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Exercise's Impact on 25-Hydroxycholesterol Levels in Vitamin D Deficient Individuals

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Abstract

Introduction: Deficiency of calciferol (vitamin D) contributes to the development of a number of chronic disorders. A significant frequency of metabolic syndrome, vitamin D insufficiency, and insufficiency exists throughout Asia. People in Pakistan also suffer from a lack and

inadequacy of vitamin D. More and more evidence suggested that exercise may have an impact on vitamin D levels. Different exercise intensities affect vitamin D levels in people.

Objectives: To research how different exercise intensities affect the proportions of 25-hydroxycholecalciferol in vitamin D deficient people.

Design of study: An integrated program with BOOT camps was run at the "Department of Physiology, BMSI, JPMC, Karachi" a year after the summary was accepted.

Participants and Methods: There were 100 participants in the study, and they were split into four groups. Individuals assigned to low intensity exercises in Group B (controls) (n=25), those assigned to moderate intensity activities in Group C (n=25), and those assigned to vigorous intensity exercises in Group D (n=25). On days 1 and 90, blood samples for the serum 25(OH) D were taken from each person. Through ELISA, vitamin D serum analysis was calculated. The SPSS 22 version was used to analyze the data that had been gathered.

Results: According to this study, the average patient age in Group A (the control) was 30 ± 4.9 , in Group B it was 28.6 ± 5.89 , in Group C it was 29.9 ± 5.09 and in Group D it was 29.8 ± 4.89 , with a p value of ($p = 0.607$). In total, 41 men (37.5%) and 50 women (62.5%) participated in our study. In the strenuous exercise intervention group, the levels of vitamin D at baseline were 14.9 ± 2.99 ng/ml with a p value of ($p = 0.79$), compared to 14.91 ± 2.89 ng/ml in the control group. They were $20.812.99$ ng/ml in the group receiving the intensive exercise intervention, with a p value of ($p = 0.000$).

Conclusion: This study discovered a relationship between various exercise intensities and persons' levels of 25(OH)D (Vit-D deficient). As a result, this study has drawn the conclusion that content of 25(OH)D increases in plasma due to vigorous activity, as opposed to control, mild, and moderate exercise. It also decreases the %age of fats in the body.

Key Words: Vitamin D, 25 (OH) D, calciferol insufficiency, Corporeal activity, Exercise

Physiology

In the epidermis, a series of processes starting with "7- dehydrocholesterol" lead to the formation of vitamin D3. After exposure of "7-dehydrocholesterol" to ultraviolet radiation, with the

wavelengths between 280 and 320 nm. It changes into “pre-vitamin D₃”, which is then turned into vitamin D₃ in the skin via “thermally induced isomerization”. Newly generated vitamin D₃ enters circulating system from skin by affixing to a protein (vitamin D binding protein). Vitamin D must go through two hydroxylation in order to activate, creating "1,25[OH]₂ D (Malik, Jan, Haq, Kaur, & Panda, 2022). Atherosclerosis patients increased the level of deficiency of calciferol. It is a biomarker for cardiovascular disorders. For the accurate diagnosis of CVD in both men and women, biomarkers such as vitamin D and serum blood glucose may be employed (Hamad & Abdourahman, 2019).

