DOI: https://doi.org/10.53350/pjmhs221610661

ORIGINAL ARTICLE

Effect of Vitamin D Supplementation on High Fat Diet Induced Thyroid Dysfunction an RCT on Mouse Model

CHAMAN NASRULLAH[1], MAIMONA TABBSUM[2], MAIMOONA NASREEN[3], SAMAN SAEED ANSARI[4], HAFIZA HINA PASHA[5]
[1]*Assistant Professor Physiology, University College of Medicine and Dentistry, University of Lahore*
[2]*Assistant Professor Physiology, Akhtar Saeed Medical and Dental College, Lahore*
[3]*Associate Professor Physiology, University College of Medicine and Dentistry, University of Lahore*
[4]*Assistant Professor Physiology, University College of Medicine and Dentistry, University of Lahore*
[5]*Assistant Professor Physiology, Shalamar Medical and Dental College, Lahore*
Correspondence to: Chaman Nasrullah

ABSTRACT

Background: Excess of dietary fats induce hyperlipidemia which causes endoplasmic reticulum (ER)stress. ER stress causes thyroid dysfunction and reduces serum thyroid hormone levels. Vitamin D supplementation increases thyroid hormone levels by reducing high fat diet induced ER stress, inflammation and autoimmunity.

Objective: This study was planned to determine the effect of vitamin D supplementation on levels of serum free and total thyroxine in male albino mice.

Methodology: In this randomized control trial, ninety healthy male albino mice were taken by consecutive, nonprobability sampling and randomly divided into three groups, each group containing 30 mice. Group A mice were given normal diet, Group B mice were given high fat diet, Group C mice were given high fat diet and vitamin D through oral gavage (100ng/kg/day) for 6 weeks. By the end of 6 weeks, blood sample of all mice was taken by cardiac puncture and serum was separated. Levels of serum total thyroxine and free thyroxine were measured by ELISA technique. Data was analyzed by using SPSS version 20.

Results: In group B mice (high fat diet group) serum total and free thyroxine levels were reduced significantly (p=0.00) as compared to group A mice (normal diet group). In group C mice (high fat diet plus vitamin D group) serum total and free thyroxine levels were increased significantly (p=0.00) as compared to group B mice (high fat diet group). The results of group C and A were also comparable and significant, with serum free and total thyroxine levels significantly high in group C (high fat diet+ vitamin D) as compared to group A(normal diet).

Conclusion: Vitamin D supplemented mice on high fat diet had increased levels of serum total thyroxine, free thyroxine as compared to the group of mice on high fat diet only.

Keywords: Vitamin D, High fat diet, Hyperlipidemia, Thyroxine.

INTRODUCTION

Prolonged consumption of diet rich in fats causes hyperlipidemia. Hyperlipidemia means increased levels of chylomicrons, low density lipoproteins (LDL), very low density lipoproteins (VLDL), triglycerides (TG) and low levels of high density lipoproteins (HDL). Hyperlipidemia is a risk factor for multiple disorders[1] like cardiovascular diseases, hyper tension, diabetes mellitus and thyroid dysfunction.[2]

Thyroglobulin is a protein synthesized by thyrocytes. Thyroid peroxidase (TPO) is required for iodination of thyroglobulin as well as coupling of tyrosine residues to form thyroxine (T_4) and tri-iodothyronine (T_3). Any disorder leading to decreased production of thyroglobulin results in decreased secretion of thyroid hormones. Endoplasmic reticulum stress (ER stress) is an important mechanism by which HFD reduces thyroid hormone levels.[3]

Endoplasmic reticulum (ER) is an important organelle which synthesizes lipids and cholesterol, stores calcium and is a site for modification of proteins after translation. Endoplasmic reticulum is also responsible for proper folding and maturation of proteins. ER stress is induced when ER is no more able to fold the proteins, unfolded proteins accumulate, or there is impaired glycosylation of the proteins. ER stress triggers a set of signaling pathways called unfolded protein response (UPR) to offset the ER load, so, unfolded protein response (UPR) is an adaptive response against ER stress. Unfolded protein response (UPR) includes increased folding of proteins, degradation of misfolded proteins by ERAD (endoplasmic reticulum associated protein degradation, a proteasome degradation pathway) and decreased secretion of proteins by the cell. If UPR fails to regain protein homeostasis, apoptosis of the cell occurs.[4] Mild ER stress promotes healing while severe or prolonged ER stress leads to apoptosis.

