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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) g[illegible] alone displayed a remarkable enhanceme[illegible]of blood pressure, glucose and insulin levels, [illegible] glycerides, cholesterol, and low-density lipop[illegible]teins with a reduction of high-d[illegible]y lipopr[illegible]teins. Additionally, all distingu[illegible]tures o[illegible] obesity were substantially i[illegible]ased. [illegible]everthe-less, the combined grou[illegible]D-+Me[illegible] demonstrated an expeditious an[illegible]esta[illegible] in all the aforesaid p[illegible]mete[illegible]pared t[illegible] (VD-) and (MetS) gr[illegible]s alone.

CONCLUSION: [illegible]he hallmar[illegible]this study, reinforces a n[illegible]ier of awar[illegible]s of the deleterious e[illegible]ct of[illegible]) on each component of (MetS). Eventually, [illegible]lementation of vitamin D [illegible] circumvent t[illegible]ements of (MetS) and n[illegible]s further validation[illegible] determination of (VD[illegible]olecu[illegible]pathway on the parameters of (M[illegible]

Key Wo[illegible]
*Vitamin [illegible]cy, Metabolic syndrome, Interleuk-[illegible] Homeost[illegible]odel assessment for insulin resistance [illegible]ex, Nuclear factor kappa β.

Introduction

An exclusive characteristic of the hormonal active configuration of vitamin D 1,25-dihydroxycholecalciferol (calcitriol) is the coordination complex or a l[illegible]d for[illegible]in D r[illegible]tor (VDR) which c[illegible]es confor[illegible]nal[illegible]anges that culminate[illegible]pic acid X-r[illegible] (RXR) heterodimer[illegible]tion.[illegible]eover, this heterodimer subsequently acts as a[illegible]scription factor, that activa[illegible]get gene exp[illegible]n at the transcription[illegible]cell level and thus, [illegible]duces protein synth[illegible][1]. Remarkably, over two hundred genes a[illegible]egulated by[illegible]citriol directly or indirectly, co[illegible]uting to a[illegible]de variety of physiological func[illegible]. Mor[illegible]er, vitamin D is currently of great c[illegible]y health interest because vita-[illegible]in D deficiency (VD-) is a common worldwide [illegible]with high prevalence[2]. Although vita-[illegible]in D[illegible]as 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 hypertrophy[2-4]. 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 functions[5]. 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 hypertension[2-7]. Seemingly, vitamin D deficiency is a major contributory factor to the highly prevalent metabolic syndrome[8]. 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.

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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 syndrome[9].

Animals

We obtained 48 adult male Wister rats approximately weighing 140-220 g from King Fahad Medical Research Center, King Abdulaziz versity, Jeddah, Saudi Arabia. Furthermore rats were kept singly in cages with all poss care, to keep the environmental conditions c stant all the time. They were kept imal ca for one week before the com ce of the experimental study with du re to p ide free access to food and water a time

Diet of the Anim

The Harlan d custom d TD87095] (vitamin D de) procured f Envigo® Company, Ind anapoli USA. This is brown chow pe deficient in v in D and comprises of 20 ctose, 15% corn h,17.6% protein, 5% 2% ca m (Ca^{+2}), and 1.25% phosphate (1

n, a regu at diet was obtained from he Gra los & mills organization, Jeddah A, and : 20% protein, 4% fat, 1% calm, 0.6% p phate, and vitamin D 2.20 IU/g.

ntal Design

The experimental animals were randomly categorized into four groups, each group comprising 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 D_2, an active form of vitamin D_2 (VDR ago Zemplar®] in a dose of 32 ng on days , 5, 8, 10, and 12 to enhance the metabol of endogenous vitamin D metabolites by c rome enzyme induction for 3 weeks[10] Subse ly, the serum levels of vitamin were mea in the third week to conf the in uction vitamin D deficiency. Th s ther ained on a vitamin D deficient diet r ar wate *ad libitum* for 8 more ks.

