

Preclinical study of vitamin D deficiency in the pathogenesis of metabolic syndrome in rats

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Abstract. – OBJECTIVE: To explore the impact of vitamin D deficiency (VD-) in the pathogenesis of metabolic syndrome (MetS).

MATERIALS AND METHODS: Models of (VD-) and (MetS) were induced in male Wister rats by dividing into four groups, group-I for the development of (VD-) by intraperitoneal injection of paricalcitol for 3 weeks, group II for (MetS) model by adding 10% fructose to their drinking water for 8 weeks, the group III for induction of combined (VD- + MetS) and group-IV as a control. Ultimately, the parameters of (VD-) and (MetS) were assessed at zero time and after 8 weeks.

RESULTS: Both (VD-) and (MetS) groups alone displayed a remarkable enhancement of blood pressure, glucose and insulin levels, triglycerides, cholesterol, and low-density lipoproteins with a reduction of high-density lipoproteins. Additionally, all distinguishing features of obesity were substantially increased. Nevertheless, the combined group (VD-+MetS) demonstrated an expeditious and substantial increase in all the aforesaid parameters compared to the (VD-) and (MetS) groups alone.

CONCLUSIONS: The hallmark of this study, reinforces a new frontier of awareness of the deleterious effect of (VD-) on each component of (MetS). Eventually, supplementation of vitamin D can circumvent the elements of (MetS) and needs further validation by determination of (VD-) molecular pathway on the parameters of (MetS).

Key Words:

Vitamin D deficiency, Metabolic syndrome, Interleukine, Homeostasis model assessment for insulin resistance index, Nuclear factor kappa β .

tion complex or a ligand for vitamin D receptor (VDR) which causes conformational changes that culminate in retinoic acid X-receptor (RXR) heterodimerization. Moreover, this heterodimer subsequently acts as a transcription factor, that activates target gene expression at the transcriptional cell level and thus, induces protein synthesis¹. Remarkably, over two hundred genes are regulated by calcitriol directly or indirectly, contributing to a wide variety of physiological functions. Moreover, vitamin D is currently of great community health interest because vitamin D deficiency (VD-) is a common worldwide condition with high prevalence². Although vitamin D has the main function on bone and skeletal health, it also has many important functions including modulation of the body's immune system, anti-inflammatory, anti-hypertensive activity, and antiproliferative effect in myocardial hypertrophy²⁻⁴. Besides, vitamin D has a role in glycemic control, insulin release, and lipid metabolism. So, all of these elucidate that vitamin D significantly impacts the body's metabolic functions⁵. Remarkably, several studies demonstrated an inadvertent and robust correlation between vitamin D deficiency and cardiometabolic risk, which invariably develops in the presence of other chronic disorders like obesity, diabetes mellitus, atherosclerosis, and hypertension²⁻⁷. Seemingly, vitamin D deficiency is a major contributory factor to the highly prevalent metabolic syndrome⁸. Thus, the basic essence directed towards our objective to conduct this research is to establish a concrete relationship between vitamin D deficiency and etiopathogenesis of cardiovascular metabolic syndrome and further to precisely clarify the underlying mechanisms and cardiovascular complications in rats. Ultimately, the outcome of combined vitamin D deficiency and metabolic syndrome were likewise investigated.

Introduction

An exclusive characteristic of the hormonal active configuration of vitamin D 1,25-dihydroxycholecalciferol (calcitriol) is the coordina-

Materials and Methods

This experimental work was done at King Fahd Center for Medical Research (KFCMR), King Abdulaziz University (KAU), Jeddah, Saudi Arabia, the KAU Research Ethical Committee granted the protocol of this animal study.

Chemical and Reagents

Paricalcitol (19-nor-1,25-dihydroxyvitamin D₂) purchased from AbbVie company, Diethyl ether, Krebs-Henseleit buffer solution, Buffered formalin, from Sigma-Aldrich® Co. (Bayouni Trading Co. Ltd., Al-Khobar, KSA).

Formulations

The Krebs-Henseleit buffer solution was used for maintaining the physiological media for the aorta and Fructose solution for induction of metabolic syndrome⁹.

Animals

We obtained 48 adult male Wister rats approximately weighing 140-220 g from King Fahad Medical Research Center, King Abdulaziz University, Jeddah, Saudi Arabia. Furthermore, the rats were kept singly in cages with all possible care, to keep the environmental conditions constant all the time. They were kept in optimal care for one week before the commencement of the experimental study with due care to provide free access to food and water all the time.

Diet of the Animals

The Harlan Teklad custom diet [TD87095] (vitamin D deficient diet) procured from Envigo® Company, Indianapolis, IN, USA. This is brown chow pellet deficient in vitamin D and comprises of 20% lactose, 15% corn starch, 17.6% protein, 5% fat, 2% calcium (Ca⁺²), and 1.25% phosphate (PO₄).

In addition, a regular rat diet was obtained from the Grain silos & flour mills organization, Jeddah KSA, and it contains: 20% protein, 4% fat, 1% calcium, 0.6% phosphate, and vitamin D 2.20 IU/g.

Experimental Design

The experimental animals were randomly categorized into four groups, each group comprising 12 rats (n= 12).

1. The control group (C) – recipient of regular diet and water *ad libitum*.

2. The vitamin D deficient group (VD) – from the first day this group of rats received the

Harlan Teklad custom diet, deficient in vitamin D, intended to develop vitamin D deficiency. These rats were injected intraperitoneally with paricalcitol [19-nor-1,25-dihydroxyvitamin D₂, an active form of vitamin D₂ (VDR agonist) Zemplar®] in a dose of 32 ng on days 1, 3, 5, 8, 10, and 12 to enhance the metabolism of endogenous vitamin D metabolites by cytochrome enzyme induction for 3 weeks¹⁰. Subsequently, the serum levels of vitamin D were measured in the third week to confirm the induction of vitamin D deficiency. The rats then remained on a vitamin D deficient diet and regular water *ad libitum* for 8 more weeks.