High fat causes oxidative stress and increases the production of free radicals which cause thyrocytes destruction by necrosis and apoptosis. Oxidative stress also initiates autoimmunity in thyroid gland leading to its destruction.[5]

Vitamin D is a fat soluble vitamin. It is required for calcium and phosphate homeostasis as well as multiple other beneficial effects on various organ systems of the body. Vitamin D has multiple genomic and nongenomic actions. Genomic actions are regulation of the gene expression. $1,25(OH)_2D_3$ acts through vitamin D receptors (VDR).[6] VDR is a member of steroid receptor family which bind ligands like vitamin D, retinoic acid, hormones of thyroid gland, sex steroidal hormones and adrenal gland steroids. VDR are located on the cell membrane or nuclear membrane. However, whether all of the actions of VDR require $1,25(OH)_2D_3$ or not, is not clear yet.[7]

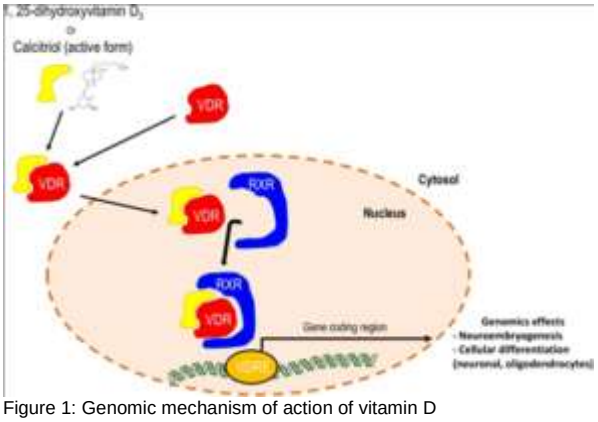

Figure 1: Genomic mechanism of action of vitamin D

$1,25(OH)_2$ D_3 after binding with VDR activates second messenger systems like protein kinase-A (PKA) and protein kinase-C (PKC) resulting in increased formation of second messengers like inositol tri phosphate (IP_3), which in turn leads to increased entry of calcium from extracellular or increased release from intracellular sources like endoplasmic reticulum. Membrane associated receptors are six to ten times more abundant than nuclear VDR. Nuclear VDR binds with $1,25(OH)_2D_3$ and makes a heterodimer complex with retinoid X receptor (RXR). This complex then binds with a specific DNA sequence called vitamin D response element (VDRE) either in or around the genes to be

targeted and activates or suppresses transcription. There are various co regulators which complex with VDR and affect the transcriptional activity of VDR.[6] (figure 1)

Vitamin D supplementation reduces ER stress. ER stress reduces vitamin D receptor (VDR) expression on the cells while vitamin D increases VDR expression.[9] ER stress activates NF-κB (nuclear factor kappa B) signaling which leads to autoimmunity in thyroid gland (Hashimoto thyroiditis) and reduces production of thyroid hormones.[10] Vitamin D supplementation reduces NF-κB expression which decreases the production of auto antibodies in thyroid gland and prevents decrease in thyroid hormone levels.[9]