3. Metabolic drome gro Me Animals were fe lar diet and fructose was added drink water (equivalent to the diet containing 48-57 fructose)[11,12]. A regular d 10% fructo ater were supplemen *ad libitum* for 8 we ks to induce metabo syndrome[9].

Combined oup (vitamin D deficiency an tabolic s rome) (VD^{-} + MetS): after rend the r vitamin D deficient, the animals 10% fructose added to their inking water and on a vitamin D deficient diet ks 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 syndrome[13].

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 assay (F. Hoffmann-La Roche ltd., Basel, Switzerland). The vitamin D level was calculated and expressed in terms of nmol/L[10].

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

Serum Ca^{+2}, PO_4^{-3} and Mg^{+2} 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 bichromatic endpoint technique, Ca^{+2} at absorbance (577-450 nm), PO_4^{-3} 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® Siemen system (acquired from Healthineers, Riyadh, KSA). However, the level of blood glucose was measured by utilization of a bichromatic (at absorbance 340 and 383 nm) endpoint technique, finally compared 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) Ammanian]. Insulin level was calculated in µIU/L.

Insulin resistance index

The insulin resistance index was measured for all tested groups by the utilization of the standard method of Matthews et al[16], with the mathematical equation:

HOMA - IR = glucose concentration (mmol/L) insulin (µIU/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
3rd week of vitamin D deficiency induction (< 12.5 nmol/l)	84.3 ± 4.19	7.33 ± 0.09***	88.24 ± 3.11	7.96 ± 0.2

***$p < 0.0001$ 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:

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

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 time zero to 3 and 8 weeks. One-way analy variance (ANOVA) followed by Tukey's HSD was performed and statistical comparisons amo the various groups were conducte a Grap Pad prism program and excel analysis (La Jolla, CA, USA). Statist signifi ce is set at $p < 0.05$.

esults

Serum Vitamin D L

By th week, the vi D deficient groups [VD- D- + MetS)] sho a significant decre in vita D level (< 12.5 nmol/l) with a p e < 0 01 compared to the baseline value (Tab

Serum Level of Calcium [Ca^{+2}], Phospha [PO_4^{-3}], and Magnesi [Mg^{+2}]

The metabolic syndro (MetS) a ne exhibited a signifi decr $p < 0.0$ at the end of 8 w (Table II) r phate levels, only th in D deficie up (VD) showed a r sign t decrease (p-value > 0.05) *vs.* each basel Moreover, the magnesiu s for all te groups showed a no gnificant decrease (p-value > 0.05) *vs.* ea baseline.

Hy tension

Measu 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.

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

		Calcium (mmol/l)		Phosphate (mmol/l)		Magnesium (mmol/l)	
	Duration	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

***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 t control group. Moreover, the vitamin D def (VD-) group and the combined (VD- + M group had significant increase in insulin resista index ($p < 0.001$) for (VD-) and (0001) f (VD- + MetS)) compared to lic syn drome (MetS) group alone.

Dyslipidemia

Triglycerides (T vel

Table VII showed th e (VD-) group produce a signi t increase in TG serum level (p .05) compar the control group where both (MetS) and + MetS) groups pro ed a sig cant increase in TG serum level (001) pared to the control group.

Total sterol vel

Table d that the total cholesterol was ificantly eased ($p < 0.05$) for the vitamin D deficient (VD-) group and ombin roup (VD- + MetS) in comparison to th roup. In n-trast, the metabolic rome ted a n ig-nificant ($p > 0.05$) n equated to on roup.