3. Metabolic syndrome group (MetS): Animals were fed a regular diet and 10% fructose was added to drinking water (equivalent to the diet containing 48-57% of fructose)^{11,12}. A regular diet and 10% fructose water were supplemented *ad libitum* for 8 weeks to induce metabolic syndrome⁹.

4. Combined group (vitamin D deficiency and metabolic syndrome) (VD + MetS): after rendering the rat's vitamin D deficient, the animals continued 10% fructose added to their drinking water and on a vitamin D deficient diet for 8 weeks to induce metabolic syndrome.

Body Weight Measurements

The body weights for rats were measured at the baseline and on the 8th week by the end of the experiment using a calibrated, sensitive weighing instrument (acquired from Al-Falak Electronic Industries Company, Jeddah, KSA) and calculated in gram (g).

Blood Samples Collection

The rats were anesthetized by utilization of diluted ether and then blood samples were aspirated from the choroid plexus by using capillary tubes and collected in a 5 ml blood tube then centrifuged at 4,500 rpm for 15 min. Blood samples were taken at Baseline, on the 3rd week to confirm vitamin D deficiency and on the 8th week of the experiment for the combined vitamin D deficient and metabolic syndrome groups.

The underlying biochemical evaluation was executed to study the effect of vitamin D deficiency in the pathogenesis of cardiovascular metabolic syndrome and the possible underlying mechanism:

1. Serum vitamin D level (assessment of vitamin D deficiency).

2. Serum calcium, phosphate & magnesium levels (assessment of mineral homeostasis).

3. Assessment of the parameters of metabolic syndrome (viz: diabetes mellitus, obesity, hyperlipidemia, and hypertension). The development of metabolic syndrome was evaluated according to the guidelines of NCEP ATP III (2001)¹³, which includes insulin resistance (increased insulin values, impairment of fasting blood glucose level (>5.5 mmol/L), hypertension (>130/85), glucose tolerance impairment and type II diabetes mellitus, enhanced abdominal obesity (increased waist circumference and BMI), hyperlipidemia (increased TG levels >1.995 mmol/L or decreased levels of HDL). Notably, the presence of a minimum of three of these parameters is labeled as having metabolic syndrome¹³.

Assessment of Vitamin D Level

Serum vitamin D level was measured at the baseline for all tested groups and on the 3rd week for the VD group and VD + MetS group. The determination of vitamin D level was done by Cobas[®] automated analyzer vitamin D total assay (F. Hoffmann-La Roche Ltd., Basel, Switzerland). The vitamin D level was calculated and expressed in terms of nmol/L¹⁰.

Assessment of Calcium (Ca^{+2}), Phosphate (PO_4^{-3}) and Magnesium (Mg^{+2}) Levels

Serum Ca^{+2} , PO_4^{-3} and Mg^{+2} levels were measured for all test groups, at the baseline and the 8th week by the end of the experiment by using Flex[®] reagent cartridge on the Dimension Vista[®] system, (obtained from Siemens Healthineers, Riyadh, KSA). Subsequently, their concentrations were measured using a bichromatic endpoint technique, Ca^{+2} at absorbance (577-450 nm), PO_4^{-3} at absorbance (340/700 nm) & Mg^{+2} at absorbance (600 and 510 nm). Their levels were calculated as mmol/L.

Hypertension

Measurement of blood pressure

Measurement of systolic and diastolic blood pressure was done using a Tail Cuff reader (Non-invasive technique) by using UGO BASILE[®] 58500 blood pressure recorder (obtained from Gemonio, Varese, Italy), at the baseline, as well as after 8 weeks for all tested groups by the technique of Widdop and Li, 1997^{14,15}.

Diabetes Mellitus

Measurement of glucose level

The serum glucose level was measured at the beginning of the experiment and the 8th week for all experimental groups with the use of Flex[®] reagent cartridge over the Dimension vista[®] Siemens system (acquired from Healthineers, Riyadh, KSA). However, the level of the glucose was measured by utilization of a bichromatic (at absorbance 340 and 383 nm) endpoint technique, finally computed as mmol/L.

Measurement of insulin concentration

The serum insulin level was measured at the baseline and the 8th week for all tested groups. This was accomplished by using RayBio[®] rat insulin ELISA [Acquired From-(CGenomix) Amman-Jordan]. Insulin level was calculated in pmol/L.

Insulin resistance index

The insulin resistance index was measured for all the tested groups by the utilization of the standardized method of Matthews et al¹⁶, with the mathematical equation:

$$\text{HOMA - IR} = \frac{\text{glucose concentration (mmol/L)} \times \text{insulin } (\mu\text{IU/L})}{22.5}$$

Dyslipidemia

Measurement of a lipid profile

The serum concentration of cholesterol, TG, HDL, and LDL was determined at the baseline and the 8th week for all tested groups. The lipid profile was determined using Flex[®] reagent cartridge on the Dimension Vista[®] SIEMENS (acquired from Healthineers, Riyadh, KSA). Serum cholesterol by polychromatic (540, 452 & 700 nm) endpoint technique, TG by bichromatic (510, 700 nm) endpoint technique, HDL was measured using bichromatic (600, 700 nm) technique, and LDL concentration was measured using a bichromatic (540, 700 nm) endpoint technique. These parameters were calculated in mmol/L.