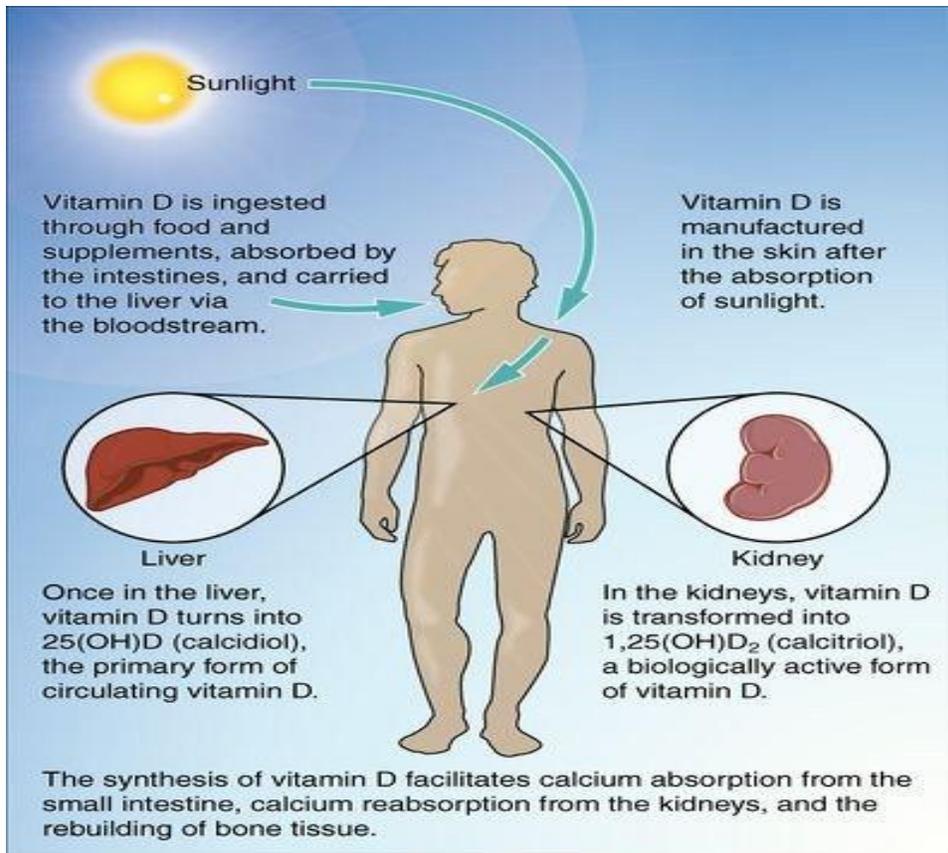


Figure 01. Synthesis of Vitamin D facilitates Calcium Absorption

Hydroxylation of vitamin D occurs at 25th position by the action of 25-hydroxylase (mitochondrial enzyme), also known as hepatic microsomal. In the kidney, "25-hydroxyvitamin D-1 alpha-hydroxylase" carries out second hydroxylation. The "1,25(OH)₂D" hormone binds to a receptor known as "vitamin D receptor" (VDR) after entry inside the cell. A heterodimer is formed when the retinoic acid X receptor and the connected vitamin D receptor work together (RXR). Heterodimer then moves on to the nucleus, where it interacts with "deoxyribonucleic acid"(DNA) and promotes the synthesis of RNA (transcription) of the vitamin D gene (Dabai, Pramyothin, & Holick, 2012). Protein linked with microtubules Although its major activity is in the cytoplasm, the autophagy regulator light chain 3 (LC3), which is prevalent in the nucleus. In an LC3-interacting region (LIR)-independent way, LC3 binds to VDR and facilitates the nuclear translocation of VDR. When high glucose is applied to HK-2 cells, LC3 encourages the

development of the VDR:retinoid X receptor (RXR) heterodimer and suppresses the production of fibrogenic genes (Li et al., 2019).

Pathogenesis

Vitamin D is largely stored in the liver and adipose tissue. It is a fat-soluble vitamin. This vitamin is produced within the body as well as supplemented from outside source. Patients at risk for deficiency should have their serum 25-hydroxyvitamin D levels measured by a trustworthy assay as the initial diagnostic procedure. Patients who are low in vitamin D should receive treatment with either vitamin D2 or vitamin D3 (Holick et al., 2011).

After exposure of UV light, the body creates "7-dehydrocholesterol", which is later changed in the bloodstream to cholecalciferol (vitamin D3). Then it is converted into "25-hydroxyvitamin D3". This happens in the liver by the enzyme "25-hydroxylase". Vitamin D3 is converted into "1,25-dihydroxyvitamin D3" in the kidney. It regulates calcium metabolism. Sunlight exposure and food sources provide vitamin D3. The sunshine vitamin, vitamin D, has played a vital role in the creation of a well calcified vertebrate framework as well as the development of a hormone with a wide range of physiologic effects (F Holick, 2011).

Vitamin D is stored in the liver. Vitamin 25 hydroxylase is subject to negative feedback control in the liver. However, this control is insufficient to prevent toxicity. The vitamin D binding receptors become saturated when significant doses of vitamin D are taken because it is deposited in the liver and adipose tissue. This leads to a rise in many other vitamin D metabolites, including 25(OH)D. There is growing evidence that vitamin D functions as an immunomodulator and lowers tenderness in IBD, strengthening the innate immune system. In addition to suppressing dendritic cell inflammation, inducing antimicrobial venture. It regulates cytokine production in favor for a response against inflammation. The active form of vitamin D, 1,25(OH)D3, operates on T cells to boost T helper (Th)2/regulatory T responses over Th1/Th17 responses (Reich, Fedorak, Madsen, & Kroeker, 2014).

According to clinical research, vitamin D is preserved in fat cells. Vitamin D may not release independently. After its deposition, fatty acids are encouraged for the making of energy. The function of the vitamin D receptor (VDR) is demonstrated by the study on skeletal muscles (anatomy). These enzymes, "25-hydroxyvitamin D-1-hydroxylase (CYP27B1)" and "cytochrome P450 family 24 subfamily members (CYP24A1)," function in combination. This indicated that the vitamin D manufacture and local regulation happens in muscle fibers. As a result, fat

percentages reduced and serum vitamin D levels rose when people exercised. The vigorous interval training provides a stronger trigger for changes in vitamin D levels (Dzik et al., 2022).

Exercise is a crucial catalyst for the mobilization of lipids from adipose tissue. It is therefore possible that physical exercise will lead to the mobilization of both stored fat and vitamin D that has been "stuck" in adipocytes. According to the findings of association studies, those who self-report engaging in more physical activity have greater levels of "25(OH)D" in their blood (Lafontan & Langin, 2009).

