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AKRAM,

ABSTRACT

Al-Nabulsi A.M.A. The Role of Vitamin D in Type 2 Diabetes Mellitus. Near East University, Graduate School of Health Sciences, Graduation Project in Medical Biochemistry Programme, Nicosia, 2018.

Diabetes mellitus is a very common disease and its prevalence is rising rapidly. Nowadays preventive approaches are concentrated on healthy nutrition and vitamin supplementation because of abundance of diabetic patients. The connection between vitamin D deficiency and diabetes mellitus is known for nearly 40 years. Although there is the convincing evidence on the preventive effect of vitamin D in infancy, clinical investigations are usually based on epidemiological and some of them give contradictory results.

The aim of this study is to find strong assess the role and the mechanisms of action of vitamin D on the synthesis and functions of both insulin and insulin receptors. Molecular and genetic mechanisms have been considered more than epidemiological surveys and statistics.

Vitamin D receptor (VDR) blocks the NF-κB activity through IKKβ protein and this interactions lessens skeletal muscle insulin resistance mediated by activated NF-κB. These results explain the mechanism of vitamin D induced decrease of insulin resistance in skeletal muscle.

Investigations on the relationship between the insulin-like growth factor binding proteins and calcitriol indicate the role and importance of vitamin D in diabetes mellitus.

Maestro et al. investigated the probable presence and location of VDREs in the promoter of insulin receptor gene (hIR). All of the transfected and wild promoters activities are increased by 60 % after treatment with and without 10-8M calcitriol. A computer search located a short sequence of 30 bp (5'CGTCGGGCCTGTGGGG-CGCCTCCGGGGGTC3') in the promoter of hIR which is the candidate sequence for activation. This discovery may probably be the strongest evidence which on molecular interactions of VDR and hIR promoter.

This review and reevaluation project has shown that vitamin D plays an important role in diabetes mellitus, and its deficiency may contribute to the pathogenesis both T1DM and T2DM.

ÖZET

Al-Nabulsi A.M.A. The Role of Vitamin D in Type 2 Diabetes Mellitus. Near East University, Graduate School of Health Sciences, Graduation Project in Medical Biochemistry Programme, Nicosia, 2018.

Diabetes mellitus sık ratlanan bir hastalıktır ve prevalansı hızla artmaktadır. Diyabetik hastaların sayısının çokluğu nedeniyle, günümüzde sağlıklı beslenme ve vitamin takviyesine ağırlık verilmektedir. D vitamin yetmezliği ile diabetes mellitus arasındaki bağlantı yaklaşık 40 yıldır bilinmektedir. D vitamininin bebeklik çağındaki diabete karşı koruyucu etkisi hakkındaki kuvvetli kanıtlara ragmen, diyabetle ilgili klinik araştırmalar genellikle epidemiyolojik yaklaşımlıdır ve bunların bir kısmı çelişkili sonuçlar vermektedir. Bu çalışmanın amacı D vitamininin hem insulin, ve hem de insulin reseptörünün sentezi ve fonksiyonları üzerindeki rolü ve mekanizmaları hakkında kuvvetli kanıtlar bulmaktır. Epidemiyolojik araştırma ve istatistiklerden çok moleküler ve genetic mekanizmalara ağırlık verilmiştir.

Vitamin D reseptörü (VDR), IKKβ protein üzerinden NF-κB'yi aktive etmektedir. Bu etkileşme, NF-κB ile active edilen iskelet kası insulin direncini azaltmaktadır. Bu sonuçlar vitamin D'nin iskelet kasında insulin direncini azaltmasının mekanizmasını aydınlatmaktadır.

İnsülin-benzeri büyüme faktörü (IGF) bağlayıcı protein ve kalsitriol arasındaki etkileşmelerin araştırılması D vitamininin diabetes mellitustaki rolü ve önemi göstermiştir.

Maestro ve arkadaşları, insan insulin reseptör geninin (hIR) promotör bölgesinde muhtemel bir vitamin D yanıt elemanının (VDRE) varlığını araştırdılar. İnsülin reseptör geninin orijinal ve transfekte edilmiş promotör dizilerinin kullanıldığı çalışmada 10-8M kalsitriol ile inkübasyonda kontrole gore %60 daha fazla aktiviteye elde edildi. Bilgisayar ile araştırma, insulin reseptör geninin promotor bölgesinde 30 bp uzunluğundaki bir VDR bağlanma bölgesini (5'CGTCGGGCCTGTGGGGCGCCTCCGGGGGTC3') ortaya çıkardı. Bu bulgu muhtemelen VDR ve hIR promotör bölgesi arasındaki moleküler etkileşme için en güçlü kanıttır.

Bu tarama ve yeniden değerlendirme projesine, D vitamininin diabetes mellitusta önemli bir rolü bulunduğu, ve eksikliğinin ise hem T1DM ve hem de T2DM'ata önemli nedenlerden biri olduğu bilgileri elde edilmiştir.

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LIST OF ABBREVIATIONS

1.	1α,25(OH)2D3	:	1-alpha–25 dihydroxycholecalciferol
2.	25-HCC	:	25-hydroxy cholecalciferol (cholecalcidiol)
3.	AA	:	Ascorbic acid
4.	ADA	:	American Diabetes Association
5.	AF-1	:	Activation function-1
6.	AF-2	:	Activation function-2
7.	AR	:	Androgens receptor
8.	aPKC	:	Atypical isoforms of Protein kinase C
9.	BMI	:	Body Mass Index
10.	CKD	:	Chronic kidney disease
11.	CYP-450	:	Cytochrome P-450
12.	CYP2R1	:	Cytochrome P-2 receptor 1
13.	DBP	:	Vitamin D binding protein
14.	DHA	:	Dehydroascorbic acid
15.	7-DHC	:	7-Dehydrocholesterol
16.	DBD	:	DNA binding domain
17.	DM	:	Diabetes Mellitus
18.	DNA	:	Deoxyribo Nucleic Acid
19.	DKA	:	Diabetic Keto-Acidosis
20.	DVBP	:	Vitamin D binding protein
21.	ERK	:	Extracellular signal-regulated kinase
22.	ER	:	Estrogen receptor
23.	GDM	:	Gestational Diabetes Mellitus
24.	GLUTs	:	Glucose transporters
25.	GLUT-4	:	Glucose transporter-4
26.	GR	:	Glucocorticoid receptor
27.	Grb-2	:	Growth factor receptor-bound protein-2
28.	IDDM	:	Insulin Dependent Diabetes Mellitus
29.	IFG	:	Impaired Fasting Glucose
30.	INS-R (IR)	:	Insulin Receptors
31.	IRS	:	Insulin receptor substrates
32.	I.O.M	:	Institute of Medicine