Obesity

The percentage of body weight gain, BMI, abdominal fat weight, adiposity index, and AC of the rats were measured as indicators of obesity^{17,18}, essentially, this was done at the start of the experiment and then at the 8th week for all tested groups. This was done using an elec-

Table I. Serum vitamin D level (nmol/l) at the baseline and on the 3rd week after induction of vitamin D deficiency.

Animal Groups Duration	Control (n = 12)	VD- (n = 12)	MetS (n = 12)	(VD- +MetS) (n = 12)
Baseline values	84.3 ± 4.19	88 ± 0.78	88.24 ± 3.11	88.15 ± 3.87
3 rd week of vitamin D deficiency induction (< 12.5 nmol/l)	84.3 ± 4.19	7.33 ± 0.09***	88.24 ± 3.11	7.96 ± 0.24***

*** $p < 0.0001$ compared to the respective baseline values.

tronic, calibrated weighing scale (acquired from Al-Falak Electronic Industries Company, Jeddah, KSA). Adiposity index was measured according to the formula:

$$\text{Adiposity index} = \frac{\text{Perennial WAT} + \text{Retroperitoneal WAT} + \text{Epididymal WAT} \times 100}{\text{Bodyweight}}$$

Statistical Analysis

All biochemical outcomes were expressed as mean ± standard error. A paired *t*-test was used to evaluate the mean change in biochemical measurements within the experimental group from time zero to 3 and 8 weeks. One-way analysis of variance (ANOVA) followed by Tukey's HSD test was performed and statistical comparisons among the various groups were conducted using a Graph-Pad prism program and excel 2016 data analysis (La Jolla, CA, USA). Statistical significance is set at $p < 0.05$.

Results

Serum Vitamin D Level

By the 3rd week, the vitamin D deficient groups [VD-& (VD- + MetS)] showed a significant decrease in vitamin D level (< 12.5 nmol/l) with a p -value < 0.0001 compared to the baseline value (Table I).

Serum Level of Calcium (Ca^{+2}), Phosphate (PO_4^{-3}), and Magnesium (Mg^{+2})

The metabolic syndrome group (MetS) alone exhibited a significant decrease ($p < 0.001$) at the end of 8 weeks (Table II). For phosphate levels, only the vitamin D deficient group (VD) showed a non-significant decrease (p -value > 0.05) vs. each baseline. Moreover, the magnesium levels for all tested groups showed a non-significant decrease (p -value > 0.05) vs. each baseline.

Hypertension

Measurement of blood pressure

A significant increase in both systolic & diastolic blood pressure ($p < 0.0001$). VD-: vitamin D deficiency group, MetS: a group of metabolic syndromes (VD- + MetS): collective group (both vitamin D deficiency and metabolic syndrome), in comparison to the control group (Table III).

Diabetes Mellitus

Glucose level measurement

The level of glucose was found to be significantly augmented ($p < 0.05$) in all the groups compared to the control group (Table IV). Moreover, the combined group (VD- + MetS) demonstrated a significant increase in glucose level ($p < 0.05$) compared to the vitamin D deficient (VD-) and metabolic syndrome (MetS) groups alone.

Table II. Serum calcium, phosphate and magnesium levels (mmol/l) at the baseline and after 8 weeks.

Mineral	Duration	Calcium (mmol/l)		Phosphate (mmol/l)		Magnesium (mmol/l)	
		Baseline	After 8 weeks	Baseline	After 8 weeks	Baseline	After 8 weeks
Animal groups	Control n = 12	2.63±0.014	2.63±0.014	2.42±0.13	2.42±0.13	0.9±0.03	0.9±0.03
	VD- n = 12	2.36±0.02	2.34±0.02	2.03±0.07	2.01±0.046	0.87±0.01	0.87±0.02
	MetS n = 12	2.74±0.03	2.49±0.02**	2.7±0.06	2.1±0.09**	0.89±0.018	0.87±0.026
	(VD- + MetS) n = 12	2.60±0.03	2.66±0.03i	2.63±0.07	2±0.07**	0.89±0.018	0.852±0.02

**Significant ($p < 0.001$) compared to baseline of each group.

Table III. Systolic and diastolic blood pressure (mmHg) measurements at baseline and after 8 weeks.

Parameter measured	Groups Duration	Control (C) n = 12	Vitamin D deficient (VD-) n = 12	Metabolic syndrome (MetS) n = 12	Combined (VD- + MetS) n = 12
Systolic blood pressure (mmHg)	Baseline	120±0.57	121.5±0.51	122±0.41	122±0.53
	After 8 weeks	120±0.57	135±0.7 ***	150.5±1.4 ***	166.33±3.8 ***
Diastolic blood pressure (mmHg)	Baseline	81±1.3	81±1.3	81±1.45	81±1.4
	After 8 weeks	81±1.3	94.17±1.7***	98.5±1.22***	111.5±2.21***

***significant ($p < 0.0001$) vs. the control group.

Insulin level measurement

Table V shows a significant increase in serum insulin level ($p < 0.0001$) for the vitamin D deficient (VD-), metabolic syndrome (MetS), and combined (VD- + MetS) groups produced compared to control groups. Interestingly, the combined group exhibited a significant increase in insulin level ($p < 0.0001$) compared to other groups.

Insulin resistance index

As illustrated in Table VI the equilibrium evaluation model for insulin resistance index (HOMA-IR) was found to be significantly augmented in all test groups ($p < 0.0001$) compared to the control group. Moreover, the vitamin D deficient (VD-) group and the combined (VD- + MetS) group had significant increase in insulin resistance index ($p < 0.001$) for (VD-) and ($p < 0.0001$) for (VD- + MetS)) compared to the metabolic syndrome (MetS) group alone.