In a study using hip-based accelerometry as an objective indicator of physical activity, serum "25(OH)D" levels increased. Recent research indicates that vitamin D status may be directly and causally influenced by exercise. Confounding factors have frequently been blamed for these correlations. For example, dynamic and energetic people spend more time outside and they have greater ratio of sun exposure than others. Traditionally, the predominant circulating form of vitamin D, 25-hydroxyvitamin D [25(OH)D], is used to measure vitamin D status (Hengist et al., 2019).

The main site of vitamin D synthesis is the adipose tissue. VDR and vitamin D processing enzymes are expressed in adipocytes, according to recent study. Additionally, a study demonstrated that vitamin D regulate the expression of adipogenic genes and the demise of adipocytes. Adipocytes are vitamin D-active at all levels. It interacts with membrane receptors, adaptor molecules, and nuclear coregulator proteins. The primary location for vitamin D production is adipose tissue. The VDR and vitamin D metabolizing enzymes are expressed by adipocytes. It has long been known that adipose tissue is where most vitamin D is produced. Adipocytes express the VDR and vitamin D metabolizing enzymes (Abbas, 2017).

Vitamin D helps to control metabolism of energy. Vit-D also controls the synthesis of uncoupling proteins through its genomic activity. In vitro, vitamin D and mVDR interact to encourage lipogenesis and suppress lipolysis. The nuclear VDR (nVDR) complexed with its heterodimer, the retinoid X receptor, is the currently recognised key regulator of the biological action of 1,25(OH)₂D₃ (RXR). The 1,25(OH)₂D₃ binds to VDR and improves VDR/RXR (Jusu, 2016)

mVDR, which is the same as the conventional nVDR, is found in the caveolae of the plasma membrane. Additionally, vitamin D directly affects leptin, a hormone that regulates appetite. Studies have shown that vitamin D regulates the synthesis of a hormone known as “adiponectin’s”, which raises insulin sensitivity. Vitamin D reduced NF-kB signaling, which reduced “cytokine production” and “inflammation” in adipose tissue. Academic studies on the part of immune cells in adipose tissue in the genesis of inflammation associated with obesity are scarce. Adiponectin, a hormone that increases insulin sensitivity, is regulated by vitamin D. Vitamin D decreased cytokine production and adipose tissue inflammation by blocking NF-B signaling (Abbas, 2017).

Operational Definitions

"Guidelines" from the American Endocrine Society

Levels of 25-Hydroxy Cholecalciferol equal to or less than 10ng/ml are considered "Vitamin D insufficiency."

When the level of 25-hydroxy cholecalciferol is between 11 and 20 ng/ml, there is insufficient vitamin D.

Levels of 25-hydroxycholecalciferol that are at least 20ng/ml are considered to be optimal vitamin D concentrations.

Hypothesis

Different exercise intensities have an impact on "25(OH)D" ranges in individuals with vitamin D insufficiency.

Methodology

This is an interventional study. In collaboration with boot camps at the "Faculty of Science (Physiology Department), BMSI, JPMC," the research has been done.

Sample

There were 100 people in the total sample. There were four groups with 25 people in each group. The size of sample is calculated using a 1:1 control to intervention ratio and the mean in "Serum

25(OH)₂D"), measured in nmol/L, between the intervention and control groups (Trubiano et al., 2020). The mean is calculated for the validation of sample size, with an online tool (Openepi.com).

Reference research used to determine sampling size

A fusion of nutrition and exercise interference affects the concentrations of "vitamin B-12," "25-hydroxy vitamin D," in serum. It also affects bone metabolism in healthy individuals (Trubiano et al., 2020).

Assessment of sample

Figure 02. Comparison of mean values for size of sample.

Input Data

Confidence Interval (2-sided)	95%		
Power	80%		
Ratio of sampling size (Group 2/Group 1)	1		
	“Group 1”	“Group 2”	Difference*
Mean Value	80.1	65.6	14.5
Standard deviation	19.2	14.6	
Variance	368.64	213.16	

Sampling size of “Group 1”	22
Sampling size of “Group 2”	22
Total sampling size	44

*Difference between the mean values

The sample size mentioned above includes one control group and one intervention group. The concluding sample size was [22 + 3 drop-out (25)] because there were four groups. One control group and three different exercise groups (intensity). Results of the "OpenEpi" (open-source) calculator using SSMean in version-3.

Recruitment of Participants

A total of 100 persons were recognized based on the standards for assortment from BOOT (Exercise) Camp "BURN OUT 40." All participants had counselling, provided agreement. It was randomly assigned to the subsequent groups.

Group A.

It is non-exercising group or control group. It included 25 people with low levels of vitamin D. Their serum vitamin D levels were between 11 and 20 ng/ml. They did not receive an exercise regimen.

Participants in Group B were told to do low-intensity exercise

There were 25 people with low levels of vitamin D (serum vitamin D levels between 11 and 20 ng/ml) who were randomized to low-intensity exercise.

