Progress in Nutrition 2020; Vol. 22, N. 2: 603-616 DOI: 10.23751/pn.v22i2.9104

Original article

Association between polymorphism in TaqI, BsmI, Apa1, DHCR7, GC, CYP27B1 and CYP24A1 genes and vitamin D deficiency in Saudi obese females

Manal Abdulaziz Binobead[1], Sahar Abdulaziz AlSedairy[1], Naveed Ahmed Syed[1,2], Periasamy Vaiyapuri Subbarayan[1,2], Tahani Aljurbua[1], Maha Abdulaziz Aljuwayd[3], Shaista Arzoo[1], Ali Abdullah Alshatwi[1,2]

[1] Department of Food and Nutrition Sciences, College of Food and Agriculture Sciences, King Saud University, Riyadh, Saudi Arabia; [2] Molecular Cancer Biology Research Lab (MCBRL), Department of Food Science and Nutrition, King Saud University, Riyadh, Saudi Arabia; [3] Ministry of Education, Riyadh, Saudi Arabia.

Summary. *Background.* Obesity is an endemic disease and obese people requires larger vitamin D intakes to achieve normal $25(OH)_2D_3$ as compared to individuals with normal weight. This study assessed the association of vitamin D levels with the single nucleotide polymorphism (SNPs) of genes related to obesity and vitamin D (i.e., TaqI, BsmI, Apa1, DHCR7, GC, CYP27B1 and CYP24A1). *Methods.* One hundred forty two women were involved in this study. The study tools included interview questionnaire, anthropometric measurements (height, weight, BMI) and blood biochemical tests (glucose, insulin, cholesterol, triglyceride, HDL, LDL, 25-Hydroxyvitamin D and parathyroid assessment and SNP analysis (VDR SNPs, DHCR7, GC, CYP27B1, CYP24A1). *Results.* As compared to normal vitamin D obese (NVD) subjects the weight, body mass index (BMI) and parathyroid hormone (PTH) was significantly higher in vitamin D deficient obese (VDD) subjects. While, total cholesterol (TC), triglyceride (TG) and vitamin D levels were significantly lower in the vitamin D deficient group compared to the normal vitamin D group. Various variants such as TaqI, BsmI, Apa1, DHCR7, GC rs4588 and CYP27B1 did not showed any significant association with the pathogenesis of vitamin D deficiency related obesity. However, GC rs7041 and CYP24A1 genotypes were significantly related with vitamin D deficiency in patients with obesity. *Conclusion.* This study suggests that polymorphisms in the GC and CYP24A1 genes are related with vitamin D deficiency in obese females. These polymorphisms may become a useful marker to predict the impending development of vitamin D deficiency in obese females. Studies have revealed that associations between genotypes of these SNPs and particular phenotypes may vary according to the following: ethnicity, interactions with environmental factors, sex, and number of participants.

Key words. Vitamin D, obesity, SNPs, genes, BMI, Saudi.

Abbreviations

HDL: high density lipoprotein; LDL: low density lipoprotein; VDR: vitamin D receptor; NVD: normal vitamin D obese subjects; VDD: vitamin D deficient obese subjects; SNP: single nucleotide polymorphisms; VDREs: vitamin D-responsive elements; BMI: body mass index; PTH: parathyroid hormone

Background

Obesity is an endemic disease and it is a foremost reason of illness and decease worldwide [1, 2]. It reduces life expectancy by 6 to 7 years [3]. The prevalence of obesity has increased between 2010 and 2014, and has tripled since 1980 [4, 5]. Globally, almost 1.9 billion adults were estimated to be overweight and almost 609 million adults were predicted to be obese in 2015,

demonstrating around 39% of the world's population [6]. It has been estimated that if the current trends continue than by the year 2030 more than half of the world population (57.8%) will either be overweight or obese [7]. In summary, the prevalence of obesity is greater in women than men, and increases with age. During the past 35 years the rates of overweight and obesity have augmented greatly to the extent that more than one-third of the world's population is now classified as overweight or obese [6]. Body mass index (BMI) is broadly used for obesity classification and nutritional assessment. However, in certain populations, such as athletes, body builders and in elderly patients it could possibly produce imprecise diagnosis. [8]. Severe obesity (BMI ≥ 40 kg/m2) decreases life expectancy by almost 20 years for men and by 5 years for women [7]. BMI ≥ 30 kg/m2 is related with lesser serum calcitriol or $25(OH)_2D_3$ levels compared to non-obese individuals. Obese individuals may need larger intakes of vitamin D to achieve $25(OH)_2D_3$ levels comparable to those of individuals with normal weight [9]. In adults obesity has been found to be related with vitamin D deficiency [10-12].

Calcitriol binds to a nuclear receptor, (vitamin D receptor; VDR), which is related with specific recognition sequences called vitamin D-responsive elements (VDREs). VDREs are usually found within 1 kilo basepair (kbp) of the start site of the target gene. The commonly occurring linked single nucleotide genetic markers (polymorphisms) at the 3' end of the VDR gene are the restriction fragment length polymorphisms (RFLPs) of BsmI, ApaI, and Taq I and the exon 2 splice site Fok polymorphism. In the absence of VDR, animals have low fat mass, resistance to high-fat-induced fat accumulation, and reduced plasma lipid levels [13-14]. Single nucleotide polymorphisms (SNPs) are abundant and scattered throughout the human genome. They have been widely used in the genetic association studies of various complex diseases such as obesity, osteoporosis, asthma, hypertension [15-17]. This study assessed the association between vitamin D status, obesity, and genes related to vitamin D (TaqI, BsmI, Apa1, DHCR7, GC, CYP27B1, and CYP24A1) in obese adult Saudi women with normal and deficient level of vitamin D.

Methods

Subjects

A total of 201 women were randomly selected from various clubs in different areas of Riyadh city. Only 142 obese women were involved in this study (94 women with vitamin D deficiency; VDD and 48 women with normal vitamin D levels; NVD) as others didn't meet the inclusion criteria (Figure 1). The study tools included an interview questionnaire, anthropometric measurements and blood biochemical tests. This study was conducted in compliance with the ethical principle of the Declaration of Helsinki. The aim of this study was explained to all participants. Written consent was obtained from the respondents involved in this research and the study abided by the principle of voluntary participation. Blood was withdrawn by a qualified nurse and subjects were assured that the information given was entirely for scientific purposes and would be kept confidential.

Inclusion criteria

Obesity, no recent surgeries, age should be between 12-70 years.

Exclusion criteria

Recent infections or surgeries, pregnancy, vitamin D supplementation

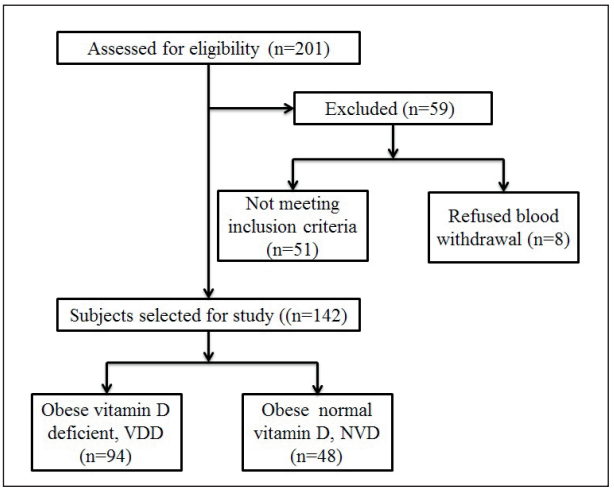

Figure 1. Flowchart of the participants throughout the study.