Dyslipidemia

Triglycerides (TG) level

Table VII and Figure 1 showed that the (VD-) group produced a significant increase in TG serum level ($p < 0.05$) compared to the control group whereas, both (MetS) and (VD- + MetS) groups produced a significant increase in TG serum level ($p < 0.001$) compared to the control group.

Total cholesterol level

Table VII showed that the total cholesterol was significantly increased ($p < 0.05$) for the vitamin D

deficient (VD-) group and the combined group (VD- + MetS) in comparison to the control group. In contrast, the metabolic syndrome illustrated a non-significant ($p > 0.05$) when equated to the control group.

HDL level

Conventionally, the level of HDL was demonstrated to be quite significantly decreased in the metabolic syndrome (MetS) group ($p < 0.05$) when compared with the control group (Table VII). However, Figure 1 showed that HDL level was increased from baseline to the 3rd week of the experiment followed by a significant decrease in HDL levels after 8 weeks for the vitamin D deficient (VD-) group ($p < 0.05$) and the combined (VD- + MetS) group ($p < 0.0001$).

LDL level

Table VII showed that LDL level was significantly increased in vitamin D deficient (VD-) group ($p < 0.001$) and the combined (VD- + MetS) group ($p < 0.0001$) while the metabolic syndrome (MetS) group showed a non-significant ($p > 0.05$) difference vs. control group.

Obesity

Percentage of weight gain

Table VIII and Figure 2A showed a significant increase in the percentage of weight gain of the vitamin D deficient (VD-) group ($p < 0.001$), metabolic syndrome (MetS) group ($p < 0.0001$), and the combined (VD- + MetS) group ($p < 0.0001$) compared to the control group.

Table IV. Serum glucose level (mmol/L) at baseline and after 8 weeks.

Parameter measured	Groups Duration	Control n = 12	Vitamin D deficient (VD-) n = 12	Metabolic syndrome (MetS) n = 12	Combined (VD- + MetS) n = 12
Serum Glucose level (mmol/L)	baseline	7.2±0.02	7.23±0.01	7.47±0.02	7.24±0.02
	After 8 weeks	7.2±0.02	8.3±0.5*	8.2±0.22*	10.2±0.8*

*Significant ($p < 0.05$) vs. control group.

Table V. Serum insulin level (Pmol/L) after 8 weeks.

Groups parameter measured	Control n = 12	Vitamin D deficient (VD-) n = 12	Metabolic syndrome (MetS) n = 12	Combined (VD- + MetS) n = 12
Insulin Level (Pmol/L)	99±6.05	379±15***	272±19***	475.5±5.9***

***Significant ($p < 0.0001$) vs. control.

Body mass index (BMI)

As shown in table VIII and Figure 2B, a significant increase in body mass index ($p < 0.001$) of all tested groups [VD-, MetS & (VD- + MetS)] compared to the control. Moreover, the combined (VD- + MetS) group showed a significant ($p < 0.001$) increase in BMI compared to each metabolic syndrome (MetS) group and vitamin D deficient (VD-) group.

Abdominal circumference (AC)

Table VIII and Figure 3 showed that the abdominal circumference was significantly ($p < 0.0001$) increased in all tested groups [(VD-, MetS & (VD- + MetS))] when compared with the control group.

Total abdominal fat

Table VIII and Figure 4 illustrated that the total abdominal fat was significantly increased for the vitamin D deficient (VD-) group ($p < 0.05$), metabolic syndrome (MetS) group ($p < 0.001$) and vitamin D deficiency with metabolic syndrome (VD- + MetS) group ($p < 0.001$) compared to the control group. Furthermore, both the metabolic syndrome (MetS) group and the combined (VD- + MetS) group produced a significant increase in total abdominal fat ($p < 0.01$) vs. the vitamin D deficient (VD-) group.

Adiposity index: Table VIII and Figure 5 showed that the adiposity index was significantly increased for the vitamin D deficient (VD-) group ($p < 0.05$), metabolic syndrome (MetS) group ($p < 0.001$) and vitamin D deficiency with metabolic syndrome (VD- + MetS) group ($p < 0.001$) compared to the control group.

Evaluation of the emerging metabolic syndrome by NCEP ATP III (2001)

Human metabolic syndrome criteria could be extrapolated to animal measurements except for the central obesity measurements (Table VIII). All test groups (VD-), (MetS), and (VD- + MetS) resulted in a significant increase in insulin resistance, glucose level, blood pressure, obesity parameters, and TG level compared to the control group as mentioned above in detail. Table VIII showed that the metabolic syndrome (MetS) and the combined (VD- + MetS) groups accomplished three criteria of metabolic syndrome (marked (√) in the table) according to NCEP-ATP III.

Discussion

A Noteworthy Skepticism Is that Vitamin D Deficiency Is Related to Metabolic Syndrome

Essentially, vitamin D is a prime nutrient prerequisite to maintain the standard health and it is an active component of the steroid nuclear hormone family^{1,5}. Low vitamin D level is linked with several diseases like osteoporosis, rickets, cancer, and cardio-metabolic complications^{2-4,19,20}. In addition, there is a great suspicion that vitamin D deficiency is related to metabolic syndrome (MetS), a complex clinical condition that comprises abdominal obesity, disturbed glucose metabolism, atherogenic dyslipidemia, and essential hypertension^{6-8,21}.

The Implication of Vitamin D in the Release and Action of Insulin

In this study, vitamin D level was significantly reduced in all three groups (VD-, MetS alone and

Table VI. Mean serum insulin and glucose levels and insulin resistance index after 8 weeks.

