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Original Article

Gender dependent association of 25-hydroxyvitamin D and circulating leptin in saudi subjects: influence of dyslipidemia

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Abstract: Background and Objective: Leptin and vitamin D play an important role in obesity development and metabolic effects; however, the association between leptin and vitamin D is not well studied in Saudi subjects. We aimed to examine gender dependent association between serum leptin and 25-OH-VitD in adult Saudi subjects. Subjects and Methods: For this cross-sectional study in a cohort of 259 Saudi adults (100 male, age: 46.4 ± 0.9 yr [mean ± SD]; BMI: 27.8 ± 0.5 Kg/m^2) and (159 female, age 46.5 ± 0.7 [mean ± SD]; BMI: 28.4 ± 0.4 Kg/m^2) anthropometrics, fasting bloods, and biochemical data were collected. Serum leptin and 25-hydroxyvitamin D (vitamin D or 25-OH-VitD) were quantified using an enzyme-linked immunosorbent assay. Results: Circulating leptin and vitamin D levels were significantly higher in females compared to male (P<0.001 and P<0.01 respectively). Visceral adiposity index (VAI), triglycerides and total cholesterol were significantly higher (P<0.05, P<0.001, and P<0.05, respectively) while HDL-cholesterol were lower (P<0.001) in male compared to female subjects. In males, vitamin D levels were positively associated with leptin (r = 0.196, P<0.05). Conclusion: Vitamin D was positively associated with serum leptin in male Saudi subjects. Additionally, male subjects were found to be dyslipidemic, which might be a responsible factor for this discordant association between vitamin D and leptin in Saudi population.

Keywords: Leptin, vitamin D, Saudi subjects, dyslipidemia

Introduction

Vitamin D is a fat-soluble vitamin with a well-known effect in regulating calcium absorption and bone mineralization [1, 2]. It is synthesized in the skin and undergoes hepatic 25-hydroxylation to form 25-hydroxyvitaminD [25-OH-VitD], a most sensitive clinical marker for vitamin D status in human [3]. Serum or plasma 25-OH-VitD level below 50 nmol/l is considered as vitamin D deficiency, which is extremely common globally and particularly in the Middle Eastern countries including Saudi Arabia [1, 4]. Besides osteoporosis, vitamin D deficiency has been also known for its role in certain forms of cancer, autoimmune disorder, cardiovascular, and metabolic dysfunctions like type 2 diabetes mellitus [4, 5].

Recent evidence suggests frequent hypovitaminosis D in adult and childhood obesity, which is generally associated with increase in number and size of adipocytes [6]. Adipose tissue exerts its metabolic effects via the secretion of a variety of bioactive molecules called adipocytokines [7]. Leptin, product of the obesity (*ob*) gene, is one such adipocytokine which play crucial role in energy metabolism [8]. It acts via its receptor in the hypothalamus to control appetite and increase in energy expenditure [8, 9]. Thus, besides hypovitaminosis D, low leptin level is also an important determining factor for the individual risk to develop obesity. In an *in vitro* study, using tissue culture model, Menendez et al. [10] found that leptin secretion by human adipose tissue is negatively and strongly inhibited by vitamin D.

