The Effect of Vitamin D_3 or Calcium on Some Endocrine, Mineral and Lipid Assays in Experimental Animals

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Abstract

The present work was designed to study the effect of dietary vitamin D_3 deficiency and supplementation with vitamin D_3 or calcium on some endocrine, mineral and lipid assays in male Albino rats for six weeks experimental period.. A total of 65 rats were divided into five comparable groups: (G_1) received the basal diet; (G_2) received the vitamin D_3 deficient- diet ; (G_3) received the vitamin D_3 deficient-diet, but given vitamin D_3 orally three times weekly for the last three weeks; (G_4) received the vitamin D_3 deficient-diet, but given vitamin D_3 orally daily for the last 2 weeks and (G_5) received the vitamin D_3 deficient-diet for four weeks then high calcium for the last 2 weeks. The present results showed a significant increase in concentrations of serum insulin, magnesium and calcium as well as a significant decrease in serum phosphorus, alkaline phosphatase (ALP) activity, cholesterol, triacylglycerol, parathyroid hormone (PTH) and thyroxine (T_4) in groups 3, 4 and 5 when compared with (G_2). From the present study, an improvement in all parameters, even the glucose-tolerance test, was concluded in G_4 followed by G_3 and finally G5

Introduction

Vitamin D_3 was isolated and identified by Windaus et al. (46). A major source of vitamin D_3 in the skin is Ultraviolet β-radiation photons which penetrate the skin and promote the production of vitamin D_3 from 7-dehydrocholesterol (18).

Vitamin D_3 elevates plasma calcium and phosphorus by intestinal calcium and phosphorus absorption, mobilization of calcium from bone and renal reabsorption of calcium; this elevation occurs to a level enough to support proper skeletal mineralization and neuromuscular junction and to prevent tetany, rickets and osteomalacia (21)

Vitamin D_3 was demonstrated to be inactive and must be metabolized to a functional form (35). The biosynthesis of 1,25 $(OH)_2 D_3$ is catalysed by 25-hydroxyvitaminD_3 1α-hydroxylase, during its degradation by induction of 25-hydroxyvitamin D_3 24-hydroxylase; these enzymes are regulated by $1,25(OH)_2D_3$ and parathyroid hormone(2).

In plasma, only a small fraction of $1,25(OH)_2D_3$ is free, while most of it is bound to the vitamin D_3 binding protein (DBP). The renal synthesis of $1,25(OH)_2D_3$ is tightly regulated by plasma levels of calicum , phosphate and PTH (7).

There is also experimental evidence of a reduction in insulin resistance and improvement in glucose levels in oral glucose tolerance tests after vitamin D supplementation in type 2 diabetic patients (12).

PTH levels were noticed to increase by a vitamin D_3 deficient diet and even more by calcium deficiency (29). Next to PTH, phosphate is the second most important physiological regulator of renal 1 α-hydroxylase. High phosphate levels decrease and low levels increase the enzyme activity. In human, phosphate restriction increases plasma $1, 25(OH)_2D_3$ by approximately 180 % and phosphate supplementation decreases 1,25 $(OH)_2 D_3$ level by 60 % (34) .

Magnesium supplementation was reported to delay the development of diabetes in a rat model of non insulin dependant diabetes mellitus, NIDDM (11).

The present study was conducted to clarify the effect of Vitamin D_3 deficiency and supplementation of Vitamin D_3 or calcium on some endocrine, mineral and lipid assays in male Albino rats as experimental animals.

Materials and Methods

1-Animals:

Sixty five male albino rats, Sprague Dawley Strain (mean weight 61g ± 5.0) were used .They were divided into five groups each of 13 rats .They were obtained from Faculty of Veterinary Medicine / Cairo University , Giza , Egypt. The animals were housed individually in stainless steel cages fitted with a wire mech bottoms and fronts in a room maintained at 25-30 °c with about 50 % relative humidity. The room was lighted on a daily photoperiod of 12h light and dark. Then they were allocated to the various experimental treatments for 6 weeks.

