

## Association of VDR gene polymorphism with insulin resistance in diabetic patients

Arash Hossein-nezhad<sup>1\*</sup>, Khadijeh Mirzaei,<sup>1</sup> Parisa Shabani,<sup>1</sup> Azam Najmafshar,<sup>1</sup> Solaleh Emamgholipour,<sup>1</sup> Mazaher Rahmani,<sup>1</sup> Bagher Larijani<sup>1</sup>

*1- Endocrinology and Metabolism Research Center, Tehran University of Medical Sciences, Tehran, Iran*

### Abstract

**Introduction:** It has been reported that vitamin D deficiency may predispose individuals to glucose intolerance and type 2 diabetes mellitus. Since FokI variant of Vitamin D Receptor (VDR) associates with alteration of receptor function which affects the bioactivity of vitamin D, we aimed to evaluate the association of FokI variant of VDR with various glucose metabolism indices among patients with diabetes mellitus type 2 (T2DM).

**Methods:** In a case series study, we recruited 105 patients with T2DM according to ADA criteria. Age at diagnosis, BMI, FBS, lipid profile, insulin levels and HbA1C were measured. HOMA-IR was calculated as index of insulin resistance. VDR genotyping was performed using PCR-RFLP method.

**Results:** Of totally 105 diabetic patients (mean age 55±10 years), 79.4% were female. Frequency of FF, ff and Ff genotypes were 71.4, 5.7 and 22.8%, respectively. In patients with Ff and ff genotype, age at diabetes onset was lower, BMI>27 and poor-controlled conditions were more frequent compared with FF genotype. In patients with ff and Ff genotypes the prevalence of obesity was higher, age of onset was significantly lower, HbA1C was higher, HOMA-IR was higher and poor-control condition was more prevalent as compared to FF genotype (P=0.04). The odds ratio and relative risk for association of ff genotype with poor-controlled condition were 2.54 (CI 95%: 1.05 to 6.17) and 1.68 (CI 95%: 0.97 to 2.89), respectively.

**Conclusion:** Our findings showed that VDR polymorphism is associated with age at diagnosis and insulin resistance in patients with type 2 diabetes mellitus.

**Keywords:** Vitamin D Receptor (VDR), Type 2 diabetes, Polymorphism, Insulin resistance

\*Corresponding Author: Endocrinology and Metabolism Research Center, 5<sup>th</sup> Floor, Shariati Hospital, North Kargar Ave., Tehran 14114, Iran, Tel: +98(21) 88220037-8, Fax: +98(21) 882220054, Email: emrc@sina.tums.ac.ir

## Introduction

According to a global survey reported in 2004, the prevalence of diabetes has been estimated 2.8% in 2000 and 4.4% in 2030, worldwide. So, the total number of people with diabetes is predicted to increase from 171 million in 2000 to 360 million in 2030, most frequently will be type 2 diabetes. Type 2 diabetes mellitus (T2DM) is a heterogeneous disease characterized by altered insulin secretion and/or insulin resistance. Insulin resistance is defined as systemic impairment of insulin function which precedes the development of hyperglycemia (1, 2).

Several observations have linked alterations in glucose concentration and also insulin resistance to vitamin D deficiency. There are some evidences that increasing vitamin D levels from 10 to 30 ng/ml could improve insulin sensitivity by 60% (3, 4); Moreover, an inverse correlation between vitamin D concentration and diabetes was observed in non-Hispanic Whites and Mexican-Americans but not in non-Hispanic Blacks (5). Finally, four-week intravenous vitamin D therapy normalized glucose intolerance and insulin sensitivity in hemodialysis patients (6). More recently, it was shown that 1, 25(OH) 2D3 affects the insulin receptor via increasing its mRNA level, the insulin receptor numbers and response in U-937 human promonocytic cells (7).

Vitamin D exerts its action through VDR which is a member of the nuclear receptor superfamily. The VDR gene, located on chromosome 12q12-q14, has at least five promoter regions, eight protein-coding exons and six untranslated exons (8, 9). The gene encodes a polypeptide that binds activated vitamin D and regulates gene expression as a vitamin D-dependent transcription factor. It acts through binding to vitamin D response elements (VDRE) in the promoter regions of target genes (7, 10). There is a report on identification of a VDRE in the IR (insulin receptor) gene promoter that could explain the transcriptional induction of this gene by vitamin D in U-937 cells. This locus might mediate the association between insulin sensitivity and vitamin D (11).