HDL level

Conventionally, the of HDL was demonstrate quite signifi y decreased in the me nic syndrome (MetS) group ($p < 0.05$) w compared with the control group (Table V However, re 1 showed that HDL level wa reased fro aseline to the 3rd week of the expe t foll d by a significant decrease in HDL le 8 weeks for the vitamin D deficient (VD-) group ($p < 0.05$) and the combined 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 easured	Groups Duration	Control n = 12	Vitamin D deficient (VD-) n = 12	Metabolic syndrome (MetS) n = 12	Combined (VD- + MetS) n = 12
Serum Glu cose 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 [illegible] total abdominal fat was significantly increas[illegible] for the vitamin D deficient (V[illegible]oup (p [illegible] 0.05), metabolic syndrome ([illegible]S) [illegible]up (p < 0.001) and vitamin D defi[illegible]cy wit[illegible]etabolic syndrome (VD- + Me[illegible]roup [illegible]) compared to the co[illegible]ol g[illegible], both the metaboli[illegible]yndrom[illegible]etS) group and the combi[illegible]VD- + Me[illegible]roup produced a signi[illegible]ease in tota[illegible]dominal fat ($p < 0.01$)[illegible]s. the [illegible]in D deficient (VD-) group.

Ad[illegible]ity index: Table [illegible]I and Figure 5 sho[illegible] that th[illegible]diposity index was significantly i[illegible]ed f[illegible]e vitamin D deficient (VD-) group (p [illegible] metabolic[illegible]ndrome (MetS) group (p < 0.00[illegible]d vita[illegible]D deficiency with metabolic [illegible]drome [illegible]MetS) group ($p < 0.001$) com[illegible]ed to the [illegible]rol group.

Evaluation of the emerging m[illegible]abolic syndrome by NCEP ATP III ([illegible]1)

Human metabolic syndr[illegible] criteri[illegible] could [illegible] extrapolated to animal m[illegible]uremen[illegible]xcept for the central obesity measu[illegible](Table V[illegible]I). All test groups (VD[illegible]MetS)[illegible](VD- + [illegible]tS) resulted in a sig[illegible]ant increase[illegible]ins[illegible] resistance, glucose[illegible]lood pressu[illegible]esity parameters, an[illegible]G le[illegible]ompared to the control group as mentioned a[illegible] in detail. Table VIII show[illegible]the metaboli[illegible]drome (MetS) and the[illegible]bined (VD- + MetS) [illegible]oups accomplished th[illegible] criteria of metabolic syndrome (marked (√) in[illegible] table) acco[illegible]g to NCEP-ATP III.

Discussion

[illegible]worthy Skepticism Is that Vitamin De[illegible]iency 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±
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 ($p < 0.0$ *vs.* contro

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 regulati remarkably authenticated[24-28].

Accumulating evidence implicates that min D preserves the release of insulin and fac tates insulin actions in the tissues e respo sive to the insulin, by controlli he cellula and extracellular calcium s[26]. Ne theless, numerous studies revealed trong he- tween hypovitaminosi D syndrome[29,30]. Like e, many demiological studies have shown that t is inverse or- relation between th vel o min D od glucose, insulin stance, an p lence of type 2 diab Thus, we w lay the emphasis, th our pr t study has unequivocally demonstrated a sign nt increase in insulin level ulin resista index (HOMA-IR) in est groups compared the control group. T nsulin level and the insulin resistance index (H MA-IR) wer gnificantly higher in the combi (VD- + M group (Table VI and VIII). This worth ding is very well corroborated by the TNF and IL-6 are immune me- tors which interfere with insulin signaling that 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 (Ca+2), Phosphate (PO_4^-

Table VIII. Metabolic synd arameter according to NCEP-ATP III.

NCE AT	Insulin resistance MA-IR	ose level > 5.5 mmol/L	Dyslipidemia TG > 1.695 mmol/L	Blood pressure > 130/85 mmHg	Central obesity	
					BMI	AC
	4.256±0.8	7.2±0.02	0.83±0.03	120±0.57	0.45±0.01	15.3±0.26
VD-	20.1 4	8.3±0.5 √	1.143±0.03	135±0.7 / 94.17±1.7 √	0.52±0.01	17.33±0.30
	14.434±0.7	8.2±0.22 √	1.74±0.09 √	150.5±1.4 / 98.5±1.22 √	0.55±0.008	17±0.23
VD- MetS	31.041±1.43	10.2±0.8 √	2.3±0.18 √	166.33±3.8 / 111.5±2.21 √	0.61±0.01	19.34±0.14