2- Experimental Design :

- **Group 1,** as control group, was given basal diet containing 25μg vitamin D_3 / kg diet and normal calcium (0.4% of diet) throughout the experimental period (1).
- **Group 2** was given vitamin D_3 deficient diet containing 5μg vitamin D_3/kg diet and normal calcium for 6 weeks (19) .
- **Group 3** was given vitamin D_3 deficient diet throughout the experiment but rats were given vitamin D_3 orally at a dose of 1μg / rat three times weekly for the last 3weeks (19).
- **Group 4** was given vitamin D_3 deficient diet throughout the experiment but rats were given vitamin D_3 orally at a dose of 1μg / rat /day for the last 2weeks (19).
- **Group 5** was given vitamin D_3 deficient diet for the first 4 weeks then rats were given high calcium (4% of diet) for the last 2 weeks.(19).

During the experimental period, diet and tap water were provided ad libitum, body weight gain and food consumption were recorded periodically

3- Blood Samples' Collection :

At the end of the experimental period, animals were fasted for 12h and anesthetized with diethyl ether. Incisions were made into the abdomen and 1 ml of blood samples were collected from the hepatic portal vein in tubes containing sodium flouride for determination of plasma glucose. Five ml of blood samples were left in tubes for 30

minutes at 37°c, then serum separated by centrifugation at 4000 r.p.m. for 15 minutes, and frozen in plastic vials and kept at –20°c for subsequent biochemical analysis.

Glucose tolerance test :

At the end of the experiment, five rats from each group were used to determine glucose tolerance test. After an overnight fasting, an initial o min blood samples were taken. Rats were given a single intraperitoneal injection of glucose load (100 mg / 100g body weight). Blood samples were collected from the orbito-sinus plexus at 30, 60, 90 and 120 minutes after the glucose load in tubes containing sodium fluoride.

4-Biochemical Parameters :

The collected samples were analyzed for the following biochemical parameters:

- **Plasma glucose** was estimated by colorimetric method - kits supplied by Diamond Diagnostic Company (44).
- **Serum insulin** was estimated by microparticle enzyme immune-assay method (42).
- **Serum magnesium** was estimated by colorimetric method - kits supplied by Gamma Treed Company (9).
- **Serum calcium** was estimated by colorimetric method - kits supplied by Gamma Trade Company (8).
- **Serum phosphorus** was estimated by colorimetric method - kits supplied by Biodiagnostic Company (15).
- **Serum alkaline phosphatase** activity was estimated by colorimetric method - kits supplied by Biodiagnostic Company (33).
- **Serum cholesterol** was estimated by colorimetric method - kits supplied by Biodiagnostic Company (4).
- **Serum triacylglycerol** was estimated by colorimetric method - Kits supplied by Biodiagnostic Company (16).
- **Serum parathyroid hormone (PTH)** was estimated by Immune-assay method (41).
- **Serum thyroxin hormone (T_4)** was estimated by immune-assay method (30) .

Statistical analysis :

Spss windows version (11.5) was used for analysis of the data. Description of presentative variables, in the form of range, means ± S.D, was done (38).

Results

There was a significant increase in body weight gain in rats which received vitamin D_3 deficient diet (G_2) than rats fed standard diet in group 1. After 45 days of experiment, significant decrease in body weight gain was observed in groups 4 and 5, when compared to group 2, while there was a significant increase in body weight gain in group 3 when compared with group 5. The results between all groups exhibited non significant differences in the mean values of feed intake (Table, 1).

There was a significant increase in fasting plasma glucose levels in group 2when compared with group 5, while there was no significant differences in plasma glucose values between groups 1, 2, 3 and 4. There was a significant increase in serum insulin in groups 3 ,4 and 5 when compared with group 2, but in groups 3 and 4 there was a significant increase in the mean value of serum insulin than group 5 (Table, 2).