Several studies have been reported on the role of VDR polymorphism in type 1 and 2 diabetes (12, 13, 14, and 15). Some studies

have proposed association of the FokI or BsmI variants with insulin sensitivity in non-diabetic Caucasian subjects (16). Another study suggested association between vitamin D receptor gene polymorphism and insulin secretion in Bangladeshi Asia (17). FokI was also demonstrated to raise susceptibility to type1 diabetes in the Uruguayan population (12). The T to C transition in exon 2 of VDR gene modifies the translation initiation site (ATG) and destroys FokI site and leads to use another ATG codon which is located 9 bp downstream. As a result, two variants of the VDR protein can be generated, the long variant containing 427 amino acids designated by f allele and the one shortened by three amino acids designated by F allele. This transition influences VDR interaction with transcriptional factor IIB (TFIIB). In contrast to f isoform, the F VDR isoform possesses more potent transcriptional activity (18, 19). Although, this polymorphism leads to obvious alteration of function, there are not adequate studies regarding its association with type2 diabetes (16), and few performed studies reported contradictory results. It may be attributed to heterogeneity of subjects about age of onset and taking insulin or any drug.

Herein, we conducted a study to determine possible association of FokI polymorphism with T2DM in a more homogeneous type 2 diabetic population.

## Methods

### Subjects

A total number of 105 diabetic patients participated in this case-series study. Participants were recruited from an outpatient clinic of Shariati Hospital, an university hospital affiliated to Tehran University of Medical Sciences from January to June 2008. All participants suffered from type 2 diabetic and were diagnosed according to ADA criteria. Because of a well-known heterogeneity of T2DM, only subjects with at least 5-year history of type 2 diabetes were recruited. The patients on insulin therapy and who with other chronic diseases were excluded. Informed written consent was obtained from all subjects before their participation in the study. The study protocol

was approved by ethics committee of EMRC (Endocrinology and Metabolism Research Center, Tehran University of Medical Sciences). All patients completed a standard questionnaire containing questions about the age at diagnosis, the treatment plan and other clinical issues. Height and weight were measured and body mass index (BMI) was calculated as weight (kg)/height (m<sup>2</sup>).

### **Biochemical measurements**

The peripheral blood was drawn from each individual after 10-12 h fasting. Serum was aliquoted following centrifuge and stored at -80 °C. All samples were run in the same assay. All measurements were performed in the EMRC laboratory of Shariati hospital.

Fasting blood glucose (FBG) by GOD/PAP method, triglyceride (TG) by GPO-PAP method, total cholesterol by Enzymatic Endpoint method, direct high-density lipoprotein-cholesterol by enzymatic clearance assay, all were done by using Randox laboratories kit (Hitachi 902). OGTT was performed according to the World Health Organization standard procedure. After overnight fasting, the subjects were given a standard glucose solution of 75 gr glucose in 250 ml of water. Blood samples were taken after 120 minutes for measuring plasma glucose concentrations by utilizing the GOD/PAP and Randox method laboratory kits (20). HbA1C was measured using HPLC method. Serum insulin concentrations were measured by ELISA method (Human insulin ELISA kit, DRG Pharmaceuticals, GmbH, Germany) minimum detectable concentration was 1.76 µIU/ml; Intra CV was 2.19% and Inter CV was 4.4%. Insulin resistance (IR) was calculated by homeostasis model assessment (HOMA). HOMA-insulin resistance (HOMA-IR) was calculated as  $\text{IRHOMA} = (\text{Fasting Plasma Glucose} \times \text{Fasting Plasma Insulin}) / 22.5$  (21). QUICKI was calculated by  $\text{ISQUICKI} = 1 / [\log (\text{fasting insulin}) + \log (\text{fasting glucose})]$  (22).

### **DNA studies**

Genomic DNA was isolated from whole blood samples using FlexiGen Kit (QIAGEN Inc. Valencia, CA) according to its protocol. The extracted DNA was stored at 4°C until it is used for PCR and RFLP analysis.