Groups Parameter measured	Control n = 12	VD- n = 12	MetS n = 12	(VD- + MetS) n = 12
Insulin (μIU/L)	14.256±0.83	54.576±2.1	39.168±1.4	68.472±0.83
Glucose (mmol/L)	7.38±0.02	8.3±0.5	8.2±0.22	10.2±0.8
HOMA-IR	4.678±0.21	20.173±2.4***	14.434±0.7***	31.041±1.43***

***Significant ($p < 0.0001$) vs. control.

Table VII. Serum cholesterol, triglycerides, HDL, and LDL levels (mmol/L) after 8 weeks.

Groups Parameter measured	Control (C) n = 12	Vitamin D deficient (VD-) n = 12	Metabolic syndrome (MetS) n = 12	Combined (VD- + MetS) n = 12
Triglyceride (mmol/L)	0.83±0.03	1.143±0.03*	1.74±0.09**	2.3±0.18**
Total cholesterol (mmol/L)	1.35±0.04	1.65±0.08*	1.4±0.04	1.76±0.07***
HDL level (mmol/L)	1.26±0.03	1.5±0.04	1.01±0.06*	1.33±0.07
LDL level (mmol/L)	0.14±0.003	0.22±0.02**	0.153±0.002	0.2±0.009***

*Significant ($p < 0.05$) vs. control group. **significant ($p < 0.001$) vs. control group. ***significant ($p < 0.0001$) vs. control group.

combined (VD- + MetS) at the end of 3 weeks (Table I). This was well illustrated by the significant increase in insulin resistance, blood glucose level, blood pressure, serum lipids, and obesity (Table III-VIII). The present results were explained based on many different mechanisms like facilitation of the synthesis and secretion of insulin directly by the metabolites of vitamin D^{22,23}. Further substantiation of the role of vitamin D is explained, based on its control over cytosolic calcium-binding protein found in the β -cells. Seemingly, vitamin D performs as a regulator of depolarization-induced insulin release by eventual calcium regulation is remarkably authenticated²⁴⁻²⁸.

Accumulating evidence implicates that vitamin D preserves the release of insulin and facilitates insulin actions in the tissues that are responsive to the insulin, by controlling the intracellular and extracellular calcium pools²⁶. Nevertheless, numerous studies revealed a strong relation between hypovitaminosis D and cardiometabolic syndrome^{29,30}. Likewise, many epidemiological

studies have shown that there is an inverse correlation between the level of vitamin D, blood glucose, insulin resistance, and the prevalence of type 2 diabetes^{31,32}. Thus, we wish to lay the emphasis, that our present study has unequivocally demonstrated a significant increase in insulin level and insulin resistance index (HOMA-IR) in all test groups compared to the control group. The insulin level and the insulin resistance index (HOMA-IR) were significantly higher in the combined (VD- + MetS) group (Table VI and VIII). This noteworthy finding is very well corroborated by the fact that TNF and IL-6 are immune mediators which interfere with insulin signaling that mediates insulin resistance^{24,33}.

The Contemplation of Electrolyte Imbalance in Diabetes and Metabolic Syndrome due to an Imbalance of Vitamin D

The reflection of underlying details of serum levels of Calcium (Ca²⁺), Phosphate (PO₄⁻

Table VIII. Metabolic syndrome parameter according to NCEP-ATP III.

NCEP ATP III	Insulin resistance HOMA-IR	Glucose level > 5.5 mmol/L	Dyslipidemia TG > 1.695 mmol/L	Blood pressure > 130/85 mmHg	Central obesity	
					BMI	AC
Control	14.256±0.8	7.2±0.02	0.83±0.03	120±0.57	0.45±0.01	15.3±0.26
VD-	20.173±2.4	8.3±0.5 √	1.143±0.03	135±0.7 /	0.52±0.01	17.33±0.30
MetS	14.434±0.7	8.2±0.22 √	1.74±0.09 √	94.17±1.7 √	0.55±0.008	17±0.23
VD- + MetS	31.041±1.43	10.2±0.8 √	2.3±0.18 √	150.5±1.4 /	0.61±0.01	19.34±0.14
				98.5±1.22 √		
				166.33±3.8 /		
				111.5±2.21 √		

VD-: Vitamin D deficiency group, MetS: a group of metabolic syndrome, (VD- + MetS): collective group (both vitamin D deficiency and metabolic syndrome).

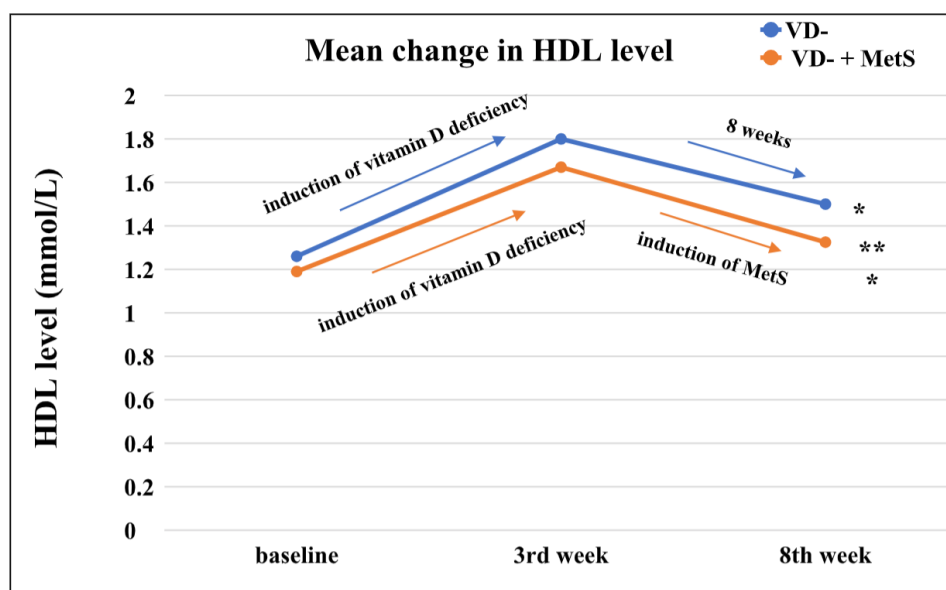


Figure 1. Mean change OF HDL level at baseline, at the 3rd week after induction of vitamin D deficiency, and after 8 weeks (n=12 rat/group). ***significant ($p < 0.0001$) vs. the level on the 3rd week, *significant ($p < 0.05$) vs. the level on the 3rd week.

