum osteocalcin, compared to those in the highest quartile.\textsuperscript{14}

An MeS-related pro-inflammatory state seems to have a determining role in the further morbidities ascribed to MeS, including diabetes\textsuperscript{53} and cardiovascular disease (CVD).\textsuperscript{54}

Assuming truncal fat being the core feature of MeS,\textsuperscript{2} the other components, notably inflammation, may emanate from the abdominal excess fat.\textsuperscript{55} However, significantly higher serum hsCRP in our subjects with MeS compared to the control group even after controlling for WC and FM indicates that systemic inflammation often accompanied by obesity may not originate solely from the adipose tissue. Lower inflammatory status, as judged by serum concentrations of CRP, has been suggested to have a protective role in the so-called “metabolically healthy but obese” phenotype against such comorbidities as cardiovascular disease (CVD).\textsuperscript{56} It has been hypothesized that chronic inflammatory stimuli together with diminished anti-inflammatory mechanisms may have a role in metabolic derangements and endothelial dysfunction in MeS.\textsuperscript{57,58} Dietary pattern may also contribute to the inflammatory process. Higher intakes of fruits and vegetables, which are rich in anti-inflammatory antioxidants, were inversely associated with serum levels of CRP and development of MeS,\textsuperscript{59} while red meat,\textsuperscript{60} high fat dairy products\textsuperscript{61} and hydrogenated oil\textsuperscript{62} consumption was associated with higher circulating inflammatory biomarkers and greater risk of MeS.

An inverse association between serum 25(OH)D and hsCRP may support the suggestion of an anti-inflammatory function of vitamin D. Though we found no significant difference in vitamin D status between women with and without MeS, 25(OH)D was the only predictor of both hsCRP and plasma glucose in the subjects with MeS.

Considering the anti-inflammatory effects of cholecalciferol,\textsuperscript{63} poor vitamin D status may contribute to the systemic inflammation often seen in obesity, thus favoring other metabolic derangements like insulin resistance and raised blood glucose. The possible anti-inflammatory effects of vitamin D must be evaluated in the controlled clinical trials.\textsuperscript{64}

This study had some limitations. Blood samples were drawn during the cold seasons, when dermal synthesis of vitamin D is minimal, so the vitamin D status of our subjects did not necessarily reflect their status for the whole year. Because of the high proportion of vitamin D deficiency in both groups, it was difficult to show the differences, if any. Moreover, only women were enrolled in the study. Therefore the relationship between vitamin D status and MeS in men remains to be studied.

In conclusion, various degrees of vitamin D deficiency had more than 85% prevalence among overweight/obese middle-aged women in Tehran who participated in this study. Although there was no significant difference in either

### Table 5 Stepwise multivariate linear regression analysis with plasma glucose as dependent variable in women with MeS

<table>
<thead>
<tr>
<th>Independent variables</th>
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<th>SE</th>
<th>β</th>
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<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>WC, BMI, FM, hsCRP, OST and 25(OH)D</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Model 1: 25(OH)D</td>
<td>-0.399</td>
<td>0.100</td>
<td>-0.553</td>
<td>-3.984</td>
<td>&lt; 0.001</td>
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Abbreviations: BMI, body mass index; FM, fat mass; hsCRP, highly sensitive C-reactive protein; OST, osteocalcin; WC, waist circumference; 25(OH)D, 25-hydroxyvitamin.
serum 25(OH)D or vitamin D status between women with and without MeS, 25(OH)D was a predictor of hsCRP, the biomarker of systemic inflammation, in women with MeS but not in the controls. Thus, vitamin D status may, at least in part, be a determining factor of systemic inflammation and the related metabolic derangements of MeS.

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Disclosure
The authors declare no conflicts of interest.

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