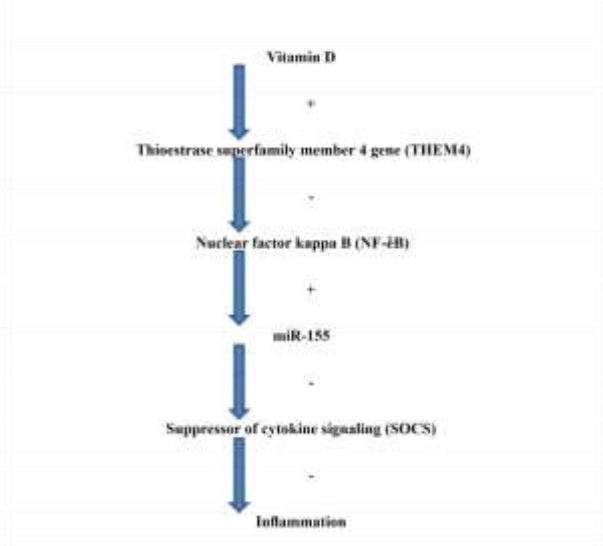

Figure 2: Mechanism by which vitamin D reduces inflammation[9]

METHODOLOGY

Study Design: Randomized control trial.
Study Place: Department of Physiology, Akhtar Saeed Medical and Dental College (AMDC), Lahore.
Sampling: Non probability consecutive sampling.
Duration: February 2021 to November 2021.
Sample Size: 90 male albino mice

Procedure: 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 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[11] was administered for 6 weeks[11] 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[12,13] through oral gavage for 6 weeks. The mice were weighted once every week to adjust the dose of vitamin D.

By the end of 6 weeks, blood sample was taken by terminal cardiac puncture technique. Serum was separated and frozen at -20°C until used for analysis. Thyroid profile was analysed by using ELISA method.
Statistical Analysis: Data was analysed by using software SPSS version 20. Mean values and standard deviations were taken for quantitative variables like total and free thyroxine. 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.
p value ≤ 0.005 was considered significant.
p value ≤ 0.001 was considered highly significant.

RESULTS

Serum free thyroxine levels were highly significantly different between three groups. (p=0.000) (Table-1 One Way ANOVA)

Serum free thyroxine was highly significantly reduced (p=0.000) in high fat diet group B as compared to normal diet group A. It was highly significantly increased (p=0.000) in high fat diet plus vitamin D group C as compared to high fat diet group B (p=0.000). It was increased significantly (p=0.001) in high fat diet plus vitamin D group C as compared to normal diet group A. (Table-2 post hoc Tukey's test)

These results showed that vitamin D supplementation in mice taking high fat diet increased serum total and free thyroxine levels as compared to mice taking only high fat diet and no vitamin D or even mice taking normal rodent chow.

Table 1: Comparison of serum free and total thyroxine levels among normal diet group (A), high fat diet group (B) and high fat diet plus vitamin D group (C) by applying one way ANOVA

Parameter measured	Group A(normal diet) (n=30)	Group B (high fat diet) (n=30)	Group C(high fat diet +vitamin D) (n=30)	p value
Serum free thyroxine(ng/dl)	1.5±0.67	0.66±0.38	2.05±0.56	0.000**
Serum total thyroxine (nmol/L)	36.74±3.52	25.25±4.94	45.74±5.13	0.000**

**p value ≤ 0.001 was considered highly significant.
* p value ≤ 0.005 was considered significant.

Table 2: Comparison of serum total and free thyroxine levels among normal diet group (A), high fat diet group (B), high fat diet plus vitamin D group (C) by applying Post hoc Tukey's test

Parameters measured	Comparison between the groups		p value
Serum Total thyroxine (nmol/L)	A	B	0.000**
	B	C	0.000**
	C	A	0.000**
Serum free thyroxine (ng/dl)	A	B	0.000**
	B	C	0.000**
	C	A	0.001*

**p value ≤ 0.001 was considered highly significant.
* p value ≤ 0.005 was considered significant.

DISCUSSION

Thyroid gland produces exclusively T_4 but T_4 is converted to T_3 in the periphery to exert its physiological functions.[14] Lipotoxicity affects many organs and thyroid gland is one of those targets. High fat diet induces lipotoxicity and affects thyroid gland functionality.[3]

Shao et al[15] showed that high fat lard diet intake in rats for 24 weeks increased body weight of rats and serum triglyceride levels. No effect on serum total cholesterol (TC) or low density lipoproteins (LDL) was seen. Total and free T_4 were reduced, TSH was increased. Triglyceride levels in the thyroid gland were also raised. Thyroid morphology showed