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

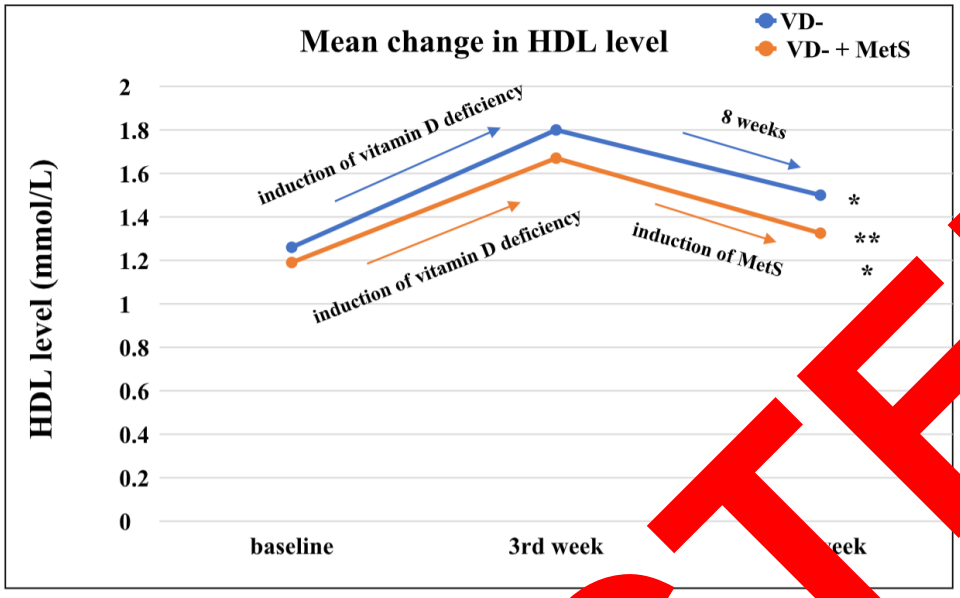


Figure 1. Mean change OF HDL level at baseline, at the 3rd week after induction of vitamin D deficiency, and after 8 weeks (n=12 rat/group). ***significant ($p < 0.0001$) *vs.* the level on the 3rd week, *significant ($p < 0.05$) *vs.* the level on the 3rd week.