Anthropometric measurements

Height and weight were used to determine BMI using the formula: BMI = weight (kg)/height^2 (m)^2 and the BMI classification for adults, based on the 1997 WHO recommendations, is as follows: normal, < 25 kg/m^2; overweight or pre-obese, 25–29.9 kg/m^2; class I obesity, 30–34.9 kg/m^2; class II obesity, 35–39.9 kg/m^2; class III obesity, ≥ 40 kg/m^2 [1].

Collection of blood samples

After an overnight fast (> 12 hours) the blood sample (8 ml) was drawn by a nurse and transferred rapidly to non-heparinized tubes for chemical analysis and in heparinized tubes for SNP analysis. Serum samples were stored at -80°C freezer for further analysis.

Biochemical assessment

Glucose

Glucose was estimated using kit (REF 037L) from United Diagnostics Industry, Dammam, and KSA on UDICHEM-300 Chemistry Analyzer [18].

Lipid Profile

Total Cholesterol (TC, REF UI24), Triglyceride (TG, REF UI59L) and HDL Cholesterol (HDLc, REF UI41HD) were estimated using kit from United Diagnostics Industry, Dammam, KSA on UDICHEM-300 Chemistry Analyzer [19,20]. LDL cholesterol was calculated using formula proposed by Friedwald et al. [21].

25-Hydroxyvitamin D Assessment

25-Hydroxyvitamin D was assessed according to the method proposed by Phinney, by kit from Roche Diagnostics (05894913190) using device Roche Analyzer Cobas e 602 Immunoassay [22].

Parathyroid hormone (PTH) Assessment

PTH was estimated according to the method proposed by Ohe et al., by kit from Roche Diagnostic's (11972103122) using device Roche Analyzer Cobas e 602 Immunoassay [23].

SNP analysis

Genomic DNA of all the enrolled subjects was isolated from 100 µL of frozen whole blood collected in EDTA-containing tubes by using DNeasy blood and Tissue kit (Qiagen, Hilden, Germany). VDR SNPs [TaqI (rs731236), Apa1 (rs7975232) and BsmI (rs1544410)], DHCR7 (rs12785878, rs3829251), GC (rs7041, rs4588), CYP27B1 (rs4646536, rs4646537) and CYP24A1 (rs2296241, rs927650) were estimated by allelic discrimination real-time PCR using predesigned TaqMan probes (Applied Biosystems, Foster City, CA, USA). The PCR comprised of a heat cycle at 95°C for 10 minutes followed by 40 cycles of denaturation at 95°C for 15 seconds and 60°C annealing/extension for 1 minute. Fluorescence detection was done at a temperature of 60°C. All assays were performed in 10 µL reactions, with the TaqMan Genotyping Master Mix in 96-well plates using an ABI 7500 instrument (Applied Biosystems). Control samples representing all possible genotypes and a negative control were included in each reaction.

Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Science (SPSS 22, Chicago IL, USA) for data analysis. Variables were presented as mean ± standard deviation (SD). Group comparisons were done using an independent Student T-test and Mann-Whitney U-test for non-normally distributed variables. The P value was considered significant if less than 0.05. The analysis of variance (ANOVA) was used to compare quantitative data. When this test was significant, comparisons between pairs were made using the Tukey-Kramer honestly significant difference test [24].

Results

Sociodemographic characteristics of study population

The characteristics of all obese subjects are shown in Table 1. A total of 142 participants with obesity were subdivided into two groups: (1) normal vitamin D group (NVD; n = 48) and (2) vitamin D deficient group (VDD; n = 94). The mean age of the NVD group was 41.42 years and that of VDD group was 33.34 years and the differences between the two groups (NVD vs VDD) were statistically significant ($p<0.05$). As expected, the VDD subjects had higher mean weight, BMI, waist circumference and parathyroid hormone levels than NVD group. However, total cholesterol (TC), triglyceride (TG), and vitamin D levels were significantly lower in the VDD group than the NVD group. High density lipoprotein (HDL), low density lipoprotein (LDL) and glucose level was higher in NVD group but statistically insignificant differences were observed between the NVD and VDD.

Table 1. Characteristics of the study population

Variables	NVD	VDD	P value
Number	48	94	
Age (years)	41.42±10.53	33.34±10.29	0.000**
Height (cm)	158.77±5.62	159.47±4.93	0.468
Weight (kg)	83.22±10.29	87.85±12.82	0.021*
BMI (kg/m^2)	33.05±3.36	34.59±4.76	0.027*
Waist circumference (cm)	91.33±8.32	93.26±9.46	0.217
Systolic blood pressure (mm)	127.04±14.16	122.09±11.57	0.027*
Diastolic blood pressure (mm)	76.69±10.16	77.14±8.98	0.787
Total cholesterol (mg/dl)	205.33±39.06	188.21±31.97	0.006**
Triglyceride (mg/dl)	124.96±61.15	103.46±59.62	0.010**
High-density lipoproteins cholesterol (mg/dl)	59.15±11.37	56.09±11.71	0.138
Low-density lipoproteins cholesterol (mg/dl)	119.02±35.47	110.65±29.86	0.143
Glucose (mg/dl)	91.91±18.64	89.35±11.05	0.321
Vitamin D (nmol/l)	89.58±11.83	42.65±17.91	0.000**
Parathyroid Hormone (pmol/l)	4.68±1.88	5.75±2.86	0.009**

Data are presented in Mean (M), standard deviation (± SD). The M values were compared using paired T-test. *P ≤ 0.05 is considered statistically significant and **P ≤ 0.01 is considered statistically significant. Abbreviations: NVD- normal vitamin D; VDD-vitamin D deficient; BMI- body mass index.

Correlations of vitamin D or body mass index with selected parameters for obesity deficiency VD patients.

The correlations of vitamin D and BMI with selected parameters for obese patients VDD group are shown in Table 2. The serum vitamin D showed a significant negative correlation with BMI ($r = -0.270$, $p<0.01$). In addition, a significant positive association of BMI with weight ($r = 0.903$, $p<0.01$), waist circumference ($r = 0.835$, $p<0.01$) and a negative association vitamin D with BMI ($r = -0.270$, $p< 0.01$) has been noted.

Distributions of SNP genotypes and odds ratios (OR) for risk of normal vitamin D vs. vitamin D deficiency in the population study

Table 3 and 4 shows the distributions of SNP genotypes and odds ratios (OR) for risk between NVD and VDD group. For the rs 1544410 SNP in the BSMI gene, genotypic for frequencies were determined as 29.2% for the TT, 52.8% for the heterozygous TC status, and 18% for the CC, in the vitamin D defi-

Table 2. Correlations of vitamin D or body mass index with selected parameters for obesity deficiency VD patients

Variables	Vitamin D		BMI	
	R	P value	R	P value
Weight	-0.198	0.072	0.903	0.000**
BMI	-0.270	0.014**	-	-
Waist circumference	-0.212	0.056	0.835	0.000**
VD	-	-	-0.270	0.014**

Spearman's correlation is done. * P value ≤ 0.05 is considered statistically significant. ** P value ≤ 0.01 is considered statistically significant. Abbreviations: VD- vitamin D; BMI- body mass index.