33.	IU	:	International Unit
34.	LBD	:	Ligand binding domain
35.	LDL	:	Low Density Lipoprotein
36.	MAP	:	Mitogen-activated protein
37.	MAPK	:	Mitogen-activated protein kinases
38.	MR	:	Mineralocorticoid receptor
39.	ng	:	Nano gram
40.	NIDDM	:	Non-Insulin Dependent Diabetes Mellitus
41.	NF-κb	:	Nuclear Factor-kB
42.	OGTT	:	Oral Glucose Tolerance Test
43.	PI3K	:	Phosphatidylinositol-3-kinase
44.	PIP2	:	Phosphatidylinositol 4, 5-bisphosphate
45.	PIP3	:	Phosphatidylinositol 3, 4, 5-trisphosphate
46.	PPAR	:	Peroxisome Proliferator-Activated Receptor
47.	PKB	:	Protein kinase B
48.	PKC	:	Protein kinase C
49.	RAR	:	Retinoic acid receptor
50.	RNA	:	Ribonucleic Acid
51.	RXR	:	Retinoid X receptor
52.	SOS	:	Son Of Sevenless gene encoding factor
53.	Shc	:	Src-homology-2-containing (Shc) proteins
54.	T1DM	:	Diabetes Mellitus Type-1
55.	T2DM	:	Diabetes Mellitus Type-2
56.	TR	:	Thyroid hormone receptor
57.	UVB	:	Ultra Violet B
58.	VDD	:	Vitamin D deficiency
59.	VDR	:	Vitamin D receptor
60.	VDDR-1	:	Vitamin D–dependent rickets type I
61.	VDDR-2	:	Vitamin D–dependent rickets type II
62.	VDREs		Vitamin D-Responsive Elements
63.	VDR-RXR	:	Vitamin D receptor-retinoic acid x-receptor complex
64.	VIT	:	Vitamin
65.	VTD	:	Vitamin-D
66.	VSMC	:	Vascular smooth muscle cells

LIST OF FIGURES

1. INTRODUCTION

Diabetes mellitus is a very common disease. It affects approximately 150,000,000 people worldwide and its prevalence rises rapidly (Poretsky, 2010). Nowadays preventive approaches are concentrated on healthy nutrition and vitamin supplementation because of abundance of diabetic patients.

The connection between vitamin D deficiency and diabetes mellitus is known for nearly 40 years (Norman et al., 1980). Cod liver oil taken during the first year of life reportedly reduced the risk of childhood-onset T1D, and a multicenter case–control study also showed an association between vitamin D supplementation in infancy and a decreased risk of T1D (Stene et al., 2000). A study found that the risk of developing T1D diminished with an intake of 2000 IU of vitamin D during the first year of life. It is also shown that the incidence of childhood diabetes was three times higher in subjects with suspected rickets (The EURODIAB Substudy 2 Study Group, 1999; Hypponen E. et al., 2001).

Clinical investigations are usually based on epidemiological studies and these studies usually give contradictory results. Some investigations on the role of vitamin D in type 2 diabetes provided convincing evidence on the protective role of the vitamin D in the prevention of insulin resistance (Zhu et al., 2005). Only few studies give experimental evidence on the action of vitamin D in diabetes mellitus.

The aim of this study is to find evidence on the role and the mechanisms of action of vitamin D on synthesis and functions of both insulin and insulin receptors. The experimentally obtained molecular evidence about the effects of vitamin D on insulin receptors and T2DM will be considered more than epidemiological surveys and statistics.

2. GENERAL INFORMATION

2.1. Vitamins

Vitamins are organic compounds occurring in small quantities in different natural foods. They are essential for growth and maintenance of good health in human beings and also in some experimental animals. Most of them are the source of the presursors of coenzymes and cofactors, so are essentil for the enzymatic reactions (Vasudevan et al., 2013).

Vitamins are classified in two classes according to physicochemical properties, transport in circulation and metabolism :

1. Water soluble vitamins (named as B complex and C): Most of the members of this class are the presursors of coenzymes and cofactors, so take an essential role in enzymatic reactions while the ascorbic acid (vitamin C) acts as an anti-oxidant.

2. The fat-soluble vitamins (A, D, E, and K): Vitamin K is a coenzyme of γ-glutamyl carboxylase and is necessary for blood coagulation. Vitamin E acts as antioxidant and radical scavenger. Vitamin A has an essential role in vision. Oxidized form of vitamin A (retinoic acid) also has specific nuclear receptors and has hormonal effects especially in the development of fetus.

Vitamin D is a unique compound among the vitamins. The active form of vitamin D, calcitriol, is a hormone and its specific nuclear receptors are widely distributed in human body.

Our study will focus on calcitriol, insulin, and insulin receptors.

2.2. Vitamin D

2.2.1. Structure and Synthesis of Vitamin D

Vitamin D derivatives are synthesized from cholesterol molecule and they have a common secosteroid structure. In secosteroids one of the rings are cut (latin word seco means “cut”).

Vitamin D may be obtained from diets or endogenous synthesis. Dietary vitamin D may be in two forms, ergocalciferol (vitamin D2, converted from ergosterol and not found in

animals), and cholecalciferol (vitamin D3, converted from 7-dehydrocholesterol and found in animals) (Figure 2.1.) (Feldman & Pike, 2011).

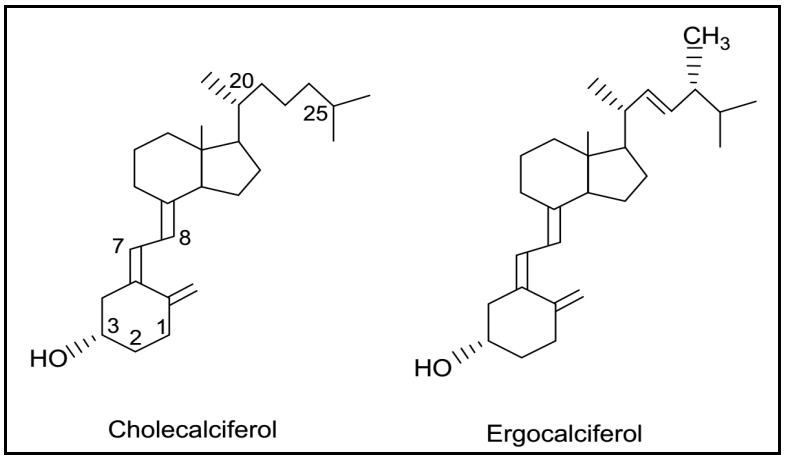


Figure 2.1. Nutritional forms of vitamin D. (Feldman & Pike, 2011).