Table 1. Age and BMI-matched general characteristics of subjects

Parameter	Males	Females	*P*-Value
N	100	159	
Obesity (%)	37.1	45.3	0.296
DMT2 (%)	27.0	25.2	0.772
Vitamin D Deficiency (<50 nmol) (%)	77.0	61.0	0.010
Hyperleptinemia	46.0	45.3	0.898
>7 ng/ml in males			
>20 ng/ml in females			
Age (years)	46.4 ± 0.9	46.5 ± 0.7	0.914
BMI (kg/m^2)	27.8 ± 0.5	28.4 ± 0.4	0.387
VAI#	5.4 ± 0.6	3.9 ± 0.5	0.032
BAI#	7.2 ± 0.6	10.4 ± 0.6	<0.001
Waist (cm)	96.5 ± 2.4	90.8 ± 2.1	0.072
Hips (cm)	105.2 ± 2.2	103.8 ± 2.0	0.640
Systolic Blood Pressure (mmHg)	117.3 ± 1.5	121.7 ± 1.5	0.038
Diastolic Blood Pressure (mmHg)	76.6 ± 0.9	78.0 ± 0.9	0.298
Glucose (mmol/l)	7.0 ± 0.3	6.7 ± 0.2	0.477
Triglycerides (mmol/l)	2.2 ± 0.1	1.5 ± 0.06	<0.001
Total Cholesterol (mmol/l)	5.3 ± 0.2	4.9 ± 0.08	0.039
HDL-Cholesterol (mmol/l)	0.78 ± 0.04	0.98 ± 0.04	<0.001
Leptin (ng/ml)#	11.9 ± 1.4	24.2 ± 2.1	<0.001
25(OH)-hydroxyvitamin D#	38.9 ± 1.9	47.5 ± 2.2	0.004

Note: Data presented as mean ± standard error; #Denotes non-Gaussian variable; significant at P<0.05.

Despite understanding the role of leptin in adiposity, gender dependent association between vitamin D status and leptin has not been well studied in Saudi subjects. Since, gender dimorphism exist in body fat deposition, distribution, and metabolism [11, 12], we aimed to investigate the gender dependent association between serum leptin and 25-OH-VitD in adult population of Saudi Arabia.

Materials and methods

Subjects and experimental design

A total of 259 Saudi subjects (100 men; 159 women), age between 35-70 years were randomly selected from different Primary Health Care Centers (PHCCs) in Riyadh, KSA. These individuals were part of the Biomarker Screening in Riyadh Project (RIYADH COHORT). All participants were provided written and informed consent prior to inclusion. Ethical approval was granted by the Ethics Committee of the College of Science Research Center, King Saud University, Riyadh, Kingdom of Saudi Arabia (KSA). All participants completed a questionnaire on demographic information, general health status, and past medical history.

Blood collection and anthropometrics measurement

Fasting blood samples (>10 h) were withdrawn by a staff nurse and centrifuged for serum isolation, and anthropometric information was gathered. The collected sera were then transferred to a prelabeled tube, stored in ice, and delivered to the Biomarkers Research Program (BRP) at King Saud University, Riyadh, KSA for immediate storage at -20°C until analysis.

Anthropometric data included height (cm); weight (kg); waist and hip circumferences (cm); sagittal abdominal diameter (SAD, cm); and systolic and diastolic blood pressure (mm Hg) (average of 2 readings 15 min. apart). Body mass index (BMI) was calculated as weight in kg divided by the height in meters squared. Visceral adiposity index (VAI) score was calculated as described [13] using the gender-specific equations: for males: {[WC/39.68 + (1.88 × BMI)] × (TG/1.03) × (1.31/HDL)}; for females: {[WC/36.58 + (1.89 × BMI)] × (TG/0.81) × (1.52/HDL)}, where TG levels are expressed in mmol/l and HDL cholesterol levels are expressed in mmol/l. Body adiposity index (BAI) was calculated using the formula [14], BAI = (((HC)/(height)) × 1.5)-1.8).

Biochemical analysis

Fasting blood glucose (FBG) and lipid profile were measured using a chemical analyzer (Konelab20XTi, Thermo Electron Corporation, Vantaa, Finland). Leptin was quantified using multiplex assay kit, which includes pre-mixed and fully customized panels that utilize the Luminex xMAP technology platform (Luminexcorp, Austin, TX, USA). Serum 25(OH)D was measured by a specific enzyme-linked immunosorbent assay (IDS, Tyne and Wear, UK). Inter and intra assay variability of this assay was 5.3% and 4.6%, respectively. In this study