Table 3 Distribution of SNP genotypes of obesity normal VD vs. obesity deficiency VD in the population study

SNP GENOTYPE	GROUP		P
	Obesity Normal VD	Obesity Deficiency VD	
BsmI rs1544410			
TT	14 (29.8%)	26 (29.2%)	0.712
TC	22 (46.8%)	47 (52.8%)	
CC	11 (23.4%)	16 (18%)	
TaqI rs 731236			
GG	13 (27.7%)	25 (28.1%)	0.710
GA	24 (51.1%)	50 (56.2%)	
AA	10 (21.3%)	14 (15.7%)	
Apa1 rs7975232			
AA	21 (44.7%)	39 (43.8%)	0.890
AC	21 (44.7%)	38 (42.7%)	
CC	5 (10.6%)	12 (13.5%)	
GC rs4588			
TT	36 (76.6%)	62 (69.7%)	0.461
TG	10 (21.3%)	21 (23.6%)	
GG	1 (2.1%)	6 (6.7%)	
GC rs7041			
CC	1 (2.1)	20 (22.5%)	0.007
CA	23 (48.9%)	36 (40.4%)	
AA	23 (48.9%)	33 (37.1%)	
CYP24A1 rs927650			
CC	23 (48.9%)	28 (31.5%)	0.074
CT	16 (34.0%)	48 (53.9%)	
TT	8 (17%)	13 (14.6%)	
CYP27B1 rs4646537			
TT	0 (0.0%)	4 (4.5%)	0.191
GT	0 (0.0%)	2 (2.25)	
GG	47 (100.0%)	83 (93.3%)	
CYP27B1 rs4646536			
GG	29 (61.7%)	55 (61.8%)	0.889
GA	15 (31.9%)	30 (33.7%)	
AA	3 (6.4%)	4 (4.5%)	
DHCR7 rs3829251			
AA	3 (6.4%)	3 (3.4%)	0.170
AG	10 (21.3%)	32 (36.4%)	
GG	34 (72.3%)	53 (60.2%)	
DHCR7 rs12785878			
GG	24 (51.1%)	37 (41.6%)	0.265
GT	16 (34.0%)	43 (48.3%)	
TT	7 (14.9%)	9 (10.1%)	

The data are presented as number (percentage). The values were compared using chi-square. *P value ≤0.05 is considered statistically significant and **P value ≤0.01 is considered statistically significant. Abbreviations: SNP- single nucleotide polymorphism; VD- vitamin D.

cient group. As compared to NVD group (29.8% and 23.4% for TT and CC respectively) the homozygous TT and CC genotype were seen in a lower percentage in VDD group (29.2% and 18% for TT and CC respectively). Data showed that TT vs TC and TT vs TC + CC genotypes did not exhibited a significant difference between the two groups (p = 0.74 and p = 0.94, respectively), and the TT genotype was not allied with the disease [OR, 0.97 (0.45–2.11); p = 0.94]. The OR were calculated for the TT homozygote [OR, 0.87 (0.38–1.98); p = 0.74], TC heterozygote [OR, 1.47 (0.58–3.68); p = 0.41] and CC homozygote [OR, 1.28 (0.47–3.49); P = 0.48] in each case.

For the rs 731236 SNP in the Taql gene, genotypic for frequencies were determined as 28.1% for the GG, 56.2% for the heterozygous GA status, and 15.7% for the AA in the VDD group. The homozygous AA genotype was seen in a lower percentage in VDD deficient group (15.4%) when compared to NVD group (21.3%). Data showed that GG vs. GA and GG vs. GA + AA genotypes did not exhibited significant difference between the VDD and NVD groups (p = 0.85 and p = 0.96 respectively), and the GG genotype was not related with the disease [OR, 1.02 (0.46–2.25); p = 0.96]. The OR was calculated for the GG homozygote [OR, 0.92 (0.40–2.11); p = 0.85], GA heterozygote [OR, 1.49 (0.58–3.83); p = 0.41], and AA homozygote [OR, 1.37 (0.48–3.93); p = 0.55] in each case.

For the rs 7975232 SNP in the Apa1 gene, genotypic for frequencies were determined as 43.8% for the AA, 42.7% for the heterozygous AC status, and 13.5% for the CC in the VDD group. The homozygous AA and heterozygous AC genotype were seen in a lower percentage in VDD group (43.8% and 42.7% for AA and AC respectively), when compared to NVD group (44.7% and 44.7% for AA and AC respectively). The genotype percentage for AA and AC genotypes among the NVD subjects were almost same, but a border line difference among subjects in VDD group has been found. Data showed that AA vs. AC and AA vs. AC + CC genotypes did not exhibited significant difference between the VDD and NVD groups (p = 0.95 and p = 0.92, respectively), and the AA genotype was not allied with the disease [OR, 0.97 (0.47–1.97); p = 0.92].

Table 4. Odds Ratio with 95% confidence interval of SNP genes in obesity normal VD vs. obesity deficiency VD patients

SNP	Genotype	Odds Ratio	95% Confidence Interval (Ci)	P
BsmI rs1544410	TT vs. TC	0.87	(0.38-1.98)	0.74
	TT vs. CC	1.28	(0.47-3.49)	0.48
	TC vs. CC	1.47	(0.58-3.68)	0.41
Dominant model	TT vs. CC + TC	0.97	(0.45-2.11)	0.94
Recessive model	CC vs. TT + TC	0.72	(0.30-1.70)	0.45
TaqI rs 731236	GG vs. GA	0.92	(0.40-2.11)	0.85
	GG vs. AA	1.37	(0.48-3.93)	0.55
	GA vs. AA	1.49	(0.58-3.83)	0.41
Dominant model	GG vs. GA + AA	1.02	(0.46-2.25)	0.96
Recessive model	AA vs. GG + GA	0.69	(0.28-1.70)	0.42
Apa1 rs7975232	AA vs. AC	1.03	(0.48-2.18)	0.95
	AA vs. CC	0.77	(0.24-2.49)	0.66
	AC vs. CC	0.75	(0.23-2.43)	0.64
Dominant model	AA vs. CC + AC	0.97	(0.47-1.97)	0.92
Recessive model	CC vs. AA + AC	1.31	(0.43-3.97)	0.63
GC rs4588	TT vs. TG	0.82	(0.35-1.93)	0.65
	TT vs. GG	0.29	(0.03-2.48)	0.26
	TG vs. GG	0.35	(0.04-3.31)	0.36
Dominant model	TT vs. TG + GG	0.70	(0.31-1.58)	0.39
Recessive model	GG vs. TT + TG	3.32	(0.39-28.47)	0.27
GC rs7041	CC vs. CA	12.78	(1.60-101.81)	0.01
	CC vs. AA	13.94	(1.74-111.33)	0.01
	CA vs. AA	1.09	(0.52-2.30)	0.82
Dominant model	AA vs. CA + CC	0.61	(0.30-1.26)	0.18
Recessive model	CC vs. AA + CA	13.33	(1.73-102.82)	0.01
CYP24A1 rs927650	CC vs. CT	0.40	(0.18-0.89)	0.02
	CC vs. TT	0.75	(0.26-2.12)	0.59
	CT vs. TT	1.85	(0.65-5.26)	0.25
Dominant model	CC vs. CT + TT	0.48	(0.23-0.99)	0.04
Recessive model	TT vs. CC + CT	0.83	(0.32-2.18)	0.71
CYP27B1 rs4646537	TT vs. GT	1.80	(0.03-121.71)	0.78
	TT vs. GG	5.12	(0.27-97.18)	0.28
	GT vs. GG	2.84	(0.13-60.49)	0.50
Dominant model	GG vs. GT + TT	0.13	(0.00-2.45)	0.17
Recessive model	TT vs. GG + GT	5.00	(0.26-94.89)	0.28
CYP27B1 rs4646536	GG vs. GA	0.95	(0.44-2.04)	0.89
	GG vs. AA	1.42	(0.30-6.79)	0.66
	GA vs. AA	1.50	(0.30-7.58)	0.62
Dominant model	GG vs. GA + AA	1.00	(0.48-2.08)	0.99
Recessive model	AA vs. GG + GA	0.69	(0.15-3.22)	0.64
DHCR7 rs3829251	AA vs. AG	0.31	(0.05-1.80)	0.19
	AA vs. GG	0.64	(0.12-3.36)	0.60
	AG vs. GG	2.05	(0.89-4.71)	0.08
Dominant model	GG vs. AG + AA	0.58	(0.27-1.25)	0.16
Recessive model	AA vs. GG + AG	0.52	(0.10-2.67)	0.43
DHCR7 rs12785878	GG vs. GT	0.57	(0.26-1.24)	0.16
	GG vs. TT	1.20	(0.39-3.65)	0.75
	GT vs. TT	2.09	(0.67-6.55)	0.20
Dominant model	GG vs. GT + TT	0.68	(0.33-1.39)	0.29
Recessive model	TT vs. GG + GT	0.64	(0.22-1.85)	0.41