The precursor 7-dehydrocholesterol is converted to previtamin D_3 (cis-secosterol) in the Malpighian layer of the epidermis is started by ultraviolet radiation (280-315 nm, UVB). Previtamin D_3 then is converted to vitamin D^3 (cholecalciferol) spontaneously (Figure 2.2.) (Vasudevan, 2013).

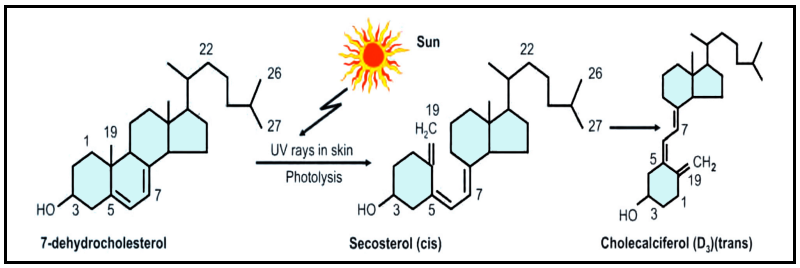


Figure 2.2. Synthesis of cholecalciferol (vitamin D_3).
Vasudevan, p. 469. 2013.

Under the UVB radiation, previtamin D_3 absorbs the solar UVB radiation and isomerized into two major photoproducts, lumisterol and tachysterol. None of these two photoproducts has any effect on calcium metabolism. Thus only 15% of the 7-dehydrocholesterol can be converted to vitamin D_3. Any further exposure will result in conversion of previtamin D_3 to lumisterol and tachysterol as well as revert back to 7-dehydrocholesterol (Wacker and Holick, 2013).

2.2.2. Metabolism of Vitamin D

Once produced, cholecalciferol specifically binds to the vitamin-D binding protein (DVBP, an alpha-2 globulin) in the circulation, which is necessary for its transport and distribution in the body. Cholecalciferol is not biologically active but is a prohormone. The hormone calcitriol, the active form vitamin, is synthesized by two consecutive hydroxylation reactions of cholecalciferol. Hepatic 25-hydroxylase (a microsomal monooxygenase, CYP2R1) catalyzes the first hydroxylation reaction to give the product 25-hydroxy cholecalciferol (25-HCC, cholecalcidiol) (Fig. 2.3.) (Vasudevan, p. 470. 2013).

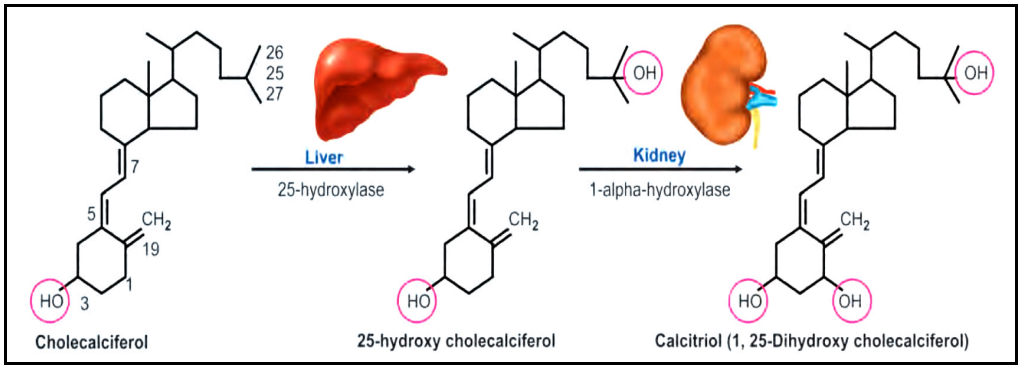


Figure 2.3. Generation of calcitriol from cholecalciferol. (Vasudevan, p. 470. 2013)

DVBP-bound 25-hydroxycholecalciferol is transported to kidneys for further hydroxylation (at the 1st position) by the catalyzing enzyme 1-alpha- hydroxylase (CYP27B1, located in mitochondria of proximal convoluted tuber of kidneys). This cytochrome P-450 enzyme requires cytochrome NADPH and ferrodoxin (an iron-sulfur protein) for the reaction. Final product is called calcitriol (1,25-dihydroxycholecalciferol) since it contains three hydroxyl groups at 1, 3 and 25 positions. The calcitriol, the end product, is the active form of vitamin and is a hormone. Both 25-hydroxycholecalciferol and calcitriol are inactivated by 24-hydroxylase (CYP24A1) in the kidney. These inactive products are converted to calcitroic acid and excreted in urine. (Vasudevan et al., 2013).

2.3. Nuclear Receptors

2.3.1. General Characteristics and Classification

A group of endocrine signaling molecules (steroids and other hormones) diffuse through the cell membrane and form hormone-receptor complexes. This complexes serve as

activators by binding to enhancers, or hormone response elements, and stimulate the transcription of their associated genes. Each hormone binds to its specific receptor, and they together activate their own gene set.

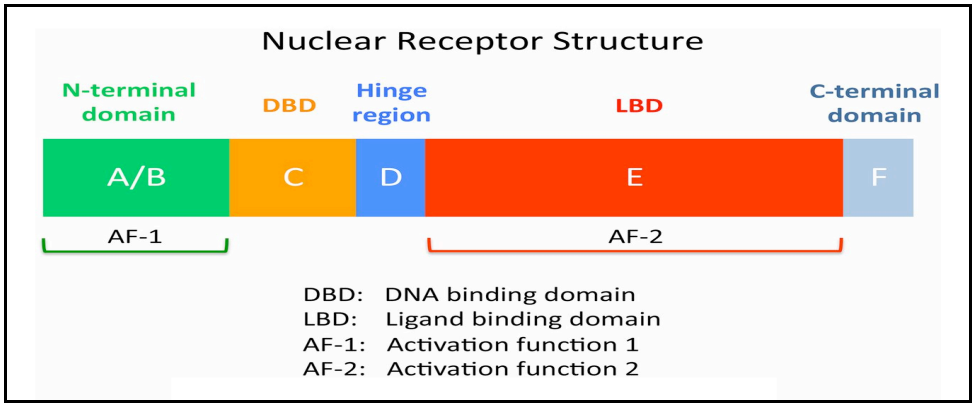


Figure 2.4. Common structure of the nuclear receptors (Baker, 2016).

A nuclear receptor consists of a variable N-terminal region (A/B), a conserved DNA-binding domain (C), a variable hinge region (D), a conserved ligand binding domain (E), and a variable C-terminal region (F) (Figure 2.4.).