The FokI polymorphism in exon 2 of the VDR gene was detected by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method previously described. Polymerase chain reaction (PCR) was carried out in a total volume of 20 µl containing 2 µL genomic DNA, 0.5 µL of each primer, 7 µL of master mix. The amplification conditions were as follows: an initial denaturation at 94°C for 5 min, followed by 35 amplification cycles (denaturation at 94°C for 30 sec, annealing at 60°C for 30 sec, and extension at 72°C for 30 sec) and a final extension at 72°C for 10 min. Then, 5 µl of the PCR product (265 base pairs) was digested in 10 µl of reaction volume containing 1 µL of FokI with the buffer supplied by the seller. The digested PCR products were resolved on 2.0% agarose gels. FokI digested the first ATG and yielded two products, 69 and 196 base pairs (f allele), but the T to C transition destroyed the FokI site (F allele).

### **Statistical analysis**

Numerical variables were reported as the mean ± SD and categorical variables were presented as percentage. All of the statistical analyses were performed using the SPSS version 15 software. Student T-test was used to compare quantitative variables. We used Chi-square to compare the qualitative variables and ANOVA (Analysis of Variance) to compare the quantitative variables. P-values less than 0.05 were considered to be statistically significant.

## **Results**

Totally 105 subjects, 21 men (20%) and 84 women (80%), with type 2 diabetes participated in this study. The values of age, BMI, WHR were  $55 \pm 10$  years,  $28.99 \pm 4.28$  kg/m<sup>2</sup> and  $0.91 \pm 0.06$ , respectively. Table 1 demonstrates demographic and biochemical characteristic of participated patients.

The distribution of genotypes in the examined population was as follows: 71.42% for FF, 5.71% for ff and 22.85% for Ff. Various parameters including BMI, HbA1C, and HOMA-IR were compared among carriers of the different genotypes.

Patients' characteristics and laboratory measurements in different genotypes are shown in Table 2. Patients with ff genotype were younger than others and in this genotype, age of diabetes diagnosis was lower than other genotypes but only the age of diabetes diagnosis in group with ff genotype was significantly lower than FF genotype ( $P=0.04$ ). Also in this genotype, fasting insulin concentration was lower than FF genotype ( $P=0.03$ ) and this finding was accompany with higher HOMA ( $P=0.02$ ) and OGTT values ( $P=0.4$ ) in ff genotype.

Homozygous subjects for the f allele presented higher BMI than heterozygous or homozygous subjects for the F allele; but this finding was not significantly significant. Also, the prevalence of obesity ( $BMI > 27 \text{ kg/m}^2$ ) in ff genotype was higher than FF genotype

( $P=0.02$ ). Based on HbA1C measurements, 55.7% of subjects were classified as poor-control ( $HbA1C > 7\%$ ) and others (43.3%) were in good-control status. There were not significant differences between genders in poor-control group.

The prevalence of poor-controlled condition in patients with FF genotype was significantly lower than patients with Ff or ff genotypes that mean were 44% (33 subjects) vs. 66.66% (20 subjects), respectively ( $P=0.04$ ). The odds ratio and relative risk for association of ff genotype with poor-controlled condition were 2.54(CI 95%: 1.05 to 6.17) and 1.68(CI 95%: 0.97 to 2.89), respectively.

In regression analysis ff genotype independent of sex, age and BMI predicted poor-control status in diabetic patients.

**Table 1. Demographic characteristic of participants in study**

Age (years)	55±10
Diagnose of T2DM (months)	67±51
BMI(kg/m <sup>2</sup> )	28.9±4.2
FBS (mg/dl)	155±54
OGTT(mg/dl)	195±69
HbA1C (%)	7.4±1.8
HOMA	5.3±2.5
Fasting Insulin (μ IU/ml)	13.9±5.9

FBS: Fasting Blood Sugar, BMI: Body Mass Index, OGTT: Oral Glucose Tolerance Test, HOMA: homeostasis model assessment.

All variables are presented as mean±SD.

**Table 2. Characteristics of patient according to genotypes**

Characteristics	Genotype		
	FF	ff	Ff
Age (years)	55±9	49±11	53±10
Diagnose of T2DM (months)	60±44	72±52	73±62
BMI(kg/m <sup>2</sup> )	29±4.5	29.8±4.9	29.1±3
FBS (mg/dl)	156±52	163±36	163±60
OGTT(mg/dl)	193±66	209±58	198±77
Hb A1C (%)*	6.9±1.4	8.3±2.4	7.6±1.8
HOMA*	4.4±2	5.6±2.1	5.5±2.5
Fasting Insulin (μIU/ml)	14.7±6.4	10.4±3.2	14.4±4

In a case series study, We used chi-square to compare the qualitative variables and ANOVA to compare the quantitative variables, n=105

\*P-value less than 0.05 is significant.