The adjusted odds ratios and 95% confidence intervals (CIs) are provided, separately for each SNP. *P value ≤ 0.05 is considered statistically significant and **P value ≤ 0.01 is considered statistically significant. Abbreviations: VD, vitamin D; SNP, single nucleotide polymorphism

For the rs 4588 SNP in the GC gene, genotypic for frequencies were determined as 69.7% for the TT, 23.6% for the heterozygous TG status, and 6.7% for the GG, in the VDD group. As compared to NVD group (76.6% for TT) the homozygous TT genotype was seen in lower percentage in VDD group (69.7%). Data showed that TT vs. TG and TT vs. TG + GG genotypes did not exhibited significant difference between the two groups ($p = 0.65$ and $p = 0.39$, respectively), and the TT genotype was not allied with the disease [OR, 0.70 (0.31–1.58)]. The OR was calculated for the TT homozygote (OR, 0.82 [0.35–1.93]; $p = 0.65$), TG heterozygote [OR, 0.35 (0.04–3.31); $p = 0.36$], and GG homozygote [OR, 0.29 (0.03–2.48); $p = 0.26$] in each case.

For the rs 7041 SNP in the GC gene, genotypic for frequencies were determined as 22.5% for the CC, 40.4% for the heterozygous CA status, and 37.1% for the AA, in the VDD group. As compared to NVD group (48.9% and 48.9% for AA and CA respectively) the homozygous AA and heterozygous CA genotypes were seen in a lower percentage in VDD group (37.1% and 40.4% for AA and CA respectively respectively). Results showed that the genotype percentage for AA and CA genotypes among the NVD group were almost same, but there was a border line difference among subjects in VDD group. A significant difference has been observed in GC-rs7041 between the two groups i.e. NVD and VDD ($p<0.05$). Data showed that AA vs. CC ($p = 0.01$), CC vs. CA + AA ($p = 0.01$) and CC vs. CA ($p = 0.01$) genotypes exhibited a significant difference between the two group, and the CC genotype was allied with the disease [OR, 13.33 (1.73–102.82); $p = 0.01$].

For the rs 927650 SNP in the CYP24A1 gene, genotypic for frequencies were determined as 31.5% for the CC, 53.9% for the heterozygous CT status, and 14.6% for the TT in the VDD group. As compared to NVD group (48.9% and 17% for CC and TT respectively), the homozygous CC and TT were seen in a lower percentage in VDD group (31.5% and 14.6% for CC and TT respectively). Data showed that CC vs. CT and CC vs. CT + TT genotypes exhibited a substantial difference between the two group ($p = 0.02$ and $p = 0.04$, respectively), and the CC genotype was related with the disease [OR, 0.48 (0.23–0.99), $p = 0.04$].

For the rs 4646537 SNP in the CYP27B1 gene, genotypic for frequencies were determined as 93.3% for the GG, 2.25% for the heterozygous GT status, and 4.5% for the TT, in VDD group. The homozygous GG genotype was seen in a lower percentage in VDD group (93.3%) when compared to NVD group (100%). It has been observed that the genotype percentage for TT and GT genotypes among the NVD patients were not present in any subjects, but there was a border line difference among subjects in VDD group. Data showed that GT vs. GG and GG vs. GT + TT genotypes exhibited insignificant differences between the NVD and VDD ($p = 0.50$ and $p = 0.17$, respectively), and the GG genotype was not allied with the disease [OR, 0.13 (0.00–2.45); $p = 0.17$]. The OR was calculated for the GG homozygote [OR, 2.84 (0.13–60.49); $p = 0.50$] and that for GT heterozygote [OR, 1.80 (0.03–121.71); $p = 0.78$] and TT homozygote [OR, 5.12 (0.27–97.18); $p = 0.28$] in each case.

For the rs 4646536 SNP in the CYP27B1 gene, genotypic for frequencies were determined as 61.8% for the GG, 33.7% for the heterozygous GA status, and 4.5% for the AA in the vitamin D deficient group. As compared to normal vitamin D group (6.4% for AA), the homozygous AA genotype was found in a lower percentage in vitamin D deficient group (4.5% for AA). Data showed that GG vs. GA and GG vs. GA + AA genotypes did not exhibit a significant difference between the two group ($p = 0.89$ and $p = 0.99$, respectively), and the GG genotype was not associated with the disease [OR, 1.00 (0.48–2.08); $p = 0.09$]. The OR was calculated for the GG homozygote [OR, 0.95 (0.44–2.04); $p = 0.89$], GA heterozygote [OR, 1.50 (0.30–7.58); $P = 0.62$] and AA homozygote [OR, 1.42 (0.30–6.79); $p = 0.66$] in each case.

For the rs 3829251 SNP in the DHCR7 gene, genotypic for frequencies were determined as 60.2% for the GG, 36.4% for the heterozygous AG status, and 3.4% for the AA in the VDD group. As compared to normal vitamin D group (72.3% for GG and 6.4%, for AA) the homozygous GG and AA genotype were seen in a lower percentage in VDD group (60.2% for GG and 3.4% for AA). Data showed that AG vs. GG and GG vs. AG + AA genotypes did not exhibited any significant difference between the groups ($p = 0.08$ and $p = 0.16$, respectively), and the GG genotype was

not related with the disease [OR, 0.58 (0.27–1.25); p = 0.16]. The OR was calculated for GG homozygote [OR, 2.05 (0.89–4.71); p = 0.08], AG heterozygote [OR, 0.31 (0.05–1.80); p = 0.19], and AA homozygote [OR, 0.64 (0.12–3.36); p = 0.60].

For the rs 12785878 SNP in the DHCR7 gene, genotypic for frequencies were determined as 41.6% for the GG, 48.3% for the heterozygous GT status, and 10.1% for the TT in the VDD group. The homozygous GG and TT were seen in lower percentages in VDD group (41.6% and 10.1%, respectively) when compared to NVD group (51.1% and 14.9%, respectively). Data showed that GG vs. GT and GG vs. GT + TT genotypes did not exhibited significant difference between the two group (p = 0.16 and p = 0.29, respectively), and the GG genotype was not allied with the disease [OR, 0.68 (0.33–1.39); p = 0.29]. The OR was calculated for GG homozygote [OR, 0.57 (0.26–1.24); P = 0.16], GT heterozygote [OR, 2.09 (0.67–6.55); p = 0.20], and TT homozygote [OR, 1.20 (0.39–3.65); p = 0.75].

Analysis of variance between genotypes and some parameters of VDD subjects

There were significant associations with two SNPs and the distribution of genotypes or risk of vitamin D deficiency in obese patients using a recessive or co-dominant model in the entire group. Some parameters (weight, BMI, waist circumference, total cholesterol, triglycerides and vitamin D level) were compared among the subjects of VDD group with obesity carriers of the different genotypes of the 10 SNPs. For these traits, insignificant differences were observed for the genotypes of any of the SNPs when subjects were analyzed as one single group (Table 5).