The nuclear receptors have been divided into three classes (Figure 2.5.) (Imai et al., 2013):

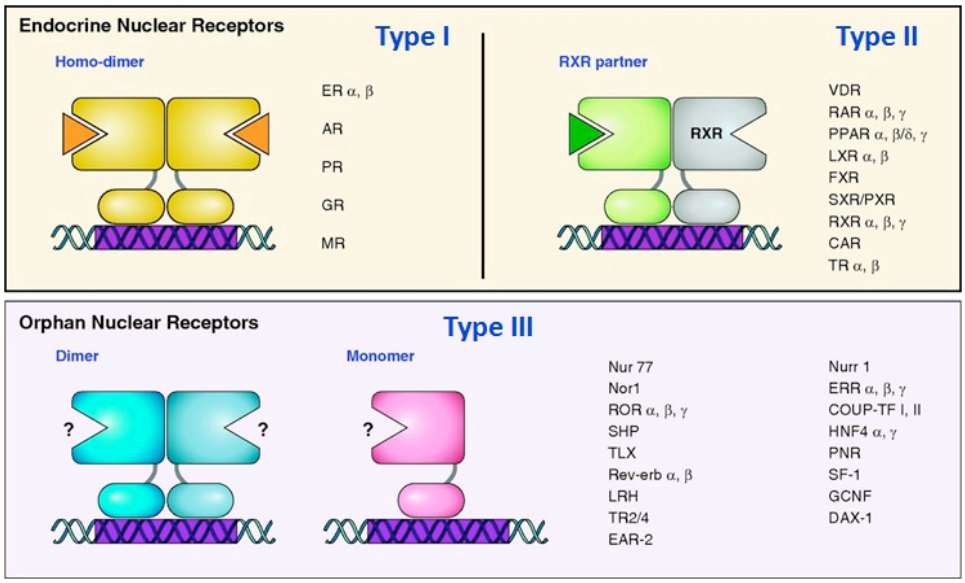


Figure 2.5. Classification and the ligands of nuclear receptors
(Imai et al.,2013)

2.3.1.1. The type I receptors: The first class contains the homodimeric receptors which includes the steroid hormone receptors, and typified by the glucocorticoid receptor. These receptors reside in the cytoplasm as coupled with another protein in the absence of their hormone ligands. When a type I receptor binds to its hormone ligand, it releases its protein partner and migrates to the nucleus, where it binds as a homodimer to its hormone response element. The members of this class includes glucocorticoid (GR), mineralocorticoid (MR), progesterone (PR), androgens (AR), and estrogen (ER) receptors (Weaver, 2012).

2.3.1.2. The type II receptors : The members of this class stay in the nucleus and is exemplified by the thyroid hormone receptor. The ligand-bound receptors form dimers with another protein called retinoid X receptor (RXR), whose ligand is 9-cis retinoic acid. These heterodimer receptors bind to their target sites in both the presence and absence of their ligands. In the absence of ligand, the binding of these type II receptors can repress transcription, whereas binding of the receptors along with their ligands can stimulate transcription. Thus, environmental conditions may determine the same protein could act as either an activator or a repressor. Thyroid hormone (T_3R), all-trans-retinoic acid (RAR), calcitriol (VDR), fatty acids and 15d-$^{\Delta 12,\ 14}$-PGJ (PPARα and PPARγ), ecdysone (EcR), and bile acids (FXR) receptors are the members of this class (Weaver, 2012).

Type I and Type II receptor classes also called endocrine nuclear receptors.

2.3.1.3. The type III receptors : They are not well understood. Their structure may be monomer or heterodimer. Since their ligands have not been identified, they are also known as “orphan receptors”. (Weaver, 2012).

2.4. Vitamin D Receptor and Hormone-Receptor Interactions

2.4.1. Distribution of Vitamin D receptors :

Vitamin D and steroid hormone target tissues and cells were investigated and and characterized with high resolution receptor microscopic autoradiography technique by Stumpf et al. (North Carolina, US). These intensive studies revealed that more than 50 tissues and cell types have vitamin D receptors (Figure 2.6.). These results explain the many metabolic effets of the vitamin D in various tissues (Stumpf WE, 2005).

2.4.2. Structure of vitamin D receptor: Vitamin D receptor (VDR) is a typical member of heterodimeric type II class nuclear receptors.

Calcitriol (1,25-dihydroxyvitamin D3) acts through the two different mechanisms :

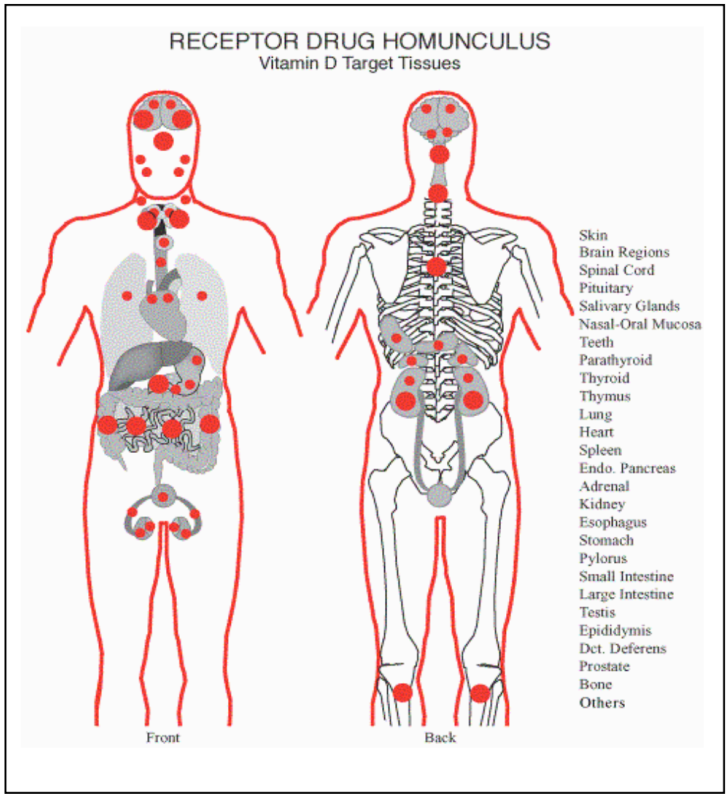


Figure 2.6. Distribution of Vitamin D receptors in target tissues (Stumpf WE, 2005).

2.4.3. Genomic Activities of Calcitriol :

In the genomic response, calcitriol binds to VDR (vitamin D receptor). The VDR forms a heterodimer with the retinoid X receptor (RXR) and binds to vitamin D response elements (VDREs) in the promoters of target genes. This usually increases the transcription, and generates downstream biological responses (Figure 2.7.) (Shin et al., 2010).