There was a significant decrease in the mean value of serum magnesium in group 2 when compared with control group1.There was a significant increase in the mean value of serum magnesium in group 3 and 4 than group 2 , while there was no significant difference between groups 2 and 5. Serum magnesium value was significantly increased in group 4 when compared with groups 3 and 5 (Table, 2).

There was a significant decrease in the mean value of calcium in group G_2 when compared with all other experimental groups.There was a significant increase in the mean value of calcium in group 4 and 5 when compared with group 3 (Table, 2).

At the end of the experiment , there was a significant increase in the mean value of phosphorus and in the activity of ALP in group 2 in comparison with all the different experimental groups, but in group 3, there was a significant increase in the mean value of ALP activity than group 4 (Table, 2).

The present study showed that there was a significant increase in the mean value of cholesterol and triacylglycerol in group 2 than group 1, but a significant decrease in the mean value of total cholesterol and triacylglycerol was noticed in all treated groups 3 ,4 and 5 when compared to group G_2 . There was a significant decrease in the mean value of cholesterol and of triacylglycerol respectively in group 4 when compared to group 3 (Table, 3).

It was found that there were no significant differences in the mean value of serum PTH and T_4 in group 2 when compared to group 1, while there was a significant decrease in the mean value of PTH and the mean value of T_4 in groups 3, 4 and 5 when compared with group 2. There was a significant increase in the mean value of PTH in groups 3 and 4 than group 5 (Table, 3).

At 30, 60, 90 and120 minutes, there was a significant increase in the mean value of plasma glucose in G_2 than did G1, but there was a significant decrease in groups 3, 4 and 5 compared to G_2. There was a significant decrease in the mean value of plasma glucose in G_4 when compared with G_3 at 30, 60 and 90 minutes (Table, 4).

Table (1) : Influence of dietary vitamin D_3 deficiency and supplementation with vitamin D_3 or high calcium on body weight and feed intake in experimental rats.

Parameters	Groups				
	(1)	(2)	(3)	(4)	(5)
Initial Weight (g)	63.30 ± 18.10	63.00 ± 10.00	59.80 ± 13.80	61.00 ± 5.00	58.30 ±5.60
Final Weight (g)	248.30 ± 22.00	264.60 ± 17.00	246.50 ± 33.00	237.90 [b] ± 23.00	227.00 [bc] ±10.30
Weight Gain(g)	185.00 ± 15.00	201.60 [a] ± 18.00	186.80 ± 29.00	176.90 [b] ± 21.00	169.00 [bc] ± 10.00
Feed Intake(g/day)	15.60 ± 0.89	15.70 ± 0.80	15.20 ± 1.40	15.03 ± 1.20	15.70 ± 0.60

a = significant difference at P< 0.05 when compared to group 1
b = significant difference at P< 0.05 when compared to group 2
c = significant difference at P< 0.05 when compared to group 3
d= significant difference at P< 0.05 when compared to group 4

Table (2) : Influence of dietary vitamin D_3 deficiency and supplementation with vitamin D_3 or high calcium on fasting plasma glucose , serum insulin,, magnesium,calcium , phosphorus and alkaline phosphatase in experimental rats.

Parameters	Groups				
	(1)	(2)	(3)	(4)	(5)
Fasting plasma Glucose(mg / dl)	113.30 ±5.70	118.90 ±13.50	110.70 ±7.50	113.20 ±10.50	105.80[b] ±7.50
Insulin (µu / ml)	2.48 ±0.21	0.69[a] ±0.005	2.2 0[b] ±0.058	2.60[bc] ± 0.17	1.30[bcd] ± 0.19
Magnesium (mg / dl)	4.03 ±0.17	3.20[a] ± 0.25	4.10[b] ± 0.45	4.55[bc] ±0.26	3.30[cd] ± 0.13
Calcium (mg / dl)	11.20 ±0.51	5.95[a] ± 0.93	8.09[b] ±0.85	9.10[bc] ± 0.39	8.98[bc] ±0.53
Phosphorus (mg / dl)	9.80 ± 0.45	12.80[a] ± 1.37	b9.63 [b] ±0.66	8.8[b] ± 0.34	8.93[b] ±0.70
Alkaline phos-phatase iu / l	113.80 ±6.80	181.63[a] ±7.90	154.95[b] ±14.3	128.04[bc] ±1.50	164.40[bcd] ±8.70