³), and Magnesium (Mg²⁺) in our results (Table II), could be emphatically attributed to electrolyte imbalance as a consequence of diabetes and metabolic syndrome due to derangement of PTH-vitamin D endocrine system^{34,35}. Interestingly, the enriched phosphate and calcium diet given to the rats in this study fails to restore the phosphate level in the combined (VD- + MetS) group due to the induction of metabolic syndrome. This observation is significantly endorsed by a streptozotocin-induced diabetes study in which rats developed hypocalcemia³⁶. In addition, our study has revealed no changes in the magnesium level in all the animal groups, nevertheless several studies demonstrated a correlation between hypomagnesemia with hyperglycemia and diabetes^{37,38}. However, magnesium diminution leads to hypocalcemia mostly due to impaired PTH release or renal tubule and skeletal resistance to the action of PTH³⁹.

Cardiovascular Effects of Vitamin D Deficiency as Risk Factors for the Development of the Metabolic Syndrome

Induction of vitamin D deficiency resulted in a noteworthy enhancement of both systolic and diastolic blood pressure in all test groups with the greatest increase in the combined group (Table III and VIII), which is a remarkable observation emerged from this study that needs to be

highlighted. This evidence is splendidly sustained by different studies indicating the expression of genetic factors complicating the myocardial and vascular functions^{40,41}. Furthermore, recent past studies reported that vitamin D-depleted male rats, developed a significant increase in blood pressure by a mechanism involving renin, and this is independent of Ca²⁺ or PTH^{29,42,43}. The induction of hypertension in the VD- group, MetS, and combined group (Table III) is further reinforced and validated indeed by the finding that vitamin D and its related metabolites can reduce contraction of the vascular tone in rats with elevated blood pressure. This is accomplished, by affecting the calcium influx and reducing the production of endothelium-derived contracting factors (EDCF)^{44,45}. Furthermore, VDR stimulation induces the production of nitric oxide (NO) in the endothelium and improves the angiogenic properties of the endothelium progenitor cells (EPC)^{43,46}. These studies strongly support that vitamin D has a key regulatory part and represents a new paradigm in blood pressure homeostasis.

Impact of Vitamin D Deficiency on Lipids and Triglycerides

A momentous highlight of our present work has revealed that vitamin D deficient group (VD-) and (VD- + MetS) induced a significant increase in TG, LDL and cholesterol levels compared to the control group (Table