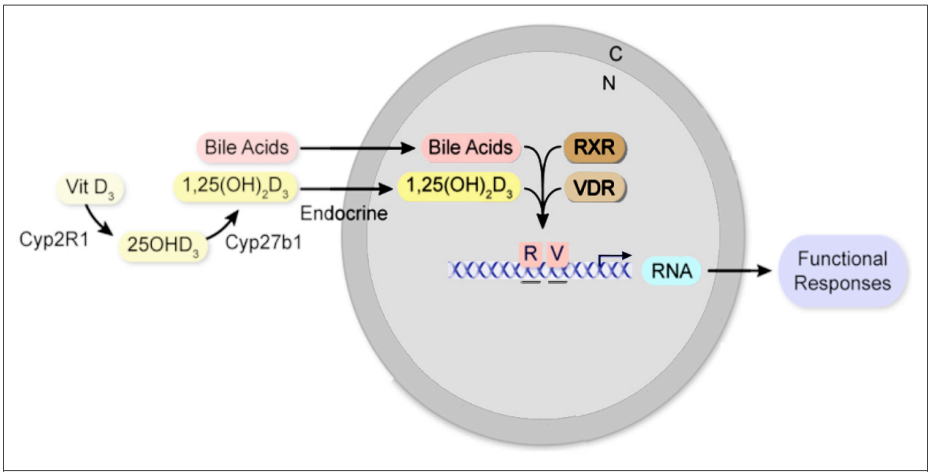


Figure 2.7. Genomic action mechanism of calcitriol (1,25(OH)2D3). C; cytoplasm, N; nucleus, R; RXR, V; VDR. (Pike et al., 2009).

2.4.4. Non-Genomic Activities of Calcitriol – VDR Complex:

Calcitriol induces non-transcriptional rapid responses which involves stimulation of transmembrane signal transduction pathways.

One of the target tissues of calcitriol is skeletal muscle. It has been shown that, avian embryonic skeletal muscle cells genomically and non-genomically respond both to the hormone. In a study it is shown that in chick myoblasts, short-term treatment (1–10 min) with calcitriol induces translocation of the VDR from the nuclear to the microsomal fraction. Capiati et al. showed the translocation process of receptors was inhibited by colchicine, genistein, or herbimycin, and proposed the involvement of microtubular transport and tyrosine kinase/s in the relocation of the receptor. These results suggest that the nuclear VDR may be the receptor that mediates the non-genomic effects of calcitriol in chick myoblasts (Capiati et al., 2002)

Rabsemen et al. investigated the mechanism of induction of growth and migration of vascular smooth muscle cells (VSMC) by calcitriol in rat aorta. They found the calcitriol (0.1 to 100 nM) induced a dose-dependent increase in VSMC migration. The stimulation of VSMCs with calcitriol with a concentration of 10 nmol/L induced a rapid and time-dependent increase in VDR-associated PI3 kinase activity.

An incubation of 10 and 30 minutes with calcitriol (1 nmol/L) increased the PI3 kinase activity by 5- and 10-fold, respectively. This study showed the requirement of the

activation of phosphatidylinositol 3-kinase (PI3 kinase), because the PI3-kinase inhibitors LY294002 (10 μM) or wortmannin (30 nM) completely abolished the calcitriol–induced migration. 5, 6-dichlorobenzimidazole riboside (a RNA polymerase inhibitor, 50 μM) did not affect calcitriol–induced VSMC migration, and this result suggests that gene transcription is not involved in this rapid response (Rebsamen et al., 2002). However, the exact molecular interaction and mechanism of activation of PI3K system by calcitriol-VDR complex is not known exactly.

Genomic and non-genomic activities of calcitriol-VDR complex are summarized as in the following (Figure 2.8.)(Shin et al., 2010).

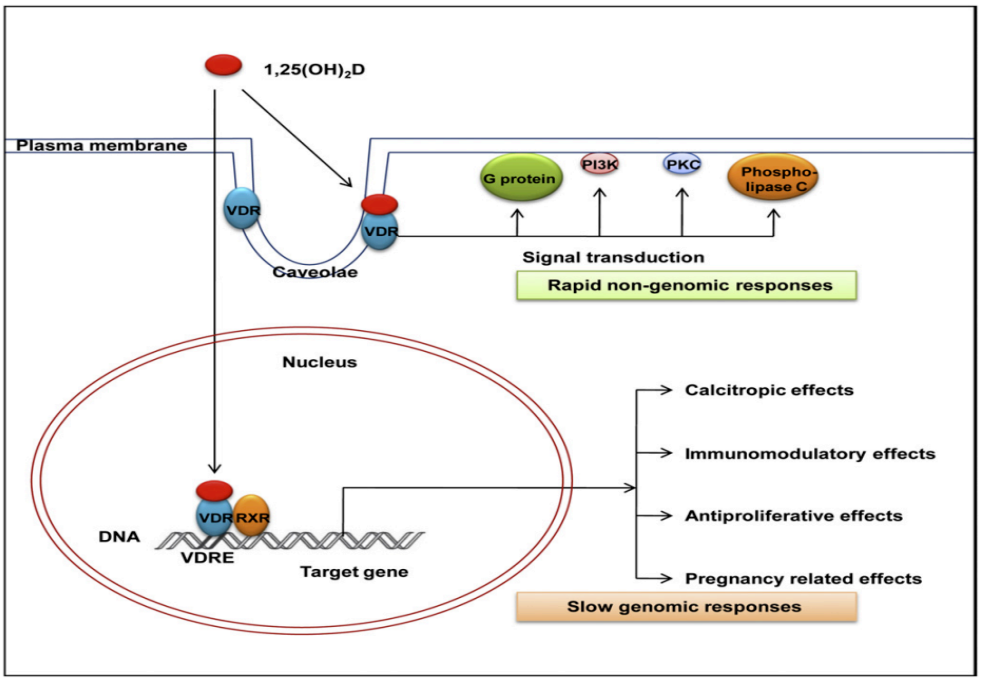


Figure 2.8. Genomic and non-genomic responses of vitamin D receptor binding to calcitriol (1, 25-$(OH)_2$D). PI3K; phosphatidylinositol-3-kinase, PKC; protein kinase C (Shin et al., 2010).

2.5. Insulin and Insulin Receptor

Insulin is a protein hormone secreted by the pancreatic islet β-cells. It is one of the most important hormones which control the metabolism of carbohydrates, lipids and proteins.