Table (3): Influence of dietary vitamin D_3 deficiency and supplementation with vitamin D_3 or high calcium on serum cholesterol, triacylglycerol, parathyroid hormone(PTH) and thyroxine(T_4), in experimental rats.

Parameters	Groups				
	(1)	(2)	(3)	(4)	(5)
holesterol (mg / dl)	216.60 ± 7.50	326.40^{a} ±13.70	246.80^{b} ±5.00	229.90^{bc} ±4.60	262.10^{bcd} ±4.10
Triacylglycerol (mg / dl)	117.40 ±6.70	151.10^{a} ± 7.30	115.90^{b} ±5.80	107.60^{bc} ±8.00	10.20^{b} ±8.00
PTH (pg / ml)	13.40 ± 1.30	14.50 ± 1.91	9.00^{b} ± 0.14	8.90^{b} ± 0.51	7.00^{bcd} ± 1.00
T_4 (ng / dl)	4.30 ± 0.19	4.01 ±0.096	3.50^{b} ± 0.33	3.60^{b} ± 0.26	3.50 ± 0.28

Table (4): Influence of dietary vitamin D_3 deficiency and supplementation with vitamin D_3 or high calcium on intraperitoneal glucose tolerance test (GTT) in experimental rats.

Time (min.)	Groups				
	(1)	(2)	(3)	(4)	(5)
0	113.20 ± 6.90	119.70 ±6.10	110.30 ± 9.30	113.00 ± 9.80	105.00^{b} ± 9.0
30	110.80 ± 4.10	209.00^{a} ± 5.00	153.10^{b} ± 4.30	143.00^{bc} ± 3.90	142.04^{bc} ± 5.20
60	162.00 ± 3.90	251.20^{a} ± 3.30	179.50^{b} ± 6.10	158.60^{bc} ± 5.90	211.20^{bcd} ± 5.80
90	132.80 ± 4.30	184.10^{a} ± 5.90	139.20^{b} ± 4.30	129.60^{bc} ± 5.30	136.80^{bd} ± 5.30
120	102.70 ± 4.00	153.50^{a} ± 4.20	132.14^{b} ± 2.40	128.20^{b} ± 4.30	136.20^{bd} ± 5.50

Discussion

The present study revealed a significant increase in the body weight gain of male Albino rats which received vitamin D_3 deficient-diet in group 2 comparing to those in groups 1, 4 and 5. Such increase may be attributed to the correlation between the vitamin D_3 deficiency and the increase in body fat mass (5). Similar results indicated that serum 25 hydroxyvitamin D_3 (25 (OH) D_3) was inversely correlated with the body mass index (BMI) and body fat mass (31). It was evident that the obesity associated with vitamin D_3 deficiency is most likely due to the decreased bioavailability of vitamin D_3 from cutaneous and dietary sources because of its deposition in body fat compartment,
So, adipose tissue is suggested to be the major storage site for vitamin D_3 in the present study and others (5; 31).

There was no significant differences in fasting plasma glucose values between groups1,2,3 and 4, a finding which came in agreement with Beaulieu et al.(6). The long period of starvation of rats (12 hours) may lead to within normal plasma glucose levels.