Homozygous subjects for the AA-genotype of the Apa1 rs7975232 SNP had a significantly lower weight (83.42 ± 11.83) than heterozygous subjects (92.24 ± 12.22; p = 0.008). Homozygous subjects for the AA-genotype had a significantly lower BMI (33.33 ± 4.54) than heterozygous subjects (36.09 ± 4.45; p = 0.040) and homozygous subjects for the AA-genotype had a significantly lower waist circumference (90.00 ± 8.63) than heterozygous subjects (96.87 ± 8.50; p = 0.006). Homozygous subjects for the CC-genotype of the GC rs7041 SNP had a significantly lower vitamin D (31.61 ± 14.21) than homozygous subjects for the AA-genotype (49.30 ± 18.22; p = 0.004). Homozygous subjects for the TT-genotype of the CYP24A1 rs927650 SNP had a significantly lower waist

Table 5. Analysis of variance between genotypes and some parameters of vitamin D deficiency VD in obesity patients

SNP Genotype	Weight	BMI	Waist	TC	TG	VD
BsmI rs1544410						
TT	88.16±12.18	34.70 ± 4.69	94.08 ± 8.95	189.65 ± 32.76	103.77 ± 70.73	41.05 ± 18.04
CC	82.51 ± 10.35	32.84 ± 3.88	88.37 ± 9.16	178.81 ± 32.32	89.75 ± 62.92	46.72 ± 19.52
TC	90.00 ± 13.70	35.37 ± 5.08	94.94 ± 9.64	191.17 ± 32.98	107.23 ± 53.78	41.02 ± 17.76
P	0.133	0.194	0.055	0.423	0.610	0.571
TaqI rs 731236						
GG	87.53 ± 12.50	34.28 ± 4.61	93.83 ± 8.86	192.88 ± 31.73	107.20 ± 71.90	42.83 ± 17.05
AA	82.53 ± 10.69	33.43 ± 3.75	88.43 ± 9.30	184.43 ± 36.29	94.50 ± 65.42	44.03 ± 19.74
GA	89.97 ± 13.38	35.30 ± 5.16	94.76 ± 9.70	187.46 ± 32.68	103.42 ± 53.45	41.01 ± 18.43
P	0.157	0.387	0.089	0.704	0.822	0.848
Apa1 rs7975232						
AA	83.42 ± 11.83[a]	33.33 ± 4.54[a]	90.00 ± 8.63[a]	183.18 ± 31.67	103.92 ± 56.79	42.27 ± 18.51
CC	90.29 ± 14.23[ab]	34.87 ± 5.85[ab]	94.27 ± 12.4[ab]	189.58 ± 33.11	79.00 ± 25.79	39.13 ± 16.66
AC	92.24 ± 12.22[b]	36.09 ± 4.45[b]	96.87 ± 8.50[b]	193.63 ± 33.73	109.81 ± 70.25	42.77 ± 18.48
P	**0.008**	**0.040**	**0.006**	0.377	0.307	0.844

GC rs4588						
TT	87.62 ± 13.06	34.37 ± 4.67	92.88 ± 9.31	190.11 ± 35.22	106.53 ± 67.63	41.70 ± 17.53
GG	89.05 ± 17.04	35.75 ± 7.31	95.33 ± 11.98	186.50 ± 16.88	124.67 ± 53.69	45.55 ± 22.50
TG	89.31 ± 11.66	35.44 ± 4.56	94.76 ± 9.96	184.33 ± 28.94	86.71 ± 30.53	42.14 ± 19.45
P	0.862	0.592	0.664	0.778	0.288	0.903
GC rs7041						
CC	90.92 ± 13.17	36.56 ± 5.65	96.45 ± 9.02	182.65 ± 29.35	98.30 ± 45.90	31.61 ± 14.21[a]
AA	89.33 ± 12.40	34.70 ± 4.19	93.66 ± 8.90	183.70 ± ± 35.67	95.27 ± 48.99	49.30 ± 18.22[b]
CA	85.44 ± 13.03	33.71 ± 4.70	91.72 ± 10.27	196.17 ± 31.05	112.89 ± 75.29	41.03 ± 17.2[ab]
P	0.250	0.104	0.209	0.191	0.449	**0.004**
CYP24A1 rs927650						
CC	89.37 ± 14.36	35.03 ± 5.45	92.96 ± 8.86[ab]	181.32 ± 37.03	84.18 ± 34.74	43.33 ± 18.18
TT	81.48 ± 13.77	32.53 ± 4.48	87.85 ± 9.31[a]	191.08 ± 32052	108.92 ± 51.06	38.67 ± 18.70
CT	89.18 ± 11.40	35.13 ± 4.44	95.38 ± 9.59[b]	192.00 ± 30.08	112.52 ± 71.84	42.17 ± 18.16
P	0.133	0.209	**0.038**	0.377	0.133	0.778
CYP27B1 rs4646537						
TT	82.27 ± 10.82	33.52 ± 3.62	91.50 ± 7.55	189.00 ± 15.81	98.25 ± 45.05	42.22 ± 16.84
GG	88.29 ± 12.89	34.76 ± 4.83	93.65 ± 9.63	189.40 ± 33.14	104.00 ± 61.79	42.04 ± 18.21
GT	92.60 ± 21.78	35.25 ± 8.84	91.50 ± 16.26	150.50 ± 24.75	74.50 ± 13.43	42.15 ± 26.66
P	0.589	0.873	0.872	0.255	0.786	1.000
CYP27B1 rs4646536						
GG	88.58 ± 12.68	34.83 ± 4.72	93.38 ± 9.16	187.04 ± 29.01[b]	99.33 ± 48.32[b]	41.88 ± 17.37
AA	84.20 ± 12.48	33.50 ± 2.95	92.25 ± 13.89	241.75 ± 42.66[a]	200.50 ± 144.7[a]	27.90 ± 5.67
GA	87.78 ± 13.64	34.67 ± 5.27	93.90 ± 10.11	184.10 ± 32.91[b]	96.97 ± 56.45[b]	44.55 ± 19.87
P	0.797	0.867	0.940	**0.003**	**0.003**	0.230
DHCR7 rs3829251						
AA	98.07 ± 18.13	39.17 ± 9.00	101.00 ± 15.87	186.00 ± 8.54	105.67 ± 24.68	52.97 ± 19.66
GG	88.98 ± 12.51	34.55 ± 4.35	94.46 ± 7.87	186.90 ± 32.63	104.57 ± 72.32	41.77 ± 17.16
AG	85.76 ± 13.10	34.45 ± 5.13	90.84 ± 10.95	191.91 ± 35.05	100.62 ± 39.19	40.69 ± 19.85
P	0.219	0.261	0.088	0.790	0.957	0.544
DHCR7 rs12785878						
GG	87.51 ± 12.94	35.03 ± 5.11	93.73 ± 10.99	191.00 ± 30.97	107.03 ± 63.59	37.66 ± 15.64[b]
TT	86.77 ± 11.81	33.35 ± 4.40	92.56 ± 7.41	175.33 ± 29.61	90.22 ± ± 58.59	58.89 ± 14.57[a]
GT	88.92 ± 13.29	34.73 ± 4.69	93.50 ± 8.81	189.12 ± 34.86	102.37 ± 58.97	41.98 ± 18.73[b]
P	0.844	0.650	0.948	0.435	0.756	**0.011**

Data are presented in (mean ± standard deviation). ANOVA P values are followed by Tukey-Kramer HSD test results presented in bold when appropriate. P value ≤ 0.05 was considered statistically significant. Abbreviations: VD, vitamin D; BMI, body mass index; TC, total cholesterol; TG, triglyceride.