## Discussion

Several studies have investigated the possibility of VDR polymorphism involvement in the pathogenesis of type 2 diabetes. aa genotype of ApaI polymorphism in non-diabetic Caucasian patients was correlated with higher prevalence of glucose intolerance and bb genotype of BsmI polymorphism was accompanied by significantly more insulin resistance (23). Another report which detected ApaI and TaqI polymorphism in Turkish population did not corroborate linkage between these two polymorphism and T2DM (24). The other one examined four polymorphisms (ApaI, BsmI, TaqI and FokI) of VDR gene in Polish population, did not provide evidence for the association of the aforementioned polymorphisms with T2DM (25). Among VDR putative polymorphisms, our study focused on FokI variant which causes functional alteration of the receptor and seems important enough to be studied.

The distribution of genotypes (FF: 71.42%, Ff: 22.85% and ff: 5.71%) was different from the previously reported frequency in T2DM subjects in Poland (FF: 27.6%, Ff: 51.6% and ff: 20.8%) (25). To our knowledge, there are not more reports on the frequency of FokI polymorphisms in type 2 diabetes but, as to other patients from different countries, genotype frequencies in postmenopausal Maltese women (FF: 60.4%, Ff: 30.7% and ff: 8.9%) (26), Indian people with pulmonary tuberculosis (FF: 65.0%, Ff: 30.0% and ff: 5.0%) (27) and also premenopausal American black women (FF: 65%, Ff: 31% and ff: 4%) were similar to ours (28). However, the distributions of genotypes in premenopausal American white women (FF: 37%, Ff: 45% and ff: 18%) (28) or in Finnish with T1DM (FF: 36.2%, Ff: 49.0% ff: 14.7%) (10) were different from our study. Heterogeneity in population of different countries and various patterns of frequency in different diseases can be important factors accounting for this discrepancy. It might also arise from possible bias related to small sample size.

Association between obesity defined as BMI>27 and VDR polymorphism in our study was in accordance with the report in French Caucasians which was explained by effect of vitamin D in adipocyte differentiation and

metabolism (29). This association was also observed in premenopausal American women and also in pregnant Iranian women in two separate studies. Obesity was suggested to be correlated with insulin resistance (3, 30).

According to our findings, insulin resistance index, calculated by HOMA-IR, is higher in the patients with f allele. Our results related to insulin resistance are in accordance with the study in Caucasians which indicated subjects with the f allele were more insulin resistant than those with homozygous F allele (16).

The difference between patients with and without F allele in terms of insulin resistance is in accordance with, on the one hand, the report that this polymorphism of the translation initiation codon affects its biological activity. As demonstrated this T to C transition leads to synthesis of a smaller protein with increased vitamin D-dependent transcriptional activation (19) and on the other hand, so many evidences confirming role of vitamin D in insulin resistance. Positive correlation between vitamin D concentration and insulin sensitivity was demonstrated following study in middle-aged men with impaired glucose tolerance and also by another study in pregnant women (3, 31). Supplementation of vitamin D improved postprandial insulin sensitivity in obese men likely to have insulin resistance (32). Furthermore, it has been shown that vitamin D improves insulin resistance in muscle cells (33). These observations along with the fact that vitamin D acts through binding to the vitamin D receptor leads tempts us to conclude that Ff/ff genotype synthesizing less active VDR protein, are prone to be more insulin resistant.

Although the exact mechanism by which this interaction occurs is unclear, identification of a vitamin D response element (VDRE) in the promoter of insulin receptor gene causing transcriptional induction of this gene by vitamin D, can be the possible mechanism of vitamin D involvement in insulin resistance (11).

Aside from the above mechanism, it is reported that vitamin D acts as a negative regulator of the renin gene and vitamin D deficiency is followed by enhanced renin production which in turn increases angiotensin II levels. The latter is proposed to cause the development of insulin resistance via



mediating various pathways in the insulin cascade (34).