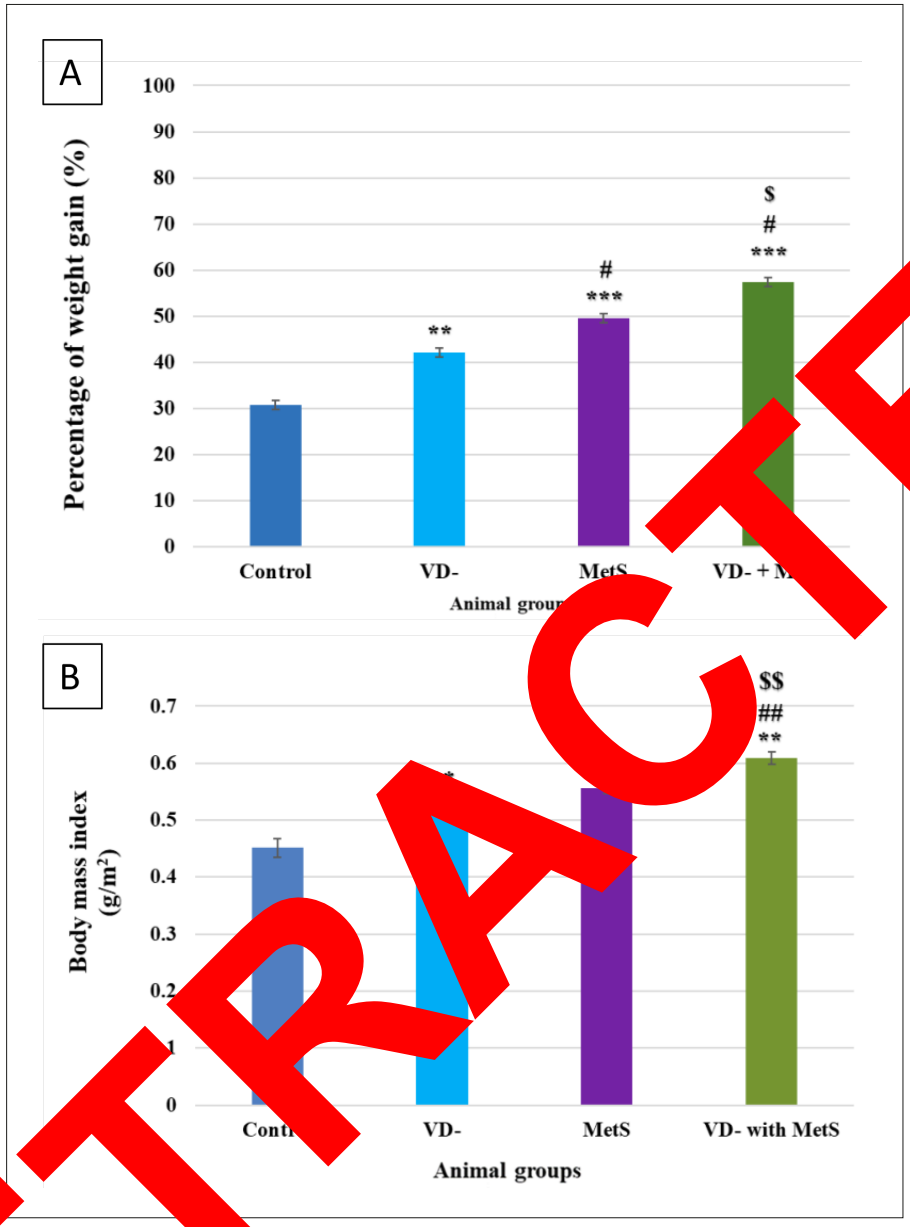
[3]), and Magnesium (Mg+2)in our results (Table II), could be emphatically attributed to electrolyte imbalance as a consequence of diabetes and metabolic syndrome due to derangement of PTH-vitamin D endocrine system[34,35]. Interestingly, the enriched phosphate and magnesium diet given to the rats in this study failed 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 hypocalcemia[36]. In addition, our study has revealed no changes in the magnesium level in all the animal groups, nevertheless several studies demonstrate a correlation between hypomagnesemia with hyperglycemia and diabetes[37,38]. However, magnesium diminution leads to hypocalcemia mostly due to impaired PTH release or renal tubular and skeletal resistance to the action of PTH[39].

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

Induction of vitamin D deficiency resulted in 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 Ca^{2+} 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



Figu Mean ± E of: (**A**) Perc tage of weight gain, (**B**) body mass index of control VD-, MetS and (VD- + MetS) groups af weeks (rat/group). ***Significant ($p < 0.0001$) *vs.* control group, **significant ($p < 0.001$) *vs.* control group, #significant ($p <$) *vs.* (VD-) group, $ significant ($p < 0.01$) *vs.* (MetS) group.

&VIII). present observation is concor- with a study involving vitamin D deficient ch reported elevated TG and total cholesterol levels. In addition, vitamin D has n impact on insulin secretion and sensitivity ich, indirectly stimulates the metabolism of ipid[47]. Moreover, there is strong evidence of an association between the state of vitamin D deficiency and insulin resistance[48]. Conversely, in the event of insulin resistance and consequent 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 inflammation[49]. 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].