The present results showed a significant decrease in the mean value of insulin in deficient Vitamin D_3-diet of group 2than the corresponding control and treated groups, a finding which came in agreement with a previous report (19) where insulin secretion was impaired in vitamin D_3 deficient rats and it was improved by dietary vitamin D_3 repletion. Several mechanisms might explain the involvement of 1,25- $(OH)_2$ D_3 in insulin secretion including either (1) An enhanced levels of serum calcium by the action of 1,25 $(OH)_2$ D_3 participation in the rise of intracellular calcium which is needed for insulin release (6, 22); or (2) A direct interaction of the 1,25 $(OH)_2$ D_3 with pancreatic islet β-cells to affect secretion of insulin (36) and the decreased pancreatic preproinsulin mRNA (22). Also, the supplementation with high calcium feeding alone during a sufficient period of time (14 days) succeded to fill intracellular calcium pool which enhanced insulin release .

Also 1, 25 $(OH)_2$ D_3 affected the synthesis of calcium binding protein (CaBP) which regulated intracellular calcium , this may facilitate the delivery of calcium mediated signals to sites regulating insulin release. The role of vitamin D_3 in regulating pancreatic CaBP was also

suggested, as in chicken (22) where pancreatic CaBP was altered with variations in vitamin D_3 and mineral status.

The present study revealed a significant decrease in the mean value of magnesium in rats having Vitamin D_3 deficient-diet of group 2 when compared to that in group 1.It has been reported that hypovitaminosis D_3 is a risk factor for type2 diabetes mellitus patients (23), a finding which suggested that there is a relationship between hypovitaminosis D_3 and insulin resistance. So, the effect of vitamin D_3 deficiency on magnesium levels occurs in indirect way like the high prevalence of hypomagnesaemia in NIDDM patients (27). Magnesium may be an important determination of insulin sensitivity in maturity onset diabetes in rats. According to Bolan et al. (10), It may induce its action by two ways (1) Magnesium increases the affinity and especially the number of insulin receptor (2) Magnesium is important for rate limiting enzyme of glycolysis. Thus, magnesium depletion may lead to decrease cellular glucose utilization contributing to insulin resistance. From the present results, there is a significant difference in serum magnesium in groups 3 and 4 supplemented with vitamin D_3 when compared with group2, a finding which might be explained on the fact that supplementation with vitamin D_3 increases the circulating levels of 1,25 $(OH)_2$ D_3 which leads to a decrease in the incidence of diabetes and modulates serum magnesium levels (11).

. The present results showed a significant decrease in the mean value of calcium in vitamin D_3 deficient group 2 comparing to that in other groups, a finding which came in consistent with a previous study (19) where the vitamin D_3 deficient group displayed significantly lower serum calcium than that in the vitamin D_3 replete and high calcium groups. The vitamin D_3 deficient rats seemed to have a decreased circulating levels of 1, 25 $(OH)_2$ D_3 which played a major role in regulation of calcium and phosphorus homeostasis by promoting their absorption in the small intestine and depressing renal calcium excretion (19).Supplementation with vitamin D_3 or high calcium diet normalized the serum calcium levels which became significantly higher than the vitamin D_3 deficient group. The present study revealed a significant increase in the mean value of phosphorus in group 2when compared with

control and treated groups. Serum phosphorus levels in rats which received vitamin D_3 deficient diet were significantly higher than groups supplemented with vitamin D_3 or high calcium diet (20).

There is a significant increase in the activity of alkaline phosphatase (ALP), in rats on vitamin D_3 deficient diet of group2 when compared to that in other groups. The serum alkaline phosphate seemed to increase in vitamin D_3 deficient rats compared to vitamin D_3 deficient rats supplemented with vitamin D_3 or high calcium diet (19). Such increase might be explained on the fact that vitamin D_3 deficiency is accompanied by hypocalcemia and bone resorption. So, the elevation of ALP in this group might be attributed to a leakage of the enzyme from bone.