(87.85 ± 9.31) than heterozygous subjects (95.38 ± 9.59; p = 0.038). Homozygous subjects for the AA-genotype of the CYP27B1 rs4646536 SNP had a significantly higher total cholesterol (241.75 ± 42.66) than heterozygous subjects (184.10 ± 32.91) or homozygous subjects for the GG-genotype (187.04 ± 29.01; p = 0.003). Homozygous subjects for the AA-genotype had a significantly higher triglyceride (200.50 ± 144.7) than heterozygous subjects (96.97 ± 56.45) or homozygous subjects for the GG-genotype (99.33 ± 48.32; p = 0.003). Homozygous subjects for the TT-genotype of the DHCR7 rs12785878 SNP had a significantly higher vitamin D (58.89 ± 14.57) than heterozygous subjects (41.98 ± 18.73) or homozygous subjects for the GG-genotype (37.66 ± 15.64; p = 0.011).

Discussion

Hypovitaminosis D is a worldwide issue, and irrespective of ethnicity and age group approximately thousand million people are at risk and have been found to be linked with higher mortality [24-26]. Even though Saudi Arabia receives a large amount of sunlight all over the year, but numerous studies have revealed the incidence of vitamin D (VD) deficiency, particularly among Saudi adults [27-29]. Various reasons such as insufficient VD dietary consumption, style of clothing, decreased solar exposure, socioeconomic status, prolonged use of anticonvulsants and corticoids, and residence has been established to be threat for hypovitaminosis D [30]. In this study comparison has been made between obese normal and obese vitamin D deficient women. In the Kingdom of Saudi Arabia obesity is increasing rapidly and WHO has reported that around 33% of total population are obese and it is more prevalent in females (39.1%) than in males (28.65%) [31]. An elucidation for obesity related vitamin D inadequacy or insufficiency might be the reduced bioavailability of $25(OH)_2D_3$. The body fat may act as a reservoir for storage of the fat-soluble vitamin D, reducing its bioavailability [32]. Serum TC and TG was significantly lower in the VDD group which is in disagreement with various studies reported in adults [33-34]. The relationship between vitamin D status and serum lipids may differ by sex and age [35]. In this study statistically insignificant differences were observed in glucose level between the two groups. Studies have stated that vitamin D exerts a protective effect against insulin such as conversion of vitamin D (by 1-alpha-hydroxylase enzyme) into its active form in β-cells [36], the presence of vitamin D receptors (VDRs) in β-cells and vitamin D-dependent calcium binding proteins in the pancreas [37]. Vitamin D plays a vital role in serum calcium homeostasis. Low calcitriol leads to reduced efficiency in intestinal calcium absorption, and the body reacts by amplifying the secretion of parathyroid hormone (PTH) [38]. As compared to the NVD group the level of PTH was significantly higher in VDD group. In various previous studies also negative correlation has been observed between PTH and $25(OH)_2D_3$ levels [39-41]. The significant negative correlation between vitamin D levels and BMI ($r = -0.270$, $p<0.01$) confirm previous findings [10-12, 42] but in disagreement with others [43, 44]. These inconsistencies highlight other confounding factors that significantly influence vitamin D levels such as interactions with environmental factors, exposure to sunlight, and vitamin supplement intake. Unlike BMI; waist circumference is relatively better indicator of visceral fat accumulation as BMI doesn't provides any information about body fat distribution [45]. A negative correlation between waist circumference and vitamin D and positive and highly significant correlation between waist circumference and BMI has been reported in this study which is in parallel to various prospective and cross-sectional studies [29, 46].

This study showed the association of vitamin D levels with the SNPs of genes related to obesity with vitamin D (TaqI, BsmI, Apa1, DHCR7, GC, CYP27B1, and CYP24A1) and other risk factors in Saudi subjects with obesity. The genomic action initiates with the interaction of 1α, $25\text{-}(OH)_2D_3$ with VDRs, followed by subsequent interaction of VDR with other transcription factors like transcription integrators such as calcium-binding proteins and co activator proteins [47]. This genomic pathway leads to changes in gene transcription [48]. Our results indicated that VDR [BsmI (rs1544410), Taql (rs731236), and ApaI (rs7975232)] SNPs were not correlated with vitamin D deficiency. The outcomes of this study are in line with other studies [49-51] but in disagreement with the finding of Santos

et al., [52] and this could be perhaps due to the difference in ethnicity between the groups. Vitamin D binding protein (DBP; also known as group-specific component, GC) transport vitamin D to the liver and it has high homology to albumin, and vitamin D metabolites are mostly transported by DBP (85–88%), and in part by albumin (12–15%) [53-54].

GC rs7041 SNP was significantly different between VDD and NVD, but similar finding was not observed in GC rs4588 SNP. The results regarding GC rs7041 are consistent with previous studies [50, 51]. However, the results are in disagreement with the various other studies [55, 56]. Similarly, the results regarding GC rs4588 are in agreement with Robien et al., [57], while in disagreement with those of Signorello et al (2011) and Xu et al., [50,58]. The significant association between vitamin D levels and the allele frequency of GC SNPs may only exist in some specific ethnic population [59, 60]. For example, GC rs7041 polymorphism was associated with $25(OH)_2D_3$ levels in Arab, and South Asian populations, but not in South East Asians [61]. These disagreements could be due to differences in ethnicity, and interactions with environmental factors.

The CYP27B1 gene is located on chromosome 12q13.1-13.3 spanning 6.66 kb on the reverse strand and it catalyzes the synthesis of 1, $25(OH)_2D_3$ (calcitriol) from $25(OH)_2D_3$ in the kidney [62]. In chronic kidney disease the CYP27B1 enzyme activity decreases which is in turn leads to low level of serum 1, 25(OH)2D. Parathyroid hormone (PTH) up regulates CYP27B1 (as part of calcium homeostasis) and fibroblast growth factor (FGF23) down-regulates CYP27B1 (as part of phosphate homeostasis) and any mutation in the CYP27B1 gene is responsible for vitamin D-dependent rickets in humans, and mice [63]. As compared to NVD group; the CYP27B1 (rs4646537, and rs4646536) SNPs were observed more frequently in VDD group; however, this difference was not significant. These results are in agreement with those of the various previous studies [50, 56, 57, 58]. Among several target genes, the 1α, 25-$(OH)_2D_3$ hormone induces in target cells the expression of the gene encoding the key effector of its catabolic breakdown, 25-hydroxyvitamin D-24-hydroxylase (CYP24A1) [64]. This guarantees weakening of the 1α, 25-$(OH)_2D_3$ biological signal inside target cells, and helps regulate vitamin D homeostasis [62]. This study showed that unlike NVD group; CYP24A1 rs 927650 SNP was significantly associated with VDD group. Our results are consistent with those of Nissen et al., [56] while in disagreement with those of Signorello et al., and Robien et al., [50, 57]. The studies reporting significant findings for CYP24A1 variants tended to be among smaller study populations, and many failed to adjust for factors known to alter serum $25(OH)_2D_3$ concentrations such as season of blood collection, ethnic differences, and supplemental/ dietary consumption of vitamin D.

Vitamin D synthesis initiates with the oxidation of cholesterol to 7-dehydrocholesterol (7-DHC) which is then transported to the skin and stowed in the cell membranes of keratinocytes and fibroblasts in the epidermis of skin [64]. Results showed that DHCR7 (rs 3829251, and rs 12785878) SNPs were observed mostly in VDD group; however, this difference was not significant. This result is in agreement with that of Nissen et al., [56]. The combined effect of these polymorphisms (VDR, CYP27B1, and DHCR7), using the most common genotype as the reference, did not show any significant relationship with vitamin D deficient risk which might be due to the small number of females studied, the recruitment of a single-sex, and with environmental factors.