In summary, our study suggested possible association between FokI polymorphism and insulin resistance. Since, our findings are compatible with the published correlation between vitamin D levels and insulin sensitivity and also direct contribution of vitamin D/VDRE in regulation of some genes involving in insulin resistance phenomenon, we postulate that FokI polymorphism of VDR can be a candidate locus conferring susceptib-

ility to T2DM. Nevertheless, in order to state a certain opinion in this case, additional studies in different populations are required.

### Acknowledgement

This research study was financially supported by a grant from Endocrinology and Metabolism Research Center, Tehran University of Medical Sciences.

### References

1. Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes. *Diabetes Care* 2004; 27:1047-1053.
2. Mirzaei k, Hossein-nezhad A, Hosseinzadeh-Attar M, Najmafshar A, Jafari N, Rahmani M, Larijani B. Relationship between genotype and serum levels of adipokines and bone mineral density in type 2 diabetes mellitus patients. *Iranian J Diabetes Lipid Disorders (electronic)* 2009; 77-86.
3. Maghbooli Z, Hossein-Nezhad A, Karimi F, Shafaei AR, Larijani B. Correlation between vitamin D-3 deficiency and insulin resistance in pregnancy. *Diabetes Metab Res Rev* 2008; 24:27-32.
4. Chiu KC, Chu A, Go VLW, Saad MF. Hypovitaminosis D is associated with insulin resistance and  $\beta$ -cell dysfunction. *Am J Clin Nutr* 2004; 79:820-5.
5. Scragg R, Sowers MF, Bell Colin. Serum 25-Hydroxyvitamin D, Diabetes, and Ethnicity in the Third National Health and Nutrition Examination Survey. *Diabetes Care* 2004; 27:2813-18.
6. Mak RHK. 1, 25-dihydroxyvitamin D3 corrects insulin and lipid abnormalities in uremia. *Kidney International* 1998; 53:1353-57.
7. Calle C, Maestro B, García-Arencibia M. Genomic actions of 1,25-dihydroxyvitamin D3 on insulin receptor gene expression, insulin receptor number and insulin activity in the kidney, liver and adipose tissue of streptozotocine-induced diabetic rats. *BMC Molecular Biology* 2008; 9:65-77.
8. Christakos S, Dhawan P, Liu Y, Peng X, Porta A. New insights into the mechanisms of vitamin D action. *Journal of Cellular Biochemistry* 2003; 88:695-705.
9. Ogunkolade B, Bouccher BJ, Prahl JM, et al. Vitamin D receptor (VDR) mRNA and VDR protein levels in relation to vitamin D status, insulin secretory capacity, and VDR genotype in Bangladeshi Asians. *Diabetes* 2002; 51:2294-2312.
10. Turpeinen H, Hermann R, Vaara S, et al. Vitamin D receptor polymorphism: not association with type 1 in the Finnish population. *European Journal of Endocrinology* 2003; 140:591-596.
11. Maestro B, Davila N, Carranza C, Calle C. Identification of a vitamin D response in the human insulin receptor gene promoter. *Journal of Steroid Biochemistry & Molecular Biology* 2003; 84:223-230.
12. Mimbacas A, Trujillo J, Gascue C, Javiel G, Cardoso H. Prevalence of vitamin D receptor gene polymorphism in a Uruguyan population and its relation to type 1 diabetes mellitus. *Genetic and Molecular Research* 2007; 6(3):534-542.
13. Guo S, Magnuson VL, Schiller JJ, Wang X, Wu Y, Ghosh S. Meta-analysis of vitamin D receptor