It was observed, from the present results, that rats in groups 3 and 4 have a significant decrease in ALP activity than those in group 2. As a result of supplementation with 1,25 $(OH)_2$ D_3, intestinal calcium absorption was noticed to increase enough to suppress bone resorption through suppression of parathyroid hormone expression in bone (40). Rats supplemented with high calcium diet in group 5, also, showed a decrease in (ALP) than that in group 2due to high calcium diet which normalizes serum calcium levels resulting in suppression in both PTH expression in bone and bone resorption, a finding which suggested that there is a relationship between parathyroid hormone, calcium, phosphorus and 1, 25 $(OH)_2$ D_3 (34)..

The present study revealed a significant increase in the mean value of cholesterol in group 2 when compared to that in the control and supplemented groups. It was evident that, in diabetes, both the rate limiting enzyme of cholesterol synthesis, B–hydroxy-B-methylglutaryl-CoA reductase, and cholesterol level seemed to increase (39). Insulin resistance was linked to high rate of cholesterol synthesis and low rate of cholesterol absorption (32; 43).Insulin may have a direct action on cholesterol synthesis as insulin is known to stimulate liver X receptors (LXRs) which upregulate lipogenesis and cholesterol synthesis. High glucose is linked to increased synthesis of cholesterol (39). From the present results, there was a pronounced reduction in cholesterol level in

groups 3, 4 and 5 when compared to group 2, a finding which might be attributed to the supplementation with either vitamin D_3 or high calcium diet enough to improve insulin sensitivity and to decrease incidence of diabetes and cholesterol levels (37).

It is clear from the present data that there is a significant increase in the mean value of triacylglycerol in group 2when compared to all different experimental groups, a finding which came in agreement with some previous reports (32) where type 2 diabetes mellitus (T2DM) had been constantly associated with over production of very low density lipoproteins (VLDL) and triacylglycerols. This was due to high levels of serum free fatty acids (FFA) which were transported to liver and re-estrified into triacylglycerols and transported out as VLDL. In rat models, β-cell dysfunction was related to increase triacylglycerols content in pancreatic islets (26).

The present results did not reveal a significant difference in the mean value of PTH between groups 2 and 1, but there was a significant decrease in groups 3, 4 and 5 when compared with group 2. This finding came in accordance to that reported earlier
(25; 28). Magnesium deficiency is the other common clinical cause of hypocalcemia and impairs PTH secretion. There was inverse correlation between serum $1,25(OH)_2\ D_3$ and PTH. Hypovitaminosis D_3 is one of the most common causes of secondary hyperparathyroidism as well as chronic renal failure.

By calcium receptors (CaR), parathyroid gland can detect the variations in calcium concentration and released PTH (13). CaR-mRNA expression, decreased in vitamin D_3 deficient group, allowed higher rate of PTH secretion to stimulate the renal 1α hydroxylase and normalize serum $1,25(OH)_2D_3$. In $1,25\ (OH)_2\ D_3$ treatment there was increased CaR mRNA and suppressed PTH. Thus, regulation of CaR expression by $1,\ 25\ (OH)_2\ D_3$ represented another potential level of regulation of calcium homeostasis by this vitamin D_3. PTH mRNA levels seemed to increase by vitamin D_3 deficient diet and to increase more by calcium deficiency (29). Because of the inverse correlation between the insulin sensitivity and plasma PTH, mild hyperparathyroidism may be the chief

mediator of the insulin resistance associated with poor vitamin D_3 status (24).

Similarly, The present study did not reveal a significant difference in the mean value of thyroxin between groups 2 and 1, but a significant decrease was noticed in groups 3 , 4 and 5 when compared with group 2. Thyroid dysfunction is frequently associated with disturbance of calcium (Ca^{++}) and inorganic phosphate (Pi) homeostasis. Thus it is apparent that thyroid hormones play an important role in mineral metabolism , can stimulate bone resorption , increase renal reabsorption of calcium and increase uptake of calcium from intestine thus serum thyroid levels increase in hypocalcemia but when calcium levels corrected by supplementation with 1,25 $(OH)_2$ D_3 or high calcium diet thyroid hormone decreased in serum (3).

