ORIGINAL ARTICLE

Effect of vitamin D supplementation on serum lipid levels in male albino mice taking high fat diet

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ABSTRACT

Background: Dietary fats lead to hyperlipidemia which causes multiple disorders. Vitamin D reduces serum lipid levels and prevents the development of hyperlipidemia.
Methodology: In this randomized control trial , 90 male albino mice were randomly taken into three groups with 30 mice in each group. Group A was given normal diet, group B high fat diet, group C high fat diet plus vitamin D (calcitriol, 100ng/kg per day) for 6 weeks. By the end of 6 weeks terminal blood sampling was taken. Serum was analysed for lipid profile by calorimetric method. Data was analysed by using SPSS version 20.
Results: Serum total cholesterol(TC), low density lipoproteins(LDL), very low density lipoproteins(VLDL) were all raised and high density lipoproteins (HDL) was reduced in high fat diet group B. TC, LDL, VLDL all were reduced and HDL was raised in high fat plus vitamin D group C as compared to group B.
Conclusion: Vitamin D prevents hyperlipidemia when administered along with high fat diet.
Keywords: Hyperlipidemia, Albino Mice and Vitamin D

INTRODUCTION

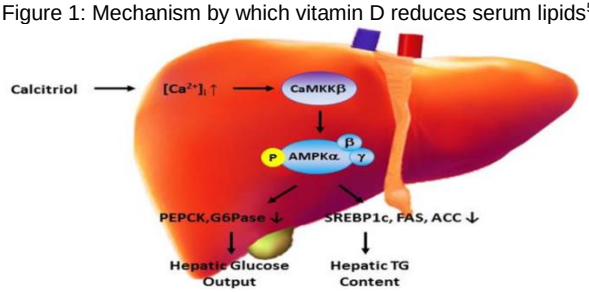
Prolonged consumption of diet rich in fats causes hyperlipidemia[1]. Hyperlipidemia means increased levels of total cholesterol (TC), low density lipoproteins (LDL), intermediate density lipoproteins(IDL), very low density lipoproteins(VLDL) and low levels of high density lipoproteins(HDL). Hyperlipidemia is a risk factor for multiple disorders like cardiovascular diseases, hypertension, diabetes mellitus[2].

Fats in the diet are absorbed from intestine in the form of chylomicrons. Increased intake of fats leads to increased production of chylomicrons. Size of chylomicron particles is also increased and all this leads to increased levels of serum lipids.

High fat diet decreases LDL receptors on liver. So, uptake of LDL by liver is decreased and serum LDL levels are increased. High fatdiet also causes upregulation of sterol regulatory elemental binding protein (SREB) which is a transcription factor and boosts up synthesis of fats by liver. High fat diet reduces HDL levels as it down regulates ABCA1 gene. ABCA is required for cholesterol reverse transport. If this transporter is not present, cholesterol is not transported from the adipose tissues and muscles to the liver and HDL levels are also reduced in the blood[3].

Vitamin D is now considered as a hormone because it has multiple actions. It is absorbed by GIT chylomicrons into lymphatics. From lymphatics it goes to circulation. Its first hydroxylation occurs in the liver and second occurs in the kidney. Calcitriol formed thus binds with vitamin D receptors. VDR are located at various sites of body and many of the effects of vitamin D are mediated by VDR[4]. Vitamin D increases expression of LDL receptors on the liver, so serum LDL levels will decrease. Vitamin D also reduces expression of SREB transcription factor in the liver and reduces fats synthesis[5](figure 1). Vitamin D also improves expression of ABCA1 gene which improves cholesterol efflux from peripheral tissues and increases serum HDL levels.

Figure 1: Mechanism by which vitamin D reduces serum lipids[5]



METHODOLGY

Present study is a randomized control trial carried at the Department of Physiology, Akhtar Saeed Medical and Dental College (AMDC), Lahore from February 2018- April 2018. Ninety (90) male albino mice were purchased from the University of Veterinary and Animal Sciences, Lahore. Age of mice was 8-10 weeks. Mice were placed in cages, 15 mice in each cage and they were kept at a temperature of 26℃ , 12:12 hour light and dark cycle, humidity of 45-65%[6]. Mice were fed commercially available standard pellet diet. They had free access to food and water. Mice were given 1 week for acclimatization, then they were randomly taken into three groups of 30 each.