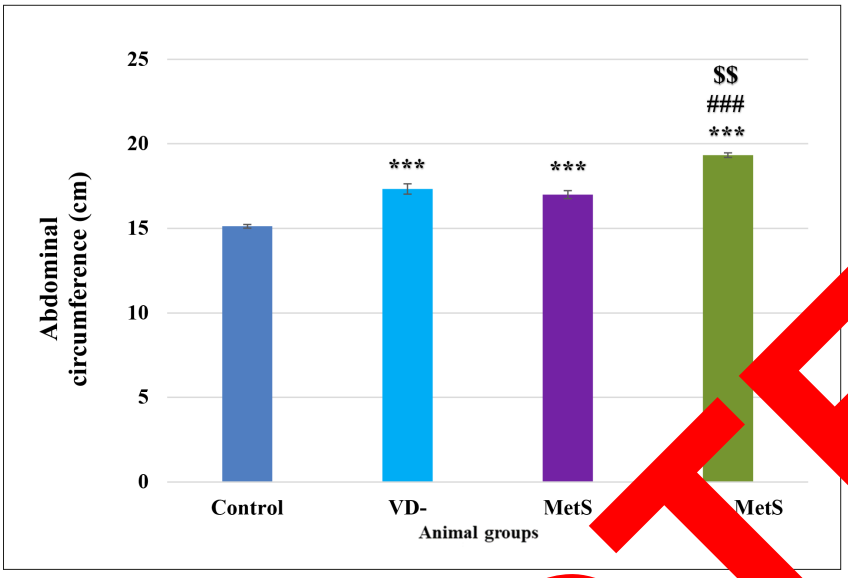


Figure 3. Mean± S.E of abdominal circumference for control, VD-, MetS [illegible] + MetS) groups a[illegible] weeks (n=12 rat/group). ***significant ($p < 0.0001$) *vs.* control group, ### significant ($p < 0.0001$) [illegible] MetS group, $$ significant ($p < 0.001$) *vs.* VD- group.

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

A distinct, noteworthy characteristic [illegible] longstanding focus of interest of metabolic s[illegible] drome is the preponderance of a[illegible]al obes[illegible] ty, an unequivocal quality no[illegible]rrelated with [illegible]min D [illegible]eficiency[52]. This association has be[illegible]orically recognized in our current study (Figure 2-5 and Table VIII) and has [illegible]rated a significant increase in AC, BMI, [illegible]rcen[illegible]ge of weight gain, adiposity index, and total abdominal fat weight in all the experimental groups. A finding of particular interest is re-