Participants in "Group C" who moderately exercised

It involved 25 participants who were allocated to moderate intensity exercise and had serum vitamin D levels between 11 and 20 ng/ml, indicating vitamin D deficiency.

Participants in Group D were instructed to engage in vigorous exercise

25 participants with low levels of vitamin D (11-20 ng/ml) were allocated to perform vigorous exercise.

Study Population

Males and females with a vitamin D deficiency and ages ranging from 18 to 39 years made up the study's sample.

Sampling Technique

Consecutive non-probability sampling

This study used a technique called sequential sampling. At the selected study location, a boot camp in an easily accessible neighbourhood of Karachi, all subjects who were willing, eligible, and available consecutively were recruited.

Randomization Technique

The two groups—the exercising group, which was further divided into three sub-groups, and the control group that did not exercise—were randomly assigned. The control group, designated "Group A," and three interventional groups, designated "Groups B," "Group C," and "Group D," were formed for the purposes of this experiment. Simple random sampling was employed via the online public random number generator made available by open "epi.com." Participants were randomly assigned to the exercise or control group after providing written informed permission. There was no blinding in this open-label randomised experiment.

Sample Selection

Inclusion Criteria	Exclusion Criteria
Either gender	Individuals with any medical condition that would contraindicate exercise
Age group 18-39 years	Individuals with renal and liver diseases
Individuals with Vitamin D insufficiency	Individuals with malignancy
Individuals who give consent	Pregnancy/ Lactation
Non-exercising individuals	Any drug affecting serum vitamin D levels or bone mineral density
	Individuals having musculoskeletal disease
	Individuals currently on any energy restricting diet, supplements or exercise program
	Individual with co-morbid condition like diabetes and hypertension

Sample Collection

On days 1 and 90 of the experiment, the participant's "median cubital vein" was punctured. After utilising aseptic techniques to clean the site, a sample of blood with a volume of roughly 10 ml was taken. Each participant's tube was first marked with a unique ID. 4 ml of blood were then put into a gold-topped vacutainer that included separating gel and clotting activator to measure serum vitamin D levels.

Detailed Consent

Each participant gave their free, informed consent to take part in the study by signing the consent form. The form was authorized by the JPMC Institutional Review Board in Karachi.

Ethical Considerations

By letter number NO.F.2-81/2021- GENL/70209/JPMC, the Institutional Review Board of the "Jinnah Postgraduate Medical Centre (JPMC)", Karachi, was contacted for the ethical approval. The collected data was treated with utmost confidentiality.

70209
NO.F.2-81/2021-GENL/ /JPMC
JINNAH POSTGRADUATE MEDICAL CENTRE
KARACHI.75510.

Dated the 22/11/2021

Dr. Sana Mahmood
Department of Physiology, BMSI
JPMC, Karachi.

Subject: *Effect of exercise on vitamin-D levels in vitamin-D insufficient individuals.*

With reference to your application / letter dated 17th June, 2021, on the subject noted above
and to say that the Institutional Review Board has approved your subject proposal.



Prof. Syed Mehboob Alam
Chairman, Institutional Review Board Committee
JPMC, Karachi.

Copy forwarded for information and necessary action to:

- Dr. Nargis Anjum, Department of Physiology, Karachi University, Karachi.
- Dr. Kousar Abbas, Assistant Prof. of Psychology BMSI, JPMC, Karachi.

Data Collection Method

The study included any members who joined "JPMC" or boot camp and who met the inclusion and exclusion criteria. Informed permission will be acquired after volunteers have been fully informed of the study's goals. On a specially created questionnaire, each participant's identify, age, gender, location, socioeconomic standing, education, career, relationship status, health information, drug use, physical evaluation (weight, stature, body fat levels, and BMI), and laboratory tests were all noted (including Vitamin D). This information was inputted onto an assessment criteria program on day 1 and day 90. Every subject was thoroughly questioned regarding compliance by the researcher during every visit.

All of the participants were given a brief explanation of the exercises and the advantages of participating in training sessions after being placed in the appropriate groups. All of them were required to stay in class the entire three months. All of the workouts were performed at night on an open field with artificial lighting. On day 1, all participants had their anthropometric measurements taken as well as blood samples drawn to check their vitamin D levels. Participants were acclimatised to exercise from day two through day five because they had previously been sedentary. The criteria for gauging exercise intensity was done by monitoring the overall heart rate through fitness trackers that every participant was wearing as provided by Boot Camp. We have three groups based on different exercise intensities. The simplest technique to gauge workout intensities is to measure total heart rate. The quickest heart rate at which anyone's heart may beat without danger is known as the maximum heart rate (MHR). to determine the highest heart rate of someone.

$$\text{MHR} = 220\text{bpm} - \text{Age of Person}$$

Through a fitness tracker, everyone's heart rate was indicated when they were working out. The TALK TEST and EXERCISE RATING SCALE were two more substitute criteria for determining exercise intensity. For our analysis, we simply took MHR into account. At day 90 of the exercise/boot camp, we took a second reading of the participants' anthropometric measurements and blood samples to check their vitamin D levels.