Group A (Normal diet group, n=30) was given normal diet for 6 weeks. (11% kcal from fat)
Group B (High fat diet group, n=30) was given high fat diet for 6 weeks. (44% kcal from fat)
Group C (high fat diet plus vitamin D group, n=30) was given high fat diet and vitamin D for 6 weeks.
High fat diet[7](table-1) was administered for 6 weeks[7] to induce hyperlipidemia in mice of group B and C. Group C mice were also given vitamin D along with high fat diet at a dose of 100ng/kg/day[8,9] through oral gavage for 6 weeks. They were weighted on weekly basis.

Components of diet	Normal diet g/100g of diet	HF diet g/100g of diet
Casein protein	16.47	16.47
L-cystine	0.18	0.18
Cornstarch	44.09	0
Maltodextrin 10	35	125
Sucrose	10	10
Cellulose	5	5
Soybean oil	4	0.00
Lard fat	0.00	20
Minerals	3.50	3.50
Vitamins	1.0	1.0
Choline bitartrate	0.25	0.25
Cholesterol	0.00	1.00
Cholic acid	0.00	0.10
Dextrinized corn starch	15.50	15.50
Lipids(g/100g)	4.1± 0.1	20.8±1.6
Energy from lipids(Kcal)	36	189
%age energy from lipids	11	44

Table-1: Composition of normal diet and high-fat (HF) diet

Solution of vitamin D was prepared in corn oil[10] by dissolving vitamin D (calcitriol) powder in corn oil. Vitamin D solution was placed at 4°**C** temperature in a tinted glass bottle to save it from sunlight. By the end of 6 weeks, blood sample was taken by terminal cardiac puncture technique. Blood was immediately taken into clot activator vials. Blood was allowed to clot in the vials. After 15 minutes, vials were centrifuged at a speed of 5000 rpm for 10 minutes. Resulting supernatant was serum. Serum was transferred to sterilized eppendorf containers (which were already labeled) with the help of pasteur pipette. Eppendorf containers of three groups were placed in three separate racks which were also properly tagged and freeze at -20°C until used for analysis. Lipid profile was analysed by using Calorimetric method.

Statistical analysis: Data was analysed by using software SPSS version 20. Mean values and standard deviations for quantitative variables like serum lipid profile, (total cholesterol, triglycerides, HDL).One way ANOVA and post hoc Tukey's tests were applied to determine the statistical significance of various parameters amongst three groups. Results were presented as mean ± SD with p-value ≤ 0.05 was considered significant.

RESULTS

The difference of serum lipid parameters between the normal diet group(A), high fat diet group (B) and high fat diet plus vitamin D group (C) was highly significant (p=0.000) as evident by applying one way ANOVA (Table-2). Comparison of serum lipid profile among groups by applying Post hoc Tukey's test showed highly significant results (p=0.000) as depicted in table -3.

Table 2: Comparison of serum lipid profile among Different diet groups

Parameters measured	Group A (n=30)	Group B (n=30)	Group C (n=30)	p value
Serum cholesterol (mg/dl)	137.27±27.98	257.10±37.13	116.80±19.58	0.000**
Serum Triglycerides (mg/dl)	131.03±13.27	228.23±47.21	108.40±11.06	0.000**
Serum high density lipoproteins (HDL)(mg/dl)	32.10±6.62	26.37±5.38	41.03±4.11	0.000**
Serum low density lipoproteins (LDL) (mg/dl)	78.97±28.17	185.39±37.31	54.10±22.08	0.000**

**Statistically highly significant.

Table 3: Comparison of serum lipid profile among groups by applying Post hoc Tukey's
**Statistically highly significant.