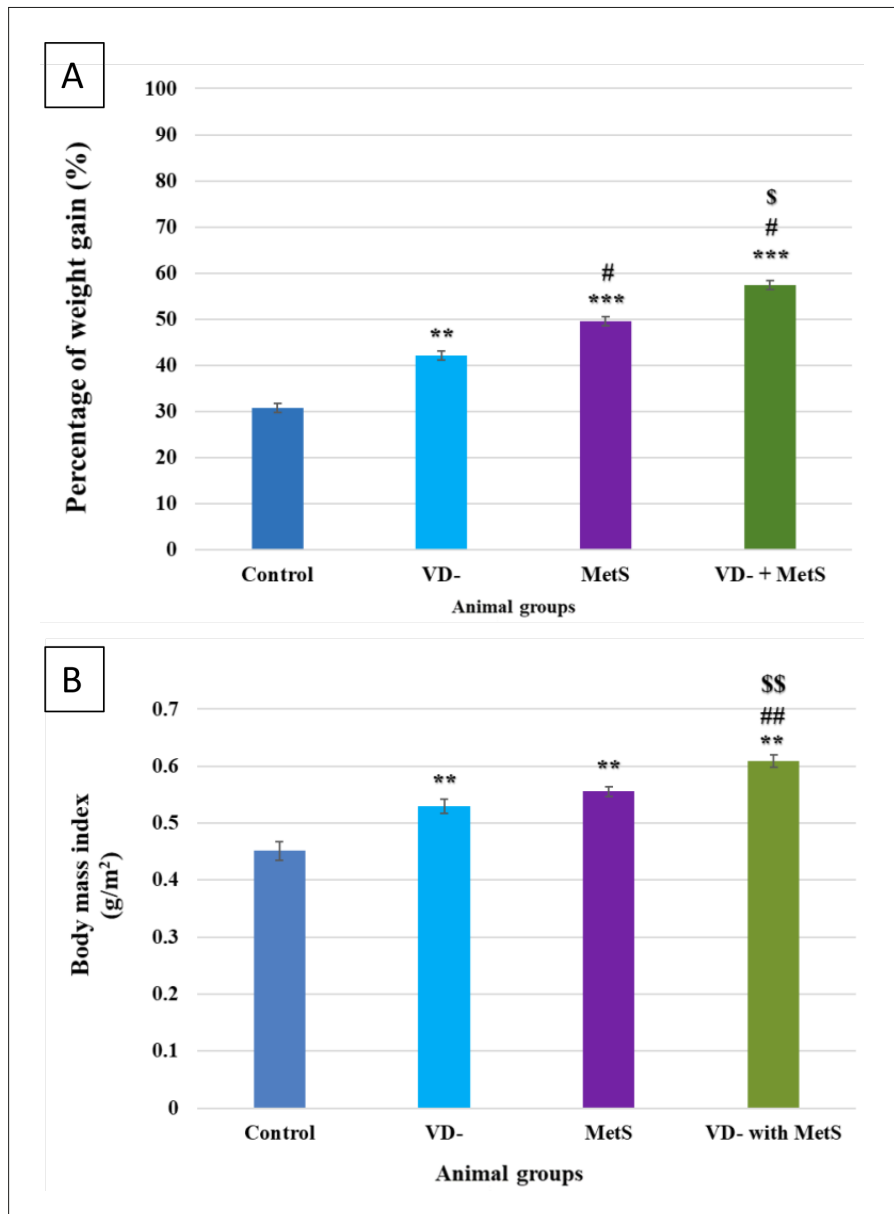


Figure 2. Mean \pm S. E of: (A) Percentage of weight gain, (B) body mass index of control VD-, MetS and (VD- + MetS) groups after 8 weeks (n=12 rat/group). ***Significant ($p < 0.0001$) vs. control group, **significant ($p < 0.001$) vs. control group, #significant ($p < 0.05$) vs. (VD-) group, \$ significant ($p < 0.01$) vs. (MetS) group.

VII&VIII). Our present observation is concordant with a study involving vitamin D deficient mice, which reported elevated TG and total cholesterol levels. In addition, vitamin D has an impact on insulin secretion and sensitivity which, indirectly stimulates the metabolism of lipid⁴⁷. Moreover, there is strong evidence of an association between the state of vitamin D deficiency and insulin resistance⁴⁸. Conversely, in the event of insulin resistance and conse-

quent hyperglycemia, the intracellular reactive oxygen species (ROS) has been observed to be substantially elevated and this accumulation of ROS, in turn activates nuclear factor-kappa B (NF- κ B) leading to liver inflammation⁴⁹. Several recent studies emphatically exhibited that vitamin D diminishes the expression of NF- κ B and conversely augments the expression of peroxisome proliferator-activated receptor alfa (PPAR- α)^{50,51}.

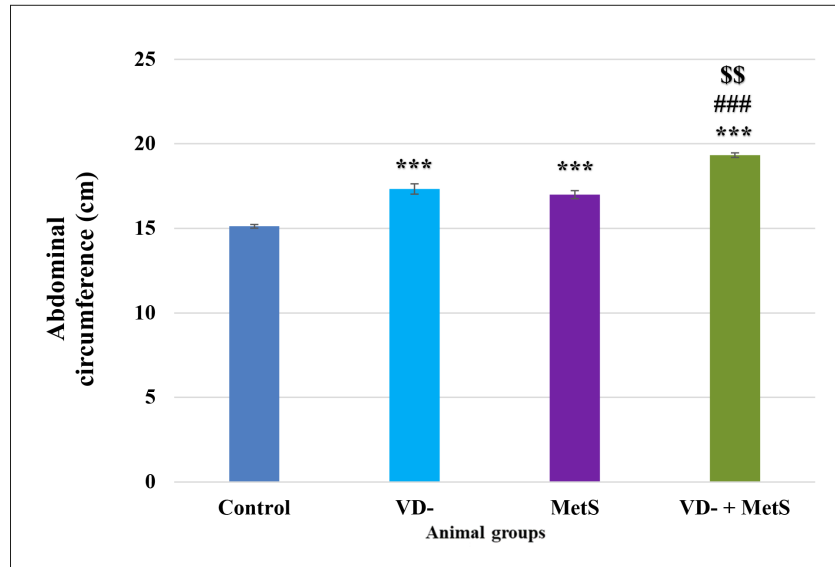


Figure 3. Mean± S.E of abdominal circumference for control, VD-, MetS and (VD- + MetS) groups after 8 weeks (n=12 rat/group). ***significant ($p < 0.0001$) vs. control group, ### significant ($p < 0.0001$) vs. MetS group, \$\$ significant ($p < 0.001$) vs. VD- group.

Attribution of Vitamin D Deficiency to the Distinctive Characteristic of Metabolic Syndrome

A distinct, noteworthy characteristic and longstanding focus of interest of metabolic syndrome is the preponderance of abdominal obesity, an unequivocal quality noticeably correlated

with vitamin D deficiency⁵². This association has been categorically recognized in our current study (Figure 2-5 and Table VIII) and has demonstrated a significant increase in AC, BMI, percentage of weight gain, adiposity index, and total abdominal fat weight in all the experimental groups. A finding of particular interest is re-

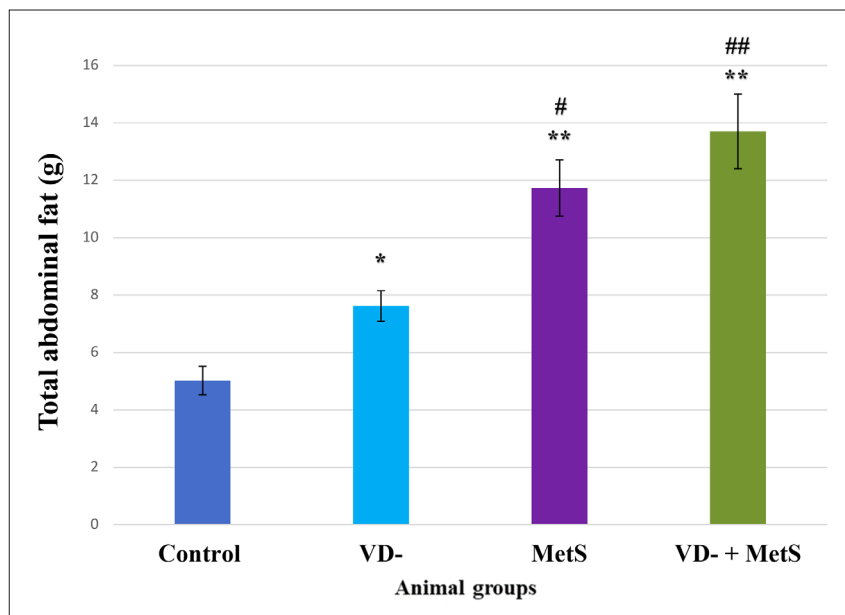


Figure 4. Mean ±S. E of total abdominal fat for control, VD-, MetS and (VD- + MetS) groups after 8 weeks (n=12 rat/group). **significant ($p < 0.001$) vs. control group, *significant ($p < 0.05$) vs. control group, #significant ($p < 0.05$) vs. VD- group, ##significant ($p < 0.001$) vs. VD- group.