- polymorphisms and type 1 diabetes: A HuGE review of genetic association studies. *Am J Epidemiol* 2006; 164:711-724.
14. Reis AF, Hauache OM, Velho G. Vitamin D endocrine system and the genetic susceptibility to diabetes, obesity and vascular disease. A review of evidence. *Diabetes Metab* 2005; 31:318-325.
  15. Palomer X, Gonzalez-Clemente M, Blanco-Vaca F, Mauricio D. Role of vitamin D in the pathogenesis of type 2 diabetes mellitus. *Diabetes Obesity and Metabolism* 2008; 10:185-197.
  16. Chiu KC, Chaung L, Yoon C. The vitamin D receptor polymorphism in the translation initiation codon is a risk factor for insulin resistance in glucose tolerant Caucasians. *BMC Med Genet* 2001; 2:2.
  17. Hitman GA, Mannan N, McDermott MF, et al. Vitamin D receptor gene polymorphism influence insulin secretion in Bangladeshi Asians. *Diabetes* 1998; 47:688-690.
  18. Jurutka PW, Remus LS, Whitfield GK, Thompson PD, et al. The polymorphic N-terminus in the human vitamin D receptor isoforms influences transcriptional activity by modulating interaction with transcription factor IIB. *Mol Endocrinol* 2000; 14:401-420.
  19. Arai H, Miyamoto K, Taketani Y, Yamamoto H, Iemori Y, Morita K, et al. A vitamin D receptor gene polymorphism in the translation initiation codon: effect on protein activity and relation to bone mineral density in Japanese women. *J Bone Miner Res* 1997; 12:915-921.
  20. Matsuda M, DeFronzo R. Insulin sensitivity indices obtained from oral glucose tolerance testing. *Diabetes Care* 1999; 22:1462-1470.
  21. Tanabe N, Saito K, Yamada Y, et al. Risk assessment by post-challenge plasma glucose, insulin response ratio, and other indices of insulin resistance and/or secretion for predicting the development of type 2 diabetes. *Inter Med* 2009; 48:401-409.
  22. Katz A, Nambi SS, Mather K, et al. Quantitative insulin sensitivity check index: a simple, accurate method for assessing insulin sensitivity in humans. *J Clin Endocrinol Metab* 2000; 85:2402-2410.
  23. Oh J, Barrett-Connor E. Association between vitamin D receptor polymorphism and type 2 diabetes or metabolic syndrome in community-dwelling older adults: The Rancho Bernardo study. *Metabolism* 2002; 51(3):356-359.
  24. Dimec F, Uzer E, Akkafa F, et al. Detection of VDR gene Apal and TaqI polymorphisms in patients with type 2 diabetes mellitus using PCR-RFLP method in a Turkish population. *Journal of Diabetes and its Complications* 2009.
  25. Maecki MT, Frey J, Moczulski D, Klupa T, et al. Vitamin D gene polymorphisms and association with type 2 diabetes mellitus in a Polish population. *Exp Clin Endocrinol Diabetes* 2003; 111:505-509.
  26. Vidal C, Grima C, Brincat M, Megally N, Xuereb-Anastasi A. Associations of polymorphisms in the vitamin D receptor gene (BsmI and FokI) with bone mineral density in postmenopausal women in Malta. *Osteoporosis International* 2003; 14:923-928.
  27. Selvaraj P, Chandra G, Kurian SM, Reetha AM, Narayanan PR. Association of vitamin D receptor gene variants of BsmI, Apal and FokI polymorphisms with susceptibility or resistance to pulmonary tuberculosis. *Current Science* 2003; 84:1564-1568.
  28. Harris SS, Eccleshall TR, Gross C, Dawson-Hughes B, Feldman D. The vitamin D receptor starts codon polymorphism (FokI) and bone mineral density in premenopausal American black and white women. *J Bone Miner Res* 1997; 12(7):1043-1048.
  29. Ye W, Reis A, Doboys-Laforgue D, Bellané-Chantelot C, Timsit J, Velho G. Vitamin D receptor gene polymorphism are associated with

- obesity in type 2 diabetic subjects with early age of onset. *European Journal of Endocrinology* 2001; 145:181-186.
30. Zajíčková K, Ofková I, Bahboub R, Křepelová A. Vitamin D receptor gene polymorphisms, bone mineral density and bone turnover: FokI genotype is related to postmenopausal bone mass. *Physiol Res* 2002; 51:501-509.
31. Tai K, Need AG, Horowitz M, Chapman IM. Vitamin D, glucose, insulin, and insulin sensitivity. *Nutrition* 2008; 24: 279-285.
32. Nagpal J, Pande N, Bhartia A. A double-blind randomized, placebo-controlled trial of the short-term effect of vitamin D3 supplementation on insulin sensitivity in apparently healthy middle-aged, centrally obese men. *Diabetic Medicine* 2008; 26:19-27.
33. Zhou QG, Hou FF, Guo ZJ, Liang M, Wang GB, Zhang X. 1, 25-Dihydroxyvitamin D improved the free fatty-acid-induced insulin resistance in cultured C2C12 cells. *Diabetes Metab Res Rev* 2008; 24: 459-464.
34. Rammos G, Tseke P, Ziakka S. Vitamin D, the renin-angiotensin system, and insulin resistance. *Int Urol Nephrol* 2008; 40:419-426.