Insulin and glucagon have an essential role in mammals and the absence of insulin

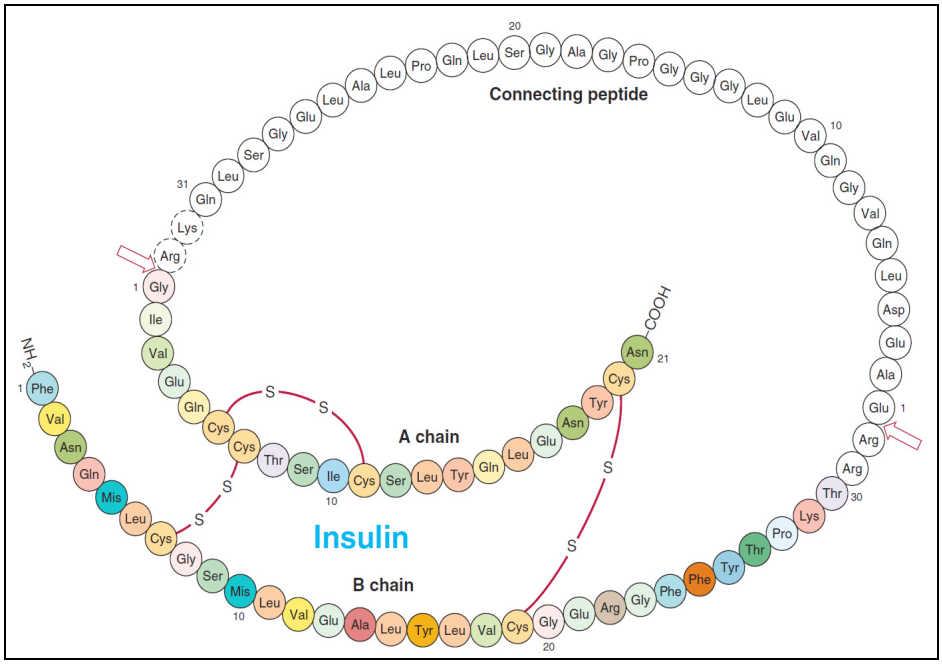
will be fatal within a few months. Insulin plays a major role in the regulation of energy substrates with glucagon, the main ones being glucose, fatty acids and ketones. The action of insulin may be summarized by its hypoglycemic effect.

Daily secretion of insulin by a normal pancreas gland secrete is about 40 - 50 units, and the fasting plasma concentration is 29-172 pmole/L (McPherson and Pincus, 2011).

2.5.1. Structure and Synthesis of Insulin

Insulin has a heterodimeric structure with one intrachain and two interchain disulfide bridges. The gene of insulin is located in the small arm of chromosome 11 and insulin is synthesized as a pre-prohormone (preproinsulin) with an approximate molecular weight of 11,500. The hydrophobic signal sequence of 23 amino acids directs the molecule into the endoplasmic reticulum of pancreatic beta- cells (Figure 2.9.).

Proinsulin, inactive form of insulin, is formed by the removal of signal sequence and then is packaged in the Golgi apparatus and transferred into membrane-bound secretory granules. The disulfide bridges of molecule are formed properly in this stage of synthesis. A series of site-specific peptide cleavages in the secretory granules gives the mature insulin (Murray et al., 2009).



2.5.2. Insulin Receptor

2.5.2.1. Structure of insulin receptor

The receptor of insulin is synthesized as a single glycosylated polypeptide and split into α and β subunits. A tetramer is assembled from these two sets of subunits by disulfide bonds. A hydrophobic domain of β subunits spans the plasma membrane and has tyrosine kinase activity in the cytoplasmic domain (Figure 2.10.). The extracellular α subunit has the insulin binding site. The binding of insulin to the α subunits of the insulin receptor induces changes in the β subunits, which promotes a quick auto-phosphorylation of tyrosine residues on each β subunit. This initiates a cascade of cell signaling responses to proteins family called insulin receptor substrates (IRS) (Bhagavan, 2011).

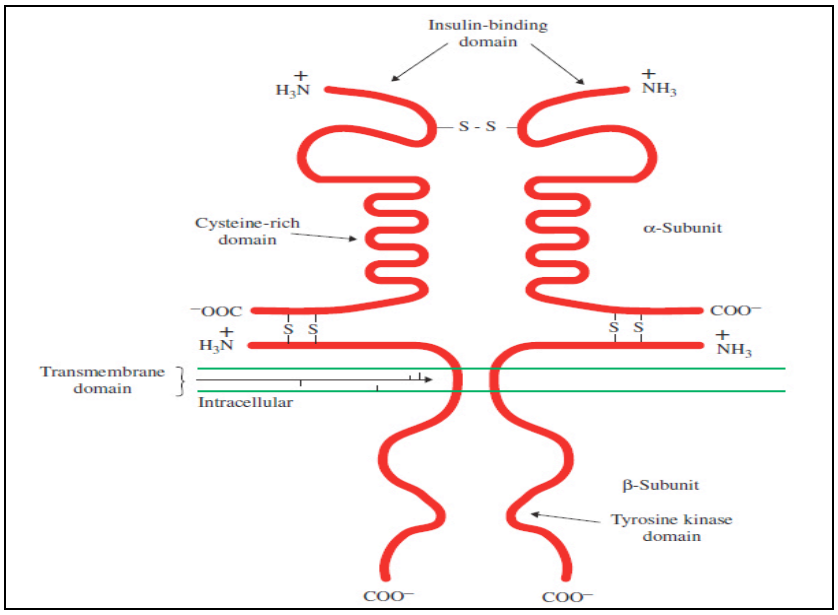


Figure 2.10. Structure of the insulin receptor (Bhagavan, 2011).

2.5.2.2. Insulin – receptor binding and signaling pathway

Binding of insulin to its receptor initiates a cascade of intracellular events in the cells. Insulin receptor is activated by auto-phosphorylation and then phosphorylates and activates the specific protein receptor substrates, insulin receptor substrate (IRS) and Src-homology-2-containing protein (Shc) (Figure 2.11).

Phosphorylation of IRS proteins interact with many other signaling proteins; a) growth factor receptor-bound protein-2 (Grb-2), and b) phosphatidylinositol -3-kinase (PI3K), so change the cellular functions. PI3K catalyzes the formation of PIP3 which activates Akt (known as protein kinase B, PKB) and atypical isoforms of protein kinase C (aPKC). Insulin also enhances the protein synthesis and glycogen synthesis, and promotes the glucose transport via the translocation of GLUT4 to the plasma membrane.

Phosphorylation of Shc promotes the formation of Shc/Grb-2/SOS complex which stimulates MAP kinase pathway, resulting in mitogenesis, cell growth, and differentiation (Mangmool S al, 2017).