CLASSIFICATION OF EXERCISES BASED ON DIFFERENT INTENSITIES

These are the exercises which were being done at Boot Camp for three different exercise groups

Every circuit was provided by 2 minutes resting phase

LOW INTENSITY EXERCISE GROUP**MODERATE INTENSITY EXERCISE GROUP****HIGH INTENSITY EXERCISE GROUP**Duration 1 hour/6 days a weekDuration 1 hour/ 6 days a weekDuration 1 hour/ 6 days a weekWarm up 8minWarm up 8minWarm up 8 min

5 squats

10 squats

25 squats

5 jacks

10 jacks

25 jacks

30 skipping ropes

50 skipping ropes

75 skipping ropes

10 high knees

20 high knees

30 high knees

10 standing mountain climbers

30 standing mountain climbers

50 standing mountain climbers

Circuit-1 12 minutesCircuit-1 12 minutesCircuit-1 12 minutes(1 set of each)(2 sets of each)(3 sets of each)

Hop leg raises x 20

Hop leg raises x 20

Hop leg raises x 20

Flutter kicks x 25

Flutter kicks x 25

Flutter kicks x 25

Grasshopper crunches x 20

Grasshopper crunches x 20

Grasshopper crunches x 20

Forward crunches x 20

Forward crunches x 20

Forward crunches x 20

Bicycle crunches x 20

Bicycle crunches x 20

Bicycle crunches x 20

90 degree crunches x 20

90 degree crunches x 20

90 degree crunches x 20

Standing mountain climbers x 50

Standing mountain climbers x 50

Standing mountain climbers x 50

Circuit-2 12 minutesCircuit-2 12 minutesCircuit-2 12 minutes(1 Set of each)(2 Set of each)(3 Set of each)

Plank 30 seconds

Plank 30 seconds

Plank 30 seconds

Burpees x 5

Burpees x 5

Burpees x 5

Push up Planks 30 Seconds

Push up Planks 30 Seconds

Push up Planks 30 Seconds

Burpees x 5

Burpees x 5

Burpees x 5

Squat Hold 30 Seconds

Squat Hold 30 Seconds

Squat Hold 30 Seconds

Burpees x 5

Burpees x 5

Burpees x 5

Circuit-3 12 minutesCircuit-3 12 minutesCircuit-3 12 minutes(1 Set of each)(2 Set of each)(3 Set of each)

Hop Squat x 10

Hop Squat x 10

Hop Squat x 10

Mountain climbers x 50

Mountain climbers x 50

Mountain climbers x 50

Jump Squat x 10

Jump Squat x 10

Jump Squat x 10

Mountain Climbers x 50

Mountain Climbers x 50

Mountain Climbers x 50

Power Jack x 10

Power Jack x 10

Power Jack x 10

Mountain Climbers x 50

Mountain Climbers x 50

Mountain Climbers x 50

Circuit-4 12 minutesCircuit-4 12 minutesCircuit-4 12 minutes(1 Set of each)(2 Set of each)(3 Set of each)

Side Stretch x 20

Side Stretch x 20

Side Stretch x 20

Bent Over Stretch x 20

Bent Over Stretch x 20

Bent Over Stretch x 20

Standing Twist Stretch x 20

Standing Twist Stretch x 20

Standing Twist Stretch x 20

Good Morning Stretch x 20

Good Morning Stretch x 20

Good Morning Stretch x 20

RESULTS

Table 01. Comparison of Age (years), Height (cm), Gender, Marital status and Occupation of Participants

Statistics	Control non- exercising (A) (n=25)	Non- Low intensity exercise (B) (n=25)	Moderate intensity exercise © (n=25)	Rigorous intensity exercise (D) (n=25)	Test Statistic	p- value
Age (years)					F-value	p-value
Age (Mean ± SD) years	31.08±5.008	29.16±6.236	31.04±5.834	30.52±5.724	0.615	0.607
Age range (year)	21-40	18-37	22 - 40	20 - 40		
Height (cm)					F-value	p-value
Height (cm)	166.780±11.31	168.608±10.067	162.919 ± 7.577	166.954±10.388	1.613	0.191
Gender					Chi-Sqaure	p-value
Male (n=41)	11	10	10	10		
Female (n=59)	14	15	15	15	0.124	0.898
Marital Status					Chi-Sqaure	p-value
Married (n=55)	12	14	16	13		
Unmarried (n=45)	13	11	9	12	1.414	0.702
Occupation					Chi-Sqaure	p-value
Indoor working (n=58)	12	18	18	10		
Outdoor working (n=26)	6	7	3	10	1.863	0.312
House wife (n=16)	7	0	4	5		

Table 02. Comparison of Body Weight (kg) Pre and Post Exercise Training in vitamin D insufficient individuals within groups