The present results reveal that impaired glucose tolerance test in G_2 but the supplementation with vitamin D_3 or high calcium diet improves glucose tolerance test .These results are in agreement with some previous studies (14; 17) who attributed the impaired glucose tolerance test in vitamin D_3 deficient diet to the impaired insulin secretion, but vitamin D_3 repletion improved glucose clearance and insulin secretion (45).

The circulating glucose was higher in hypocalcemia and vitamin D_3 depleted rats after glucose challenge than in 1,25 $(OH)_2$ D_3 supplementation (6). Calcium supplementation improved the response to glucose challenge in vitamin D_3 depleted animals , GTT was conducted 3,7 and 14 days after high calcium feeding in hypocalcemic vitamin D_3 depleted rats . Insulin response to glucose challenge was not significantly increased by 3 days of calcium feeding compared with hypocalcemic vitamin D_3 depleted groups but insulin response was significantly higher after 14 days of calcium supplementation .Insulin levels were not signifiacntly different between 1,25 $(OH)_2$ D_3 and high calcium treated animals . Also, no significant difference between control rats and rats supplemented with 1,25 $(OH)_2$ D_3 .

From the present study, an improvement in all parameters, even the glucose-tolerance test, was concluded in group 4 followed by group 3 and finally group 5.

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تاثير فيتامين د٣أو الكالسيوم على بعض الغدد الصماء, الاملاح المعدنية و الدهون في حيوانات التجارب

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تمت هذه الدراسة البيولوجية على ذكور الفئران البيضاء والبالغ عددهم خمسة وستين من النوع الالبينو, تم تقسيمهم الى خمسة مجموعات مختلفة . تحتوى كل مجموعة على ١٣ فأر. وكان متوسط أوزان الفئران ٦١ جم ± ٥,٠٠ . وتم تقسيمهم كالاتى: المجموعة الاولى: الضابطة غذيت على الوجبة القياسية , المجموعة الثانية: غذيت على وجبة منخفضة فى فيتامين د٣ لمدة ستة اسابيع, المجموعة الثالثة: غذيت على وجبة منخفضة فى فيتامين د٣ ولمدة ستة اسابيع و تم اعطاءها فيتامين د٣ عن طريق الفم ثلاث مرات اسبوعيا فى الثلاث اسابيع الاخيرة , المجموعة الرابعة: غذيت على وجبة منخفضة فى فيتامين د٣ لمدة ستة اسابيع و تم اعطائها فيتامين د٣ عن طريق الفم يوميا فى الاسبوعين الاخرين., المجموعة الخامسة: غذيت على وجبة منخفضة فى فيتامين د٣ لمدةالاربع اسابيع الاولى ثم تم اعطائها وجبة عالية فى الكالسيوم لمدة الاسبوعين الاخيرين.

وقد وجد أن التدعيم بفيتامين د٣ او الكالسيوم فى مجموعات (٣),(٤)و(٥) ادى الى زيادة ذات دلالة احصائية فى مستوى الانسولين و الماغنسيوم والكالسيوم بالمصل ونقص ذو دلالة احصائية فى مستوى الفوسفور, نشاط انزيم الفوسفاتيز القلوى ,الكوليستيرول, الجليسيريدات الثلاثية, هرمون الغدة الجار درقية وهرمون الثيروكسين بالمصل عند مقارنتهم بالمجموعة (٢).

الخلاصة انه قد حدث تحسن فى جميع القياسات البيوكيمائية فى مجموعة (٤) يليها (٣) و اخيرا(٥)، وايضا تحسن قياس مستوى منحنى السكر فى هذه المجاميع عند مقارنتهم بالمجموعة (٢).