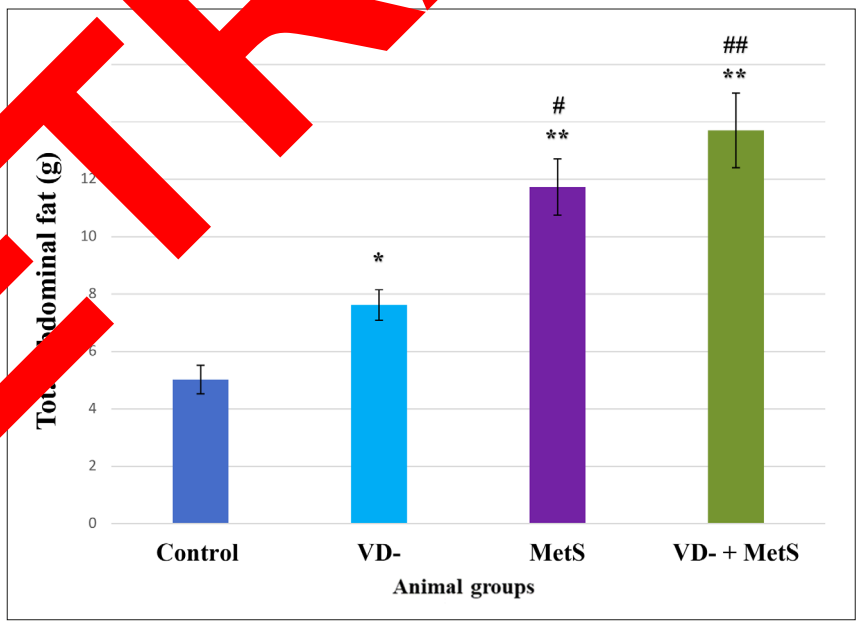


Figure 4. Mean ±S. E of total abdominal fat for control, VD-, MetS and (VD- + MetS) groups after 8 weeks (n=12 rat/group). **significant ($p < 0.001$) *vs*. control group, *significant ($p < 0.05$) *vs*. control group, #significant ($p < 0.05$) *vs.* VD- group, ##significant ($p < 0.001$) *vs.* VD- group.

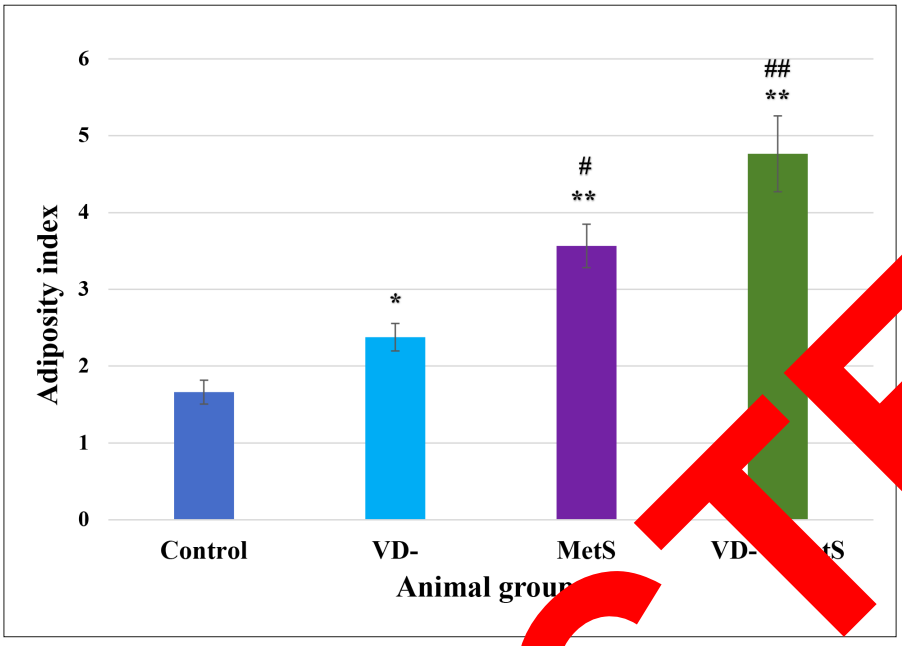


Figure 5. Mean ± S.E. of adiposity index for control, VD-, MetS (VD- + MetS) groups after 8 weeks (n=12 rat/group). **significant ($p < 0.001$) *vs.* control group, *significant ($p < 0.05$) control group, significant ($p < 0.01$) *vs.* VD- group, ##significant ($p < 0.001$) *vs.* VD- group.

lated to the mean increase in BMI and AC fo (VD-) group and (MetS) group which were si ilar, while a stronger effect was i ted in t combined group than in either up e (Fig ure 2-4 and Table VIII). T indin ndoubt- edly reinforces the delete effe min D deficiency on metab ic sy ly, a reassuring cr sectiona dy acquired from the insulin tance athero otic study of Hispanic a ol dults exhib a higher level of calci ol and in turn, is inversely related t body mass (BMI) and param- eters sceral and subcu us adiposity[53,54]. Un vocall higher risk of metabolic syn- d ob , increased waist circumference, and nic dys demia were found to be trongl ociate th low calcidiol levels and h PTH ations[52,55]. The agglomeration bdomina pose tissue leads to the progres- f dyslipidemia, hyperglycemia, and hyper-

Correlation of Hypovitaminosis D ith 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 subjects[57]. The basic essence of

l mechanisms recommended to explain e co lation 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 actions[58].

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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