Groups	Statistic	Body Weight (Kg)		Mean of Difference (kg)	t-value	p-value	95 % CI ** of difference	
		Pre Training (Day-1)	Post Training (Day-90)				L..C.L	U..C.L
Control Non-exercising (A)	n	25	25	0.16	0.411	0.685	0.636	0.952
	Mean	85.98	86.14					
	±SD	13.59	13.04					
Low intensity exercise (B)	n	25	25	-1.27	9.636	0.001*	0.999	1.544
	Mean	85.92	84.65					
	±SD	11.26	11.39					
Moderate intensity exercise (C)	n	25	25	-2.48	14.017	0.001*	2.113	2.843
	Mean	84.29	81.81					
	±SD	16.06	15.53					
Rigorous intensity exercise (D)	n	25	25	-4.01	12.879	0.001*	3.365	4.649
	Mean	90.08	86.08					
	±SD	17.18	17.22					

* : Significant difference

**C.I: Confidence Interval

Table 03. Comparison of Body Fat (%) Pre and Post exercise Training in vitamin D Insufficient individuals within groups

Groups	Statistic	Body Fat (%)		Mean of Difference (Body Fat %)	t-value	p-value	95 % CI** of difference	
		Pre Training (Day-1)	Post Training (Day-90)				L.C.L	U.C.L
Control Non-exercising (A)	n	25	25					
	Mean	41.44	40.72	0.716	0.268	0.791	-4.791	6.223
	±SD	9.61	9.06					
Low intensity exercise (B)	n	25	25					
	Mean	36.61	41.71	-5.106	1.972	0.06	-10.45	0.238
	±SD	9.83	12.10					
Moderate intensity exercise (C)	n	25	25					
	Mean	46.25	41.90	4.347	1.216	0.235	-3.033	11.73
	±SD	12.589	10.760					
Rigorous intensity exercise (D)	n	25	25					
	Mean	45.95	40.61	5.345	1.52	0.141	-1.91	12.6
	±SD	11.08	9.51					

Table 04. Comparison of Body Mass Index (Kg/m-sq) Pre and Post Exercise Training in Vitamin D insufficient individuals within groups

Groups	Statistic	Body Mass Index (Kg/m-sq)		Mean Difference (kg/m-sq) (Pre to Post)	t-value	p-value	95 % CI ** of difference	
		Pre Training (Day-1)	Post Training (Day-90)				L.C.L	U.C.L
Control Non-exercising (A)	n	25	25					
	Mean	30.69	30.39	-0.30	2.651	0.014*	0.067	0.54
	±SD	5.24	5.38					
Low intensity exercise (B)	n	25	25					
	Mean	30.39	29.90	-0.48	6.913	0.001*	0.339	0.627
	±SD	4.44	4.46					
Moderate intensity exercise (C)	n	25	25					
	Mean	31.91	30.65	-1.25	4.438	0.001	0.670	1.836
	±SD	6.11	5.62					
Rigorous intensity exercise (D)	n	25	25					
	Mean	32.88	30.46	-2.42	6.329	0.001	1.63	3.207
	±SD	7.17	6.63					

Table 05. Comparison of serum 25(OH)D (ng/dl) concentrations Pre and Post Exercise Training in vitamin D insufficient individuals within groups

Groups	Statistic	Vit-D (ng/dl)		Mean Difference (ng/dl) (Pre to Post)	t-value	p-value	95 % CI** of difference	
		Pre Training (Day-1)	Post Training (Day-90)				L.C.L	U.C.L
Control Non-exercising (A)	n	24	24					
	Mean	15.93	15.94	0.0050	0.277	0.784	-0.042	0.032
	± SD	2.80	2.76					
Low intensity exercise (B)	n	25	24					
	Mean	15.40	16.55	1.1486	5.871	0.001*	-1.590	-0.765
	± SD	2.53	2.90					
Moderate intensity exercise (C)	n	25	24					
	Mean	15.14	18.05	2.9145	17.068	0.001*	-3.116	-2.443
	± SD	2.22	2.38					
Rigorous intensity exercise (D)	n	25	24					
	Mean	15.00	20.75	5.7462	37.582	0.001*	-8.93	-5.31
	± SD	3.11	2.99					