Conclusion

This study suggests that polymorphisms in the GC and CYP24A1 genes are related with vitamin D deficiency in obese females. These polymorphisms may become a useful marker to predict the impending development of vitamin D deficiency in obese females. Studies have revealed that associations between genotypes of these SNPs and particular phenotypes may vary according to the following: ethnicity, interactions with environmental factors, sex, and number of participants. Future studies are required in larger populations with both sex, and anthropometric-based data will be required to elucidate the interactions of the gene variants (TaqI, BsmI, ApaI, DHCR7, GC, CYP27B1, and CYP24A1) with these traits in other diseases. In addition, it should concentrate on the interactions of these genes in cells.

Acknowledgement

Authors would like to thank all participants in this study. This research project was supported by "Research Center of the Female Scientific and Medical Colleges", Deanship of Scientific Research, King Saud University.

References

1. WHO. World Health Organization. Obesity epidemic puts millions at risk from related diseases. Press release 1997; 46:12.
2. Must A, Dallal GE, Dietz WH. Reference data for obesity: 85th and 95th percentiles of body mass index (wt/ht2) and triceps skinfold thickness. Am J Clin Nutr 1991; 53: 839–846.
3. Finkelstein EA, Fiebelkorn IC, Wang G. National medical spending attributable to overweight and obesity: how much, and who's paying? Health Aff (Millwood) 2003; 22 Suppl W3: 219–226.
4. World Health Organization: Health 2020: A European Policy Framework Supporting Action Across Government and Society for Health and Well-Being. Malta, WHO, 2012.
5. NCD Risk Factor Collaboration: Trends in adult body-mass index in 200 countries from 1975 to 2014: A pooled analysis of 1698 population-based measurement studies with 19.2 million participants. Lancet 2016; 387: 1377–1396.
6. Chooi YC, Ding C, Magkos F. The epidemiology of obesity. Metabolism 2019; 92:6–10.
7. Kelly T, Yang W, Chen CS, Reynolds K, He J. Global burden of obesity in 2005 and projections to 2030. Int J Obes 2008; 32:1431–1437.
8. Chittawatanarat K, Pruenglampoo S, Kongsawasdi S, Chuatrakoon B, Trakulhoon V, Ungpinitpong W, Patumanond J. The variations of body mass index and body fat in adult Thai people across the age spectrum measured by bioelectrical impedance analysis. Clin Interv Aging 2011; 6: 285–294.
9. EMRO. Regional Health Systems Observatory. EMRO World Health Organization, 2006.
10. Young KA, Engelman CD, Langefeld CD, Hairston KG, Haffner SM, Bryer-Ash M, Norris JM. Association of plasma vitamin D levels with adiposity in Hispanic and African Americans. J Clin Endocrinol Metab 2009; 94: 3306–3313.
11. Konradsen S, Ag H, Lindberg F, Hexeberg S, Jorde R. Serum 1, 25-dihydroxy vitamin D is inversely associated with body mass index. Euro J Nutr 2008; 47: 87–91.
12. Arunabh S, Pollack S, Yeh J, Aloia, JF. Body fat content and 25-hydroxyvitamin D levels in healthy women. J Clin Endocrinol Metab 2003; 88: 157–161.
13. Jones G, Strugnell SA, DeLuca HF. Current understanding of the molecular actions of vitamin D. Physiol Rev 1998; 78: 1193–1231.
14. Wong KE, Szeto FL, Zhang W, Ye H, Kong J, Zhang Z, Sun XJ, Li YC. Involvement of the vitamin D receptor in energy metabolism: regulation of uncoupling proteins. Am J Physiol Endocrinol Metab 2009; 296: E820–E828.
15. Lin RC, Wang XL, Dalziel B, Caterson ID, Morris BJ. Association of obesity, but not diabetes or hypertension, with glucocorticoid receptor N 363 S variant. Obes Res 2003; 11: 802–808.
16. Tamura K, Suzuki M, Arakawa H, Tokuyama K, Morikawa, A. Linkage and association studies of STAT6 gene polymorphisms and allergic diseases. Int Arch Allergy and Immunol 2003; 131: 33–38.
17. Yamada Y, Ando F, Niino N, Shimokata H. Association of polymorphisms of interleukin-6, osteocalcin, and vitamin D receptor genes, alone or in combination, with bone mineral density in community-dwelling Japanese women and men. J Clin Endocrinol Metab 2003; 88: 3372–3378.
18. Holvey DN. The Merck manual of diagnosis and therapy. Merck, Sharp & Dohme Research Laboratories; 12th Edition / 1st Printing edition. 1972; pp: 1960.
19. Tietz NW. Fundamentals of clinical chemistry, Philadelphia, WB Saunders, Second edition. 1970; pp:496
20. Fossati P, Principle L. Estimation of the concentration of triglyceride in plasma and liver. Clin Chem 1982; 28: 2077–2081.
21. Friedwald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972; 18:499–502.
22. Phinney KW. Development of a standard reference material for vitamin D in serum. Am J Clin Nutr 2008; 88: 511S-512S.
23. Ohe MN, Santos RO, Kunii IS, Abrahão M, Cervantes O, Carvalho AB, Vieira JGH. Usefulness of intraoperative PTH measurement in primary and secondary hyperparathyroidism: experience with 109 patients. Arq Bras Endocrinol Metabol 2006; 50: 869–875.
24. Holick MF, Chen TC. Vitamin D deficiency: a worldwide problem with health consequences. Am J Clin Nutr 2008; 87: 1080S-1086S.
25. Christie FT, Mason L. Knowledge, attitude and practice regarding vitamin D deficiency among female students in Saudi Arabia: a qualitative exploration. Int J Rheum Dis 2011; 14: 22–29.
26. Pilz S, Grübler M, Gaksch M, Schwetz V, Trummer C, Hartaigh BÓ, Verheyen N, Tomaschitz A, März W. Vitamin D and Mortality. Anticancer Res 2016: 36:1379–1387.
27. Al-Turki HA, Sadat-Ali M, Al-Elq AH, Al-Mulhim FA, Al-Ali AK. 25-Hydoxyvitamin D levels among healthy Saudi Arabian women. Saudi Med J 2008; 29: 1765–1768.
28. Ardawi M-S, Qari M, Rouzi A, Maimani A, Raddadi R. Vitamin D status in relation to obesity, bone mineral density, bone turnover markers and vitamin D receptor genotypes in healthy Saudi pre-and postmenopausal women. Osteoporos Int 2011; 22: 463–475.
29. Ardawi M-S, Sibiany A, Bakhsh T, Qari M, Maimani A. High prevalence of vitamin D deficiency among healthy