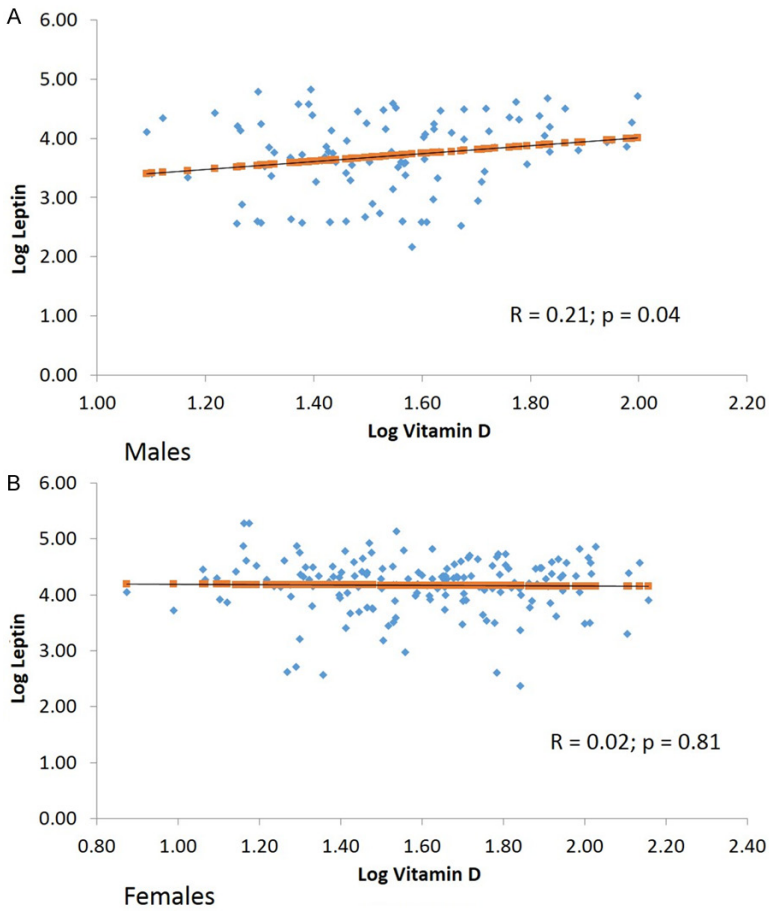

Figure 1. Association of log Leptin versus log 25(OH) vitamin D in (A) Males and (B) Females; significant at P<0.05.

hyperleptinemia defined as leptin level more than 7 ng/ml in males and >20 ng/ml in females [15] while vitamin D deficiency defined as less than 50 nmol in both male and female [16].

Statistical analysis

Data analyses were carried out using the Statistical Package for Social Sciences software (SPSS 16.0; SPSS Inc, Chicago, Illinois, USA). Data were expressed as mean ± standard deviation. Kolmogorov-Smirnov was performed to test continuous variables for normality. All non-Gaussian parameters were logarithmically or square root transformed to normalize prior to correlations and linear regression analyses. Independent Student's t-test was employed to compare means between groups of normally distributed data. *P* values <0.05 were considered statistically significant.

Results

Table 1 describes the anthropometric and biochemical parameters of male and female subjects included in the study. Females had significantly higher vitamin D and circulating leptin level as compared to male (P<0.01 and P<0.001, respectively). Importantly in males; VAI, triglycerides and total cholesterol were significantly higher (P<0.05, P< 0.001, and P<0.05, respectively) while HDL-cholesterol were lower (P<0.001) compared to females.

After Pearson correlation it was observed that vitamin D level was positively associated with leptin (r = 0.21, P<0.05) in male, but not in female (**Figure 1A** and **1B**). **Table 2** shows gender dependent association between vitamin D and leptin with metabolic and anthropometric profile. In male, leptin was negatively associated with FBG (r = -0.20, P<0.05) while vitamin D was positively associated with HDL-Cholesterol (r = 0.21, P<0.05). In female, vitamin D level was positively associated with age (r = 0.19, P<0.05) and systolic blood pressure (r = 0.30, P<0.001) while negatively associated with total cholesterol (r = -0.19, P<0.05).