2.6. Diabetes Mellitus (DM) and classification

Diabetes mellitus (DM) is a group of common metabolic disorders with a characteristic of the phenotypes of hyperglycemia. Complex interaction of genetics and environmental factors may cause several distinct types of DM.

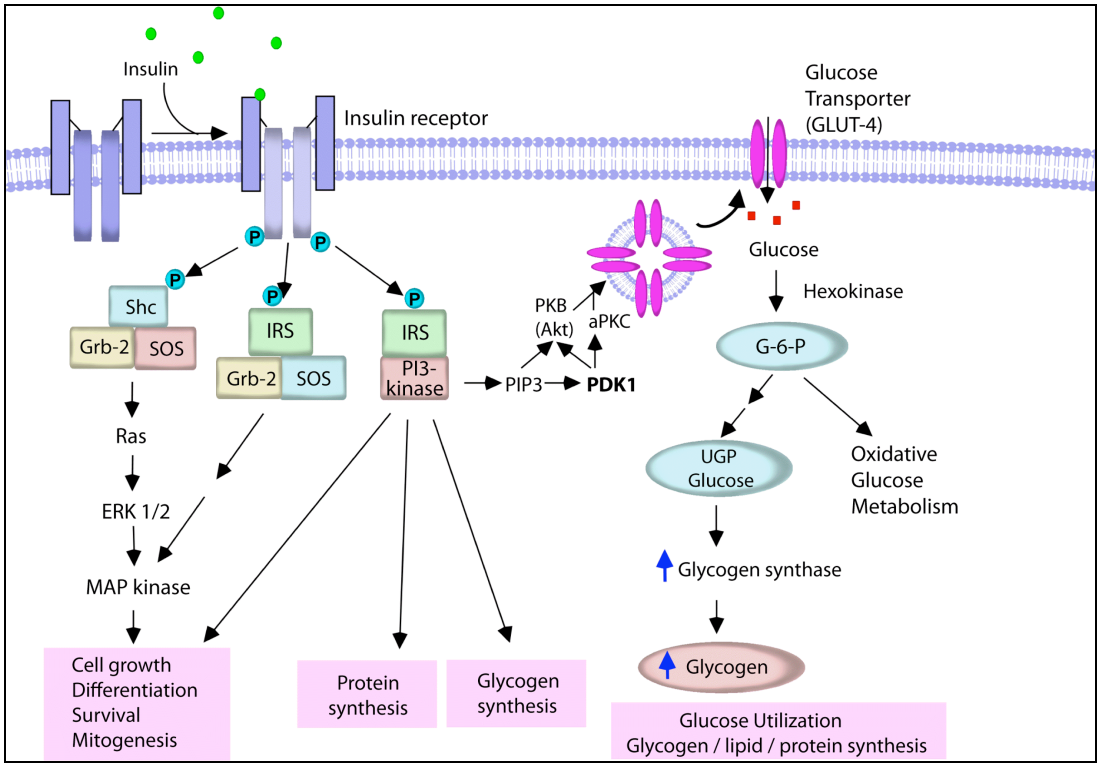


Figure 2.11. Insulin signaling pathway ((Mangmool S al, 2017).

Depending on the etiology of the disease, contributing factors of hyperglycemia may

be reduced insulin secretion, decreased glucose utilization, and increased glucose production. DM-associated metabolic dysregulations may cause the serious secondary pathophysiologic changes in multiple organ systems.

Incidence of DM is increasing worldwide and going to be a leading cause of morbidity and mortality in the near future (Powers A, 2013).

DM is classified as type 1 and type 2 according to genotypic and phenotypic characteristics. In type 1 DM (T1DM) is the result of complete or near-total insulin deficiency. Type 2 DM (T2DM) is heterogeneous and can be characterized by variable degrees of insulin resistance, impaired insulin secretion, and increased glucose production.

3. VITAMIN D AND DIABETES MELLITUS

3.1. Evidence from The Studies for The Vitamin D - Diabetes Mellitus relationship

There are many clinical and epidemiological studies which inverstigate the relationship between vitamin D deficiency and diabetes mellitus (T1DM and T2DM). The largest cross-sectional study to date, using data from the National Health Nutrition Examination Survey (NHANES) (n: 4492) showed the serum calcidiol (25(OH)D) concentrations were inversely associated with prevalence of diabetes in a dose-dependent pattern in United Sates (Scragg et al., 2004).

A gene expression study showed that CYP27B1 mRNA expression was lower in type 1 diabetic patients compared to healthy persons. This may be an evidence for the role of CYP27B1 (1- αhydroxylase) against type 1 diabetes (Ramos-Lopez et al., 2007).

Some in vitro and in vivo studies showed that impaired glucose-mediated insulin secretion due to vitamin D deficiency in rat beta cells can be restored by vitamin D supplementation (Norman AW et al,1980), (Cade C, 1986), (Kadowaki et al., 1984).

Epidemiological investigations may give conflicting results and do not present an interaction mechanism. Polymorphism based investigations seem to be more convincing.

3.2. The Molecular Evidence for The Vitamin D - Diabetes Mellitus relationship

Maestro et al. demonstrated that treatment with calcitriol for 24 hours increased in a dose-dependent manner the levels of the two major insulin receptor (IR) mRNAs (11 and 8.5 Kb) present in U-937 human promonocytic cells. The maximum values of mRNA levels (1.8-fold 11 Kb; 1.4-fold 8.5 Kb) are obtained with the addition of 10(-8) M calcitriol. In these cells an increase of both IR capacity and insulin responsiveness for glucose transport are also obtained (Maestro et al., 2000).

Maestro research group investigated the probable presence and location of VDREs (vitamin D response elements) in the promoter of insulin receptor gene (hIR). Three plasmids [phIR (-1819)-GL2 (wild type promoter), phIR (-1473)-GL2, and phIR (-876)-GL2] were constructed by linking the (-1819 to -271 bp), (-1473 to -271 bp), and (-876 to -271 bp) fragments of the hIR promoter to luciferase pGL2 basic vector. These plasmids

were used for transfecting the U-937 cell. The cells were treated or untreated with 10^{-8} M calcitriol for 24h. Luciferase activity were used as the indicator of hIR promoter enhancement. While the activity of wild promoter was increased 1.6-fold by the calcitriol, the activities of other two plasmids were enhanced 1.6- and 1.7-fold.

The shortest region extending -876 to -271 of the hIR promoter appears to contain VDREs and to be sufficient for induction by calcitriol. A computer search of candidate sequence located between -761 and -732 bp (5'CGTCGGGCCTGTGGGG-CGCCTCCGGGGGTC3'). Electrophoretic mobility shift assays revealed the specific recognition of this sequence by VDR.