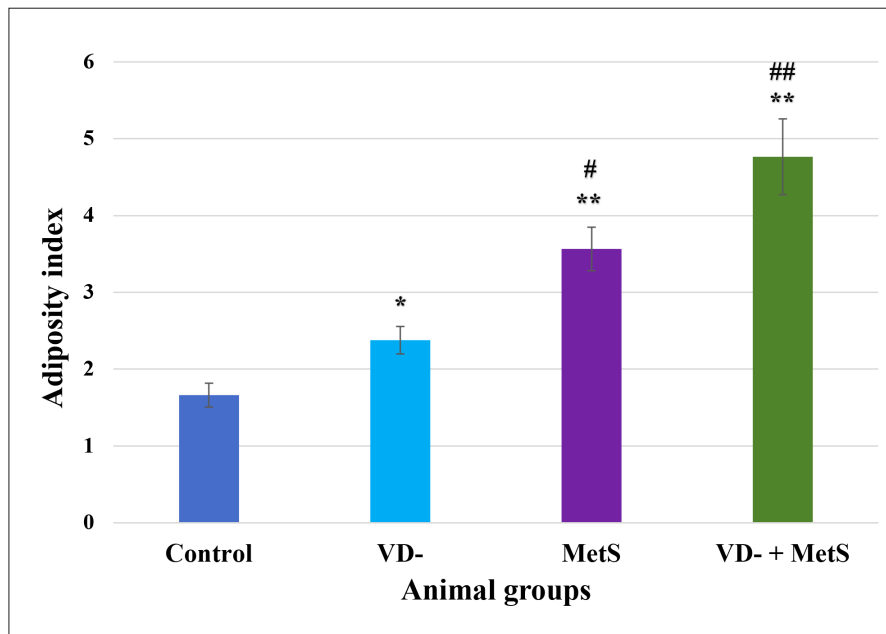


Figure 5. Mean \pm S.E. of adiposity index for control, VD-, MetS and (VD- + MetS) groups after 8 weeks (n=12 rat/group). **significant ($p < 0.001$) vs. control group, *significant ($p < 0.05$) vs. control group, # significant ($p < 0.01$) vs. VD- group, ##significant ($p < 0.001$) vs. VD- group.

lated to the mean increase in BMI and AC for the (VD-) group and (MetS) group which were similar, while a stronger effect was illustrated in the combined group than in either group alone (Figure 2-4 and Table VIII). This finding undoubtedly reinforces the deleterious effect of vitamin D deficiency on metabolic syndrome. Intriguingly, a reassuring cross-sectional study acquired from the insulin resistance atherosclerotic study of Hispanic and black adults exhibited a higher level of calcidiol and this, in turn, is inversely related to the body mass index (BMI) and parameters of visceral and subcutaneous adiposity^{53,54}. Unequivocally, a higher risk of metabolic syndrome, obesity, increased waist circumference, and atherogenic dyslipidemia were found to be strongly associated with low calcidiol levels and high PTH concentrations^{52,55}. The agglomeration of abdominal adipose tissue leads to the progression of dyslipidemia, hyperglycemia, and hypertension⁵⁶.

Correlation of Hypovitaminosis D with Inflammatory Markers and Obesity

Another focus of interest of this study is that hypovitaminosis D is related to increased inflammatory markers such as: TNF and C-reactive protein in obese subjects⁵⁷. The basic essence of

the several mechanisms recommended to explain the correlation between vitamin D deficiency and obesity incorporates the requisitioning of vitamin D in adipose tissue, which is attributable to its inherent lipophilic property and diminished synthesis of calcidiol in the liver as a result of hepatic steatosis or to the proinflammatory cytokines inhibitory actions⁵⁸.

Thus, the present study has illustrated a new frontier of awareness that vitamin D deficiency can induce different components of metabolic syndrome; hypertension, diabetes mellitus, insulin resistance, dyslipidemia, and obesity.

Conclusions

The basic hallmark of this study has emphatically validated that hypovitaminosis D could worsen the progression of each component of the metabolic syndrome. Further studies are required to elucidate the underlying mechanism and molecular pathways of vitamin D deficiency in the progression of metabolic syndrome. Eventually, healthcare providers should supplement vitamin D to minimize the variety of elements of metabolic syndrome.

Conflicts of Interest

We declare no conflict of interest.

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Ethics Approval

King Abdulaziz University (KAU), Jeddah, Saudi Arabia, the KAU Research Ethical Committee granted the protocol of this animal study.

Authors' Contribution

S.K. Mahjoub: formal analysis, experimental work, investigation, M.A.A. Sattar Ahmad: conceptualization, data curation, supervision, and methodology F.O. Kamel: visualization, original draft preparation, M. Alseini: methodology and statistical analysis, L.M. Khan: interpretation of data for the work, writing review and editing, validation, and software. All authors have read and agreed to the published version of the manuscript.

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