By splitting subjects into, with and without hyperleptinemia, significantly higher level of vitamin D was observed in males with hyperleptinemia, as compared to without hyperleptinemia (P<0.01) (**Figure 2A**). Furthermore, by dividing subjects into with or without 25-OH-VitD deficiency, significantly higher level of leptin was observed in 25-OH-VitD sufficient as compared to vitamin D deficient male (P<0.05) (**Figure 2B**).

Discussion

The present study demonstrates gender dependent association of circulating leptin and vita-

Table 2. Gender dependent association of vitamin D and leptin with metabolic and anthropometric parameters

Parameter	Males		Females	
	Vitamin D	Leptin	Vitamin D	Leptin
Age (years)	0.13	-0.11	0.19*	0.02
BMI (kg/m^2)	0.07	0.18	0.07	-0.08
VAI	-0.23*	-0.07	-0.12	0.02
BAI	-0.06	0.15	-0.07	0.12
Waist (cm)	0.15	0.02	-0.05	-0.08
Hips (cm)	-0.01	0.20	-0.19	0.07
Systolic Blood Pressure (mmHg)	0.02	0.02	0.30**	0.02
Diastolic Blood Pressure (mmHg)	0.09	-0.02	0.11	-0.04
Glucose (mmol/l)	0.01	-0.20*	0.08	-0.14
Triglycerides (mmol/l)	-0.10	-0.10	0.10	-0.02
Total Cholesterol (mmol/l)	-0.08	-0.10	-0.19*	-0.08
HDL-Cholesterol (mmol/l)	0.21*	-0.11	-0.02	-0.02

Note: Data presented as coefficient (R); *Denotes significance at <0.05; **Denotes significance at <0.01.

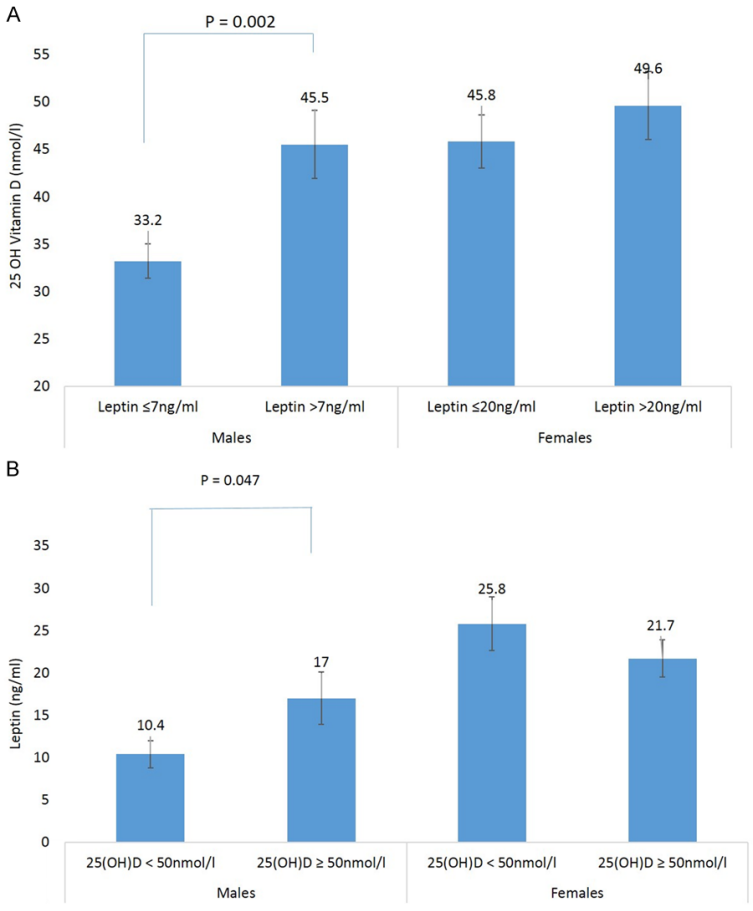

Figure 2. A. 25(OH) Vitamin D levels in males and females with or without hyperleptinemia; significant at P<0.05. B. Leptin levels in males and females with or without 25(OH) vitamin D deficiency; significant at P<0.05.

min D. Positive association between vitamin D and circulating leptin level was observed in males. Importantly, VAI, triglycerides and total cholesterol were significantly higher (P< 0.05, P<0.001, and <0.05, respectively), while HDL-cholesterol were lower (P<0.001) in males compared to females. Furthermore, by splitting subjects into with and without hypovitaminosis D, significantly higher level of leptin was observed in vitamin D sufficient male as compared to counterpart. Furthermore, by dividing the subjects into with and without hyperleptinemia, significantly higher level of vitamin D was observed in hyperleptinemic male compared to without hyperleptinemia.

We observed significantly higher level of leptin in females as compared to males. Elevated level of leptin in females can be best explained by the gender related differences in the body fat deposition. Since, leptin, the product of the obesity (*ob*) gene [17], is synthesized and secreted by adipocytes hence its production may relate to inter-individual obesity. Studies in humans have shown a strong positive correlation between serum leptin concentrations and the percentage of body fat [18, 19]. Also, it is well established that females generally have a higher percentage of body fat than male [20]. In this study a higher BAI was observed in females compared to males, BAI represents a better index of body adiposity [14]. In addition to gender based fat accumulation, sex hormone might also play a significant role in endogenous leptin production by adipose cells. Saad et al. [21] observed approximately 40% higher le-

ptin level in female than men at any level of adiposity, which strengthen the assumption of the role of sex hormone for elevated leptin in females.

We observed higher level of vitamin D in females compared to males, which contradicts the previous findings where females were found to be lower in vitamin D content [5, 22-24]. However, few studies on Saudi population found women to be higher in vitamin D level especially in post-menopausal condition [25, 26]. The possible reason for this discrepancy may relate to the fact that it is still a common practice among many clinicians that they prescribe vitamin D supplementation without assessing the exact vitamin D status in patients. In an estimate even many clinicians tend to prescribe 1 gm Calcium and 10-20 mg of vitamin D for postmenopausal women as a protection against osteoporosis [26]. Our observation of positive association between vitamin D and age in the female individual supports the assumption of unregulated vitamin D prescription by clinician in postmenopausal condition.

Our finding indicates that vitamin D level was positively associated with serum leptin in males. Current observation is not in agreement with earlier findings, where negative association was observed between leptin and vitamin D [27, 28]. These clinical findings were supported by an *in vitro* study conducted by Menendez et al. [10] showing inhibition of leptin secretion by vitamin D. In contrast, another study performed by Kong et al. in mice model [8], observed stimulated mRNA expression and secretion of leptin by vitamin D. Inconsistencies with these findings pointing towards some other factors which possibly influenced the association of vitamin D and leptin in male subjects. In the present study, higher level of total cholesterol, triglycerides and low level of HDL-cholesterol was demonstrated in males compared to females. Clinical evidences indicated that modulation in the level of these lipid markers have been considered as a metabolic dysfunction [29]. Furthermore, we observed higher VAI in male compared to female subjects. VAI, a newly-derived measure of visceral adiposity is also a well-validated predictive power for metabolic alteration and cardiovascular disease [30]. Thus, an imbalance in the lipid markers which are generally accepted features of metabolic dysfunctions could be an important modifier in the association of vitamin D and leptin. Accumulating evidence suggests that vitamin D deficiency may also contribute to the development of the metabolic dysfunction [31]. Hence, our observation of lower vitamin D level in male subjects might be another reason behind this discordant association in male Saudi subjects.

There were certain limitations of this study. Firstly, the measurement of cause and effect relation was not possible because of the cross sectional design of the study. Secondly, we were not having detailed information regarding participant's use of vitamin D supplement and sunscreen. However, our study suggests gender dimorphism in the association of vitamin D and leptin in Saudi subjects which was largely influenced by dyslipidemic condition of the male subjects.

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Disclosure of conflict of interest

None.

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