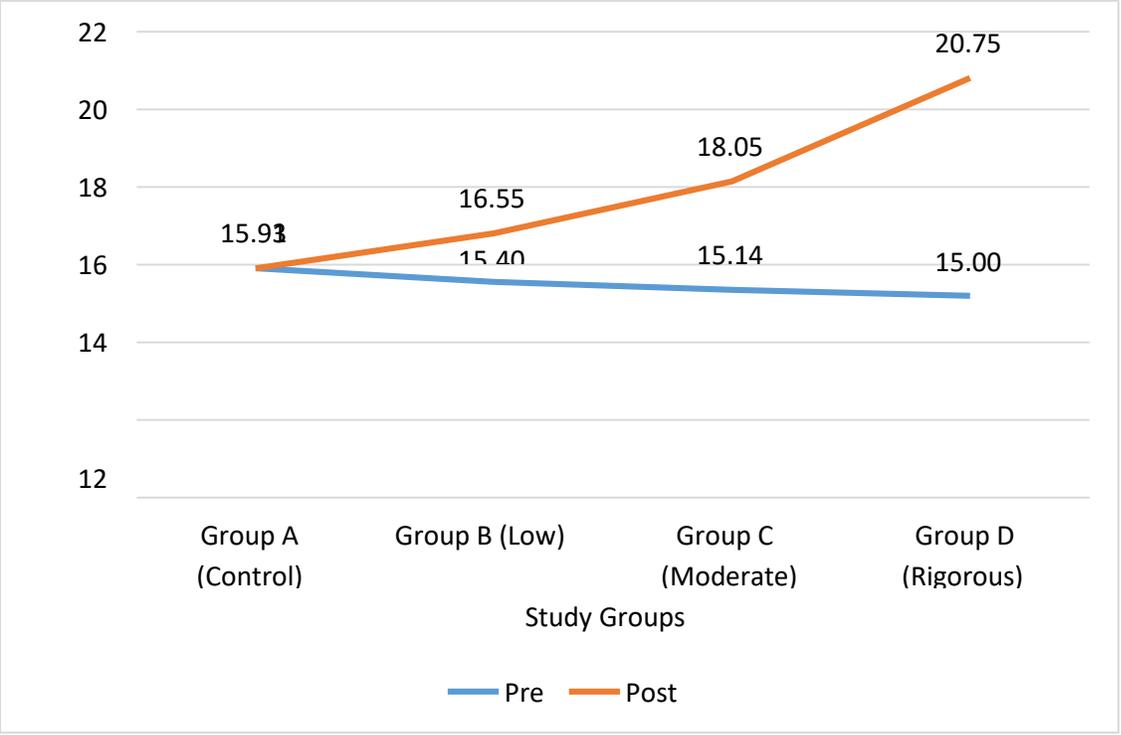


Figure 03. Comparison of ranges of serum 25(OH) D concentrations pre-training (day-1) and post-training (day-90) in vitamin D insufficient individuals

Groups	Statistic	Vit-D (ng/dl)	
		Pre Training (Day-1)	Post Training (Day-90)
Control Non-exercising (A)	N	24	24
	Mean	15.93	15.94
	± SD	2.80	2.76
Low intensity exercise (B)	N	25	24
	Mean	15.40	16.55
	± SD	2.53	2.90
Moderate intensity exercise (C)	N	25	24
	Mean	15.14	18.05
	± SD	2.22	2.38
Rigorous intensity exercise (D)	N	25	24
	Mean	15.00	20.75
	± SD	3.11	2.99
A vs B	t-value	0.702	0.746
	p-value	0.486	0.459
A vs C	t-value	1.101	2.846
	p-value	0.277	0.007*
A vs D	t-value	1.102	5.793
	p-value	0.276	0.001*
B vs C	t-value	0.385	1.969
	p-value	0.702	0.055*
B vs D	t-value	0.497	4.942
	p-value	0.622	0.001*
C vs D	t-value	0.182	3.450
	p-value	0.857	0.001*

Table 06. Comparison of serum 25(OH)D ng/dl concentrations pre and post exercise training in Vit-D insufficient individuals between groups

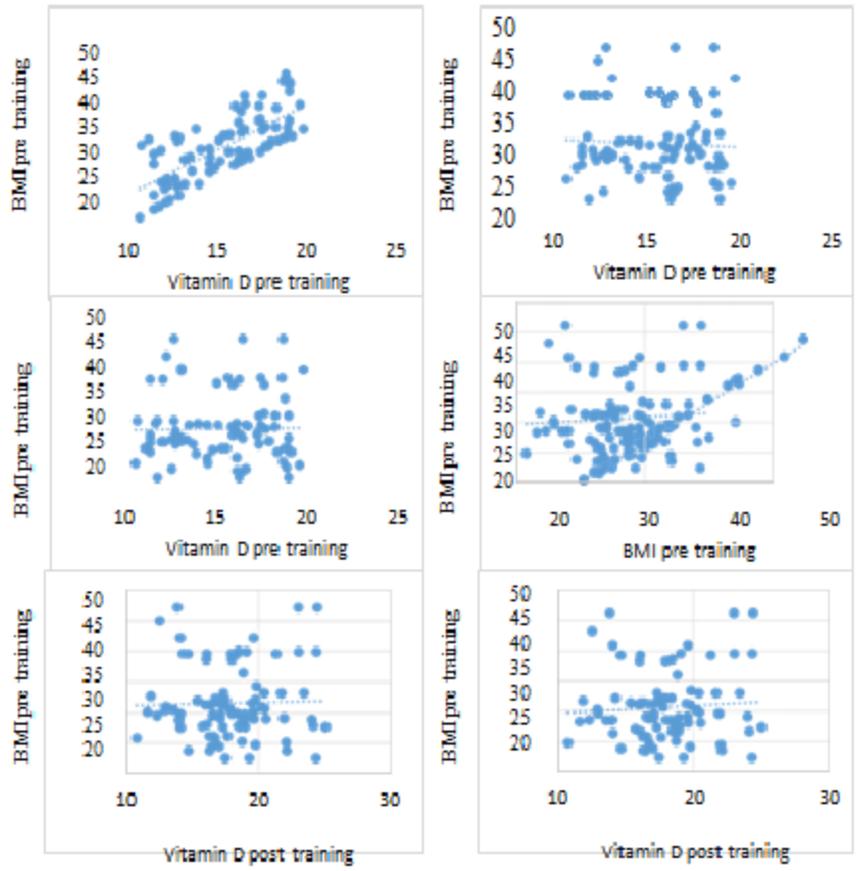


Figure 04. Results of correlation between vitamin D levels and BMI

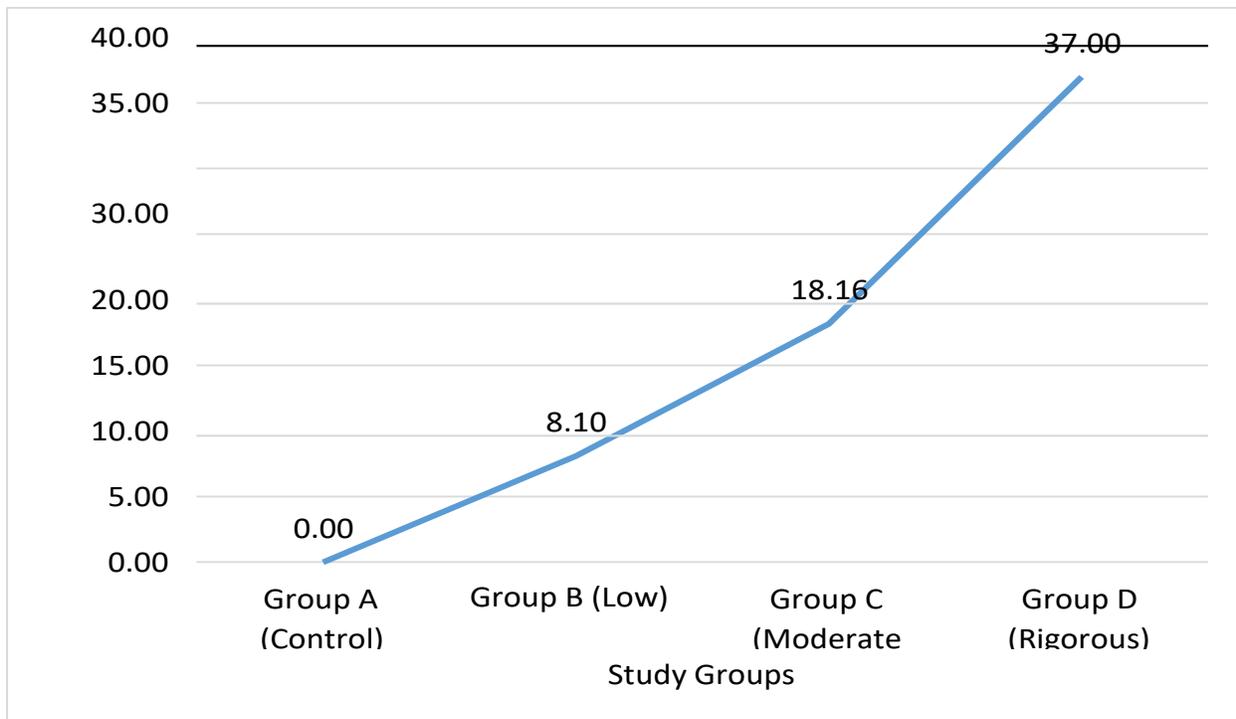


Figure 05. Results of % change in vitamin D levels (ng/ml) in study groups

Conclusion

The average patient age was found to be 31.07 in Group A (the control), 29.8 in Group B, 31.09 in Group C, and 30.52 in Group D, with a p value of ($p = 0.607$). 50 women (62.5%) and 41 men (37.5%) in total took part in our study. As opposed to 15.7 ng/ml in the control group, the baseline vitamin D levels in the group receiving the rigorous activity intervention were 15.8 ng/ml with a p value of ($p = 0.69$). Vitamin D levels after training were 14.9 ng/ml in the control group and 20.8ng/ml in the group receiving the rigorous exercise intervention, with a p value of ($p = 0.000$), respectively. This study found a link between different exercise intensities and people's levels of 25(OH)D who are vitamin D deficient. As a result, we deduce that intense exercise, as compared to control, mild, and moderate exercise, raises plasma levels of 25(OH)D while lowering body fat percentage.

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