Saudi Arabian men: relationship to bone mineral density, parathyroid hormone, bone turnover markers, and lifestyle factors. Osteoporos Int 2012; 23: 675–686.
30. Alzaheb RA, Al-Amer O. Prevalence and predictors of hypovitaminosis D among female university students in Tabuk, Saudi Arabia. Clin Med Insights Women's Health 2017; 10: 1179562X17702391.
31. World Health Organization. Noncommunicable diseases country profiles 2011 WHO global report, 2011.
32. Wortsman J, Matsuoka LY, Chen TC, Lu Z, Holick MF. Decreased bioavailability of vitamin D in obesity. *Am J Clin Nutr* 2000; 72:690–693.
33. Ramiro-Lozano JM, Calvo-Romero JM. Effects on lipid profile of supplementation with vitamin D in type 2 diabetic patients with vitamin D deficiency. Ther Adv Endocrinol Metab 2015; 6: 245–248.
34. Botella-Carretero JI, Alvarez-Blasco F, Villafruela JJ, Balsa JA, Vázquez C, Escobar-Morreale HF. Vitamin D deficiency is associated with the metabolic syndrome in morbid obesity. Clin Nutr 2007; 26: 573–580.
35. Steger FL. Associations between vitamin D status and blood lipid parameters in healthy, older adults. Iowa State University Capstones, Graduate Theses and Dissertations. 2013; 1–85.
36. Ishida H, Norman AW. Demonstration of a high affinity receptor for 1, 25-dihydroxyvitamin D3 in rat pancreas. Mol Cell Endocrinol 1988; 60:109–117.
37. Bland R, Markovic D, Hills CE, Hughes SV, Chan SL, Squires PE, Hewison M. Expression of 25-hydroxyvitamin D3-1alpha-hydroxylase in pancreatic islets. J Steroid Biochem Mol Biol 2004; 89–90:121–125.
38. Heaney RP. Toward a physiological referent for the vitamin D requirement. J Endocrinol Invest 2014; 37: 1127–1130.
39. Steingrimsdottir L, Gunnarsson O, Indridason OS, Franzson L, Sigurdsson G. Relationship between serum parathyroid hormone levels, vitamin D sufficiency, and calcium intake. JAMA 2005; 294: 2336–2341.
40. Adami S, Viapiana O, Gatti D, Idolazzi L, Rossini M. Relationship between serum parathyroid hormone, vitamin D sufficiency, age, and calcium intake. Bone 2008; 42: 267–270.
41. Oelzner P, Müller A, Deschner F, Hüller M, Abendroth K, Hein G, Stein G. Relationship between disease activity and serum levels of vitamin D metabolites and PTH in rheumatoid arthritis. Calcif Tissue Int 1998; 62: 193–198.
42. Jorde R, Sneve M, Emaus N, Figenschau Y and Grimnes G. Cross-sectional and longitudinal relation between serum 25-hydroxyvitamin D and body mass index: the Tromsø study. Eur J Nutr 2010; 49: 401–407.
43. Al-Elq AH, Sadat-Ali M, Al-Turki HA, Al-Mulhim FA, Al-Ali AK. Is there a relationship between body mass index and serum vitamin D levels? Saudi Med J 2009; 30: 1542–1546.
44. Stein EM, Laing EM, Hall DB, Hausman DB, Kimlin MG, Johnson MA, et al. Serum 25-hydroxyvitamin D concentrations in girls aged 4–8 y living in the southeastern United States. Am J Clin Nutr 2006; 83: 75–81.
45. Rankinen T, Kim SY, Pérusse L, Després JP, Bouchard C. The prediction of abdominal visceral fat level from body composition and anthropometry: ROC analysis. Int J Obes Relat Metab Disord 1999; 23:801–809.
46. Fung GJ, Steffen LM, Zhou X, Harnack L, Tang W, Lutsey PL et al. Vitamin D intake is inversely related to risk of developing metabolic syndrome in African American and white men and women over 20 y: the coronary artery risk development in young adults study. Am J Clin Nutr 2012; 96: 24–29.
47. Jenster G, Spencer TE, Burcin MM, Tsai SY, Tsai MJ, and O'Malley BW. Steroid receptor induction of gene transcription: a two-step model. Proc Natl Acad Sci USA 1997; 94: 7879–84.
48. Bouillon R, Carmeliet G, Verlinden L, van Etten E, Verstuyf A, Luderer HF et al. Vitamin D and human health: lessons from vitamin D receptor null mice. Endocr Rev 2008; 29: 726–76.
49. Ferreira F, Duarte JA. Accuracy of Body Mass Index, Waist Circumference and Body Surface Index to Diagnose Overweight and Obesity in Adolescents. Arch Exercise Health Dis 2013; 4: 299–306.
50. Signorello LB, Shi J, Cai Q, Zheng W, Williams SM, Long J, Cohen SS, Li G, Hollis BW, Smith JR, Blot WJ. Common variation in vitamin D pathway genes predicts circulating 25-hydroxyvitamin D levels among African Americans. PloS One 2011; 6: e28623.
51. Nissen J, Rasmussen LB, Ravn-Haren G, Andersen EW, Hansen B, Andersen R, Mejborn H, Madsen KH, Vogel U. Common variants in CYP2R1 and GC genes predict vitamin D concentrations in healthy Danish children and adults. PloS One 2014; 9: e89907
52. Santos BR, Mascarenhas LP, Satler F, Boguszewski MC and Spritzer PM. Vitamin D deficiency in girls from South Brazil: a cross-sectional study on prevalence and association with vitamin D receptor gene variants. BMC Pediatrics 2012; 12: 62.
53. Bikle DD. Vitamin D: role in skin and hair. In: Feldman D., Pike J.W., Glorieux F.H., editors. Vitamin D. Elsevier Academic Press; Boston, MA: 2005; pp. 609–630.
54. Cooke NE, Haddad JG. Vitamin D binding protein (Gcglobulin). Endocr Rev 1989; 10: 294–307.
55. Nimitphong H, Saetung S, Chanprasertyotin S, Chailurkit LO, Ongphiphadhanakul B. Changes in circulating 25-hydroxyvitamin D according to vitamin D binding protein genotypes after vitamin D_3 or D_2 supplementation. Nutr J 2013; 12: 39.
56. Nissen J, Vogel U, Ravn-Haren G, Andersen EW, Madsen KH, Nexø BA, and Rasmussen LB. Common variants in CYP2R1 and GC genes are both determinants of serum 25-hydroxyvitamin D concentrations after UVB irradiation and after consumption of vitamin D3–fortified bread and milk during winter in Denmark. Am J Clin Nutr 2015; 101: 218–227.
57. Robien K, Butler LM, Wang R, Beckman KB, Walek D,

Koh WP, Yuan JM. Genetic and environmental predictors of serum 25-hydroxyvitamin D concentrations among middle-aged and elderly Chinese in Singapore. Br J Nutr 2013; 109: 493–502.
58. Xu X, Mao J, Zhang M, Liu H, Li H, Lei H and Gao M. Vitamin D deficiency in Uygurs and Kazaks is associated with polymorphisms in CYP2R1 and DHCR7/NADSYN1 genes. Med Sci Monit 2015; 21:1960–1968.
59. Batai K, Murphy AB, Shah E, Ruden M, Newsome J, Agate S, Dixon MA, Chen HY, Deane LA, Hollowell CM, Ahaghotu C, Kittles RA. Common vitamin D pathway gene variants reveal contrasting effects on serum vitamin D levels in African Americans and European Americans. Hum Genet 2014; 133: 1395–1405.
60. Zhang MC, Li HX, Liu HM, Lei H, Han L, Gao M, Mao JF, Xu XJ. Serum vitamin D is low and inversely associated with LDL cholesterol in the Kazak ethnic population: a cross-sectional study. Med Sci Monit 2014; 20: 1274–1283.
61. Elkum N, Alkayal F, Noronha F, Ali MM, Melhem M, Al-Arouj, Bennakhi A, Alsmadi O, Abubaker J. Vitamin D insufficiency in Arabs and South Asians positively associates with polymorphisms in GC and CYP2R1 genes. PLoS One 2014; 9: e113102.
62. Basit, S. Vitamin D in health and disease: a literature review. Br J Biomed Sci 2013; 70: 161–172.
63. Panda DK, Miao D, Tremblay ML, Sirois J, Farookhi R, Hendy GN, et al. Targeted ablation of the 25-hydroxyvitamin D 1alpha-hydroxylase enzyme: evidence for skeletal, reproductive, and immune dysfunction. Proc Natl Acad Sci USA 2001; 98: 7498–7503.
64. Omdahl JL, Bobrovnikova EA, Choe S, Dwivedi PP, and May BK. Overview of regulatory cytochrome P450 enzymes of the vitamin D pathway. Steroids 2001; 66: 381–389.

Correspondence:
Manal Abdulaziz Binobead
College of Food Science & Agriculture,
Department of Food Science & Nutrition,
King Saud University
Riyadh, Saudi Arabia.
Email: mbinobead@ksu.edu.sa