This was the first identification of a VDRE in the hIR gene promoter (Maestro et al., 2003).

4. CONCLUSION

The connection between vitamin D deficiency and diabetes mellitus is known for nearly 40 years. Norman et al. showed that vitamin D deficiency inhibited the pancreatic secretion of insulin (Norman et al., 1980).

This is not a surprise, because the nuclear receptos of vitamin D has been detected in many tissues. Vitamin D has a variety of non-skeletal actions, including those on glucose metabolism (Stumpf WE, 2005).

Vitamin D is thought to have both direct (through activation of the vitamin D receptor) and indirect (via regulation of calcium homeostasis) effects on various mechanisms related to the pathophysiology of T2DM. These effects include pancreatic beta-cell dysfunction, impaired insulin action, and systemic inflammation (Holick, 2010).

Some mechanisms about the protective role of the vitamin D on the prevention of insulin resistance are reported (Zhu et al., 2005).

Vitamin D receptor protein binds to IKKβ protein, blocking TNFα-induced IKK complex formation and NF-κB activity (Chen et al., 2013). Suppression of the NF-kB activation by vitamin D seems to lessen skeletal muscle insulin resistance mediated by activated NF- kB (Wei Y et al, 2008). These results explain the mechanism of vitamin D induced decrease of insulin resistance in skeletal muscle.

Investigations on relationship between the insulin-like growth factor binding proteins and calcitriol indicated the role and importance of vitamin D in diabetes mellitus (Matilainen et al., 2005).

Decreasing of vitamin D with increasing of glucose level is related with insulin resistance, which means decreased insulin secretion from beta cell. (Rana, 2011)

It is shown that hypovitaminosis D and reduced IGF-1 are associated, individually, with metabolic syndrome. Physiological interactions between vitamin D and IGF-1 are investigated first time by Hyppönen et al. Data for the study were collected from 6,810 British white subjects in the 1958 cohort, surveyed during 2002–2004 (age 45 years). Both 25(OH)D and IGF-1 were inversely associated with metabolic syndrome and these results suggest that metabolic syndrome prevalence is lowest when both 25(OH)D and IGF-1 are

high (Hyppönen et al.,2008).

Maestro et al. investigated the probable presence and location of VDREs in the promoter of insulin receptor gene (hIR). They constructed some luciferase pGL2 basic vector-bound plasmids which contain the different length sequences (1549, 1203, and 606 bp) of hIR promoter. These plasmids are used for transfecting the human promonocytic cells U-937. All of the transfected and wild promoters activities are increased 60 % after treatment with and without 10^{-8} M calcitriol. A computer search located a short sequence of 30 bp (5'CGTCGGGCCTGTGGGG-CGCCTCCGGGGGTC3') in the promoter of hIR which is the candidate sequence for activation. This discovery may be probably the strongest evidence which indicate molecular interactions of VDR and hIR promoter (Maestro et al., 2003).

Effects of vitamin D in insulin target cells are summarized in (Figure XX). In peripheral insulin-target cells, vitamin D may directly enhance insulin sensitivity in several ways. The calcitriol enters the insulin-responsive cells from the circulation and interacts with the vitamin D receptor-retinoic acid x-receptor complex (RXR-VDR).

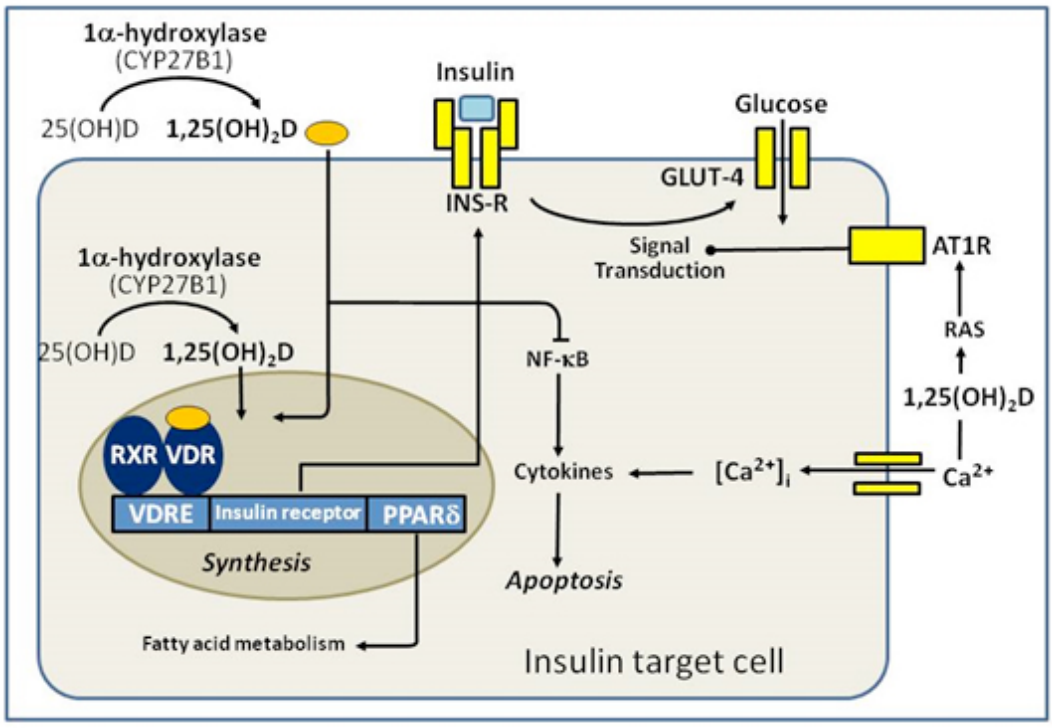


Figure 4.1. The effects of vitamin D in insulin target cells (Holick , 2010).

The complex binds to a vitamin D response element (VDRE), which is found in the human insulin receptor gene promoter, to enhance the transcriptional activation of the insulin receptor gene and increase the synthesis of insulin receptors (INS-R) which act to promote glucose uptake via the glucose transporter 4 (GLUT-4) receptor and / or by activating peroxisome proliferator–activated receptor delta (PPAR-δ), a transcription factor implicated in the regulation of fatty acid metabolism in skeletal muscle and adipose tissue. The effects of vitamin D may be mediated indirectly via regulating extracellular calcium (Ca2+), calcium flux through the cell and intracellular calcium (Ca2+). Vitamin D may promote beta cell survival by modulating the generation (through down regulation) of nuclear factor-kB.

We can conclude that vitamin D is one of the essential factors for the synthesis of both insulin and insulin receptor and an important preventive factor for diabetes mellitus.

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