Parameters measured	Comparison between the groups		p value
Serum cholesterol (mg/dl)	A	B	0.000**
	B	C	0.000**
	C	A	0.021*
SerumTriglyceride (mg/dl)	A	B	0.000**
	B	C	0.000**
	C	A	0.009*
Serum high density lipoproteins(mg/dl)	A	B	0.000**
	B	C	0.000**
	C	A	0.000**
Serum low density lipoproteins (mg/dl)	A	B	0.000**
	B	C	0.000**
	C	A	0.005*

DISCUSSION

In the current study, high fat diet increased serum lipids and vitamin D supplementation reduced serum lipids. Quach et al[11] showed that intraperitoneal injections of vitamin D or its analogues every alternate day for 8 days in mice on high fat diet reduced hepatic and serum cholesterol levels. They showed that HFD increased mRNA expression of small heterodimer partner (SHP) protein, which is a nuclear receptor and a transcriptional regulator of various proteins involved in cholesterol metabolism in the liver, whereas vitamin D supplementation by down regulating SHP expression, increased cholesterol metabolism in the liver and decreased hepatic cholesterol levels. Vitamin D also increased production of bile acids. High doses of $25(OH)D_3$ induced hypercalcemia and hepatic toxicity as manifested by increased ALT levels. In current study HFD raised serum lipids while vitamin D reduced them. We used $1,25(OH)_2D_3$ which was less likely to induce hypercalcemia and hepatic toxicity as it was immediately removed from circulation as compared to other vitamin D analogues which had increased plasma half life.[11]

A study conducted by Zhu CG et al[12] showed that vitamin D supplementation protected against hyperlipidemia in mice. Vitamin D supplementation in high fat diet group reduced all lipid parameters and raised High density lipoprotein levels. Vitamin D (calcitriol) was given intra peritoneally twice every week at a dose of 5 µg/kg. Serum calcium and phosphate levels were not affected in vitamin D supplemented group showing that this dose of vitamin D was non toxic. Histology of liver was also analyzed. High fat diet induced inflammation and lipid accumulation in the liver which was improved by vitamin D supplementation.[12] The results of our study matched this study and HFD model was also same.

Liu et al[13] came out with different and unexpected results. They prepared a high fat diet plus vitamin D deficient mice model (HFD+VDD) by depriving the mice of dietary vitamin D and UV light exposure. After 14 weeks, they measured serum TC, TG, VLDL. In high fat diet plus vitamin D deficient mice, results were quite unexpected, as body weight, liver weight, hepatic triglycerides, serum total cholesterol, triglycerides, very low density lipoproteins levels all were reduced as compared to HFD mice who had no vitamin D deficiency. High fat diet upregulated mRNA expression of enzymes involved in hepatic fatty acid uptake and synthesis, while vitamin D deficiency reduced all these effects induced by high fat diet. It seemed that vitamin D deficiency had beneficial effects on serum and hepatic lipids. In our study, high fat diet increased serum lipids while vitamin D supplementation in mice taking high fat diet reduced serum lipid levels. High fat diet model in both studies was same but in their study they induced vitamin D deficiency while we gave vitamin D supplementation. Their study showed that vitamin D deficiency might not be the only factor which was determining the fate of lipids.[13]

Ramiro-Lozano et al[14] investigated 28 patients with type 2 diabetes and vitamin D deficiency with serum 25(OH)D levels less than 20 ng/ml. They were given calcifediol 16000 IU/week for 8 weeks. In all patients serum 25(OH)D levels raised more than 20 ng/ml. In all these patients, serum total cholesterol reduced significantly after vitamin D supplementation. Serum LDL, TG reduced but results were not significant statistically. There was no effect on serum HDL levels.

No doubt this study gives us important results and highlights the important effects of vitamin D supplementation on serum lipid profile but before recommending vitamin D supplementation in humans, further animal studies and human trials are mandatory to find out the safe and effective dose of vitamin D having lipid lowering effect.

CONCLUSION

We concluded from present study that vitamin D reduces serum lipid levels when administered to mice on high fat diet.

Conflict of interest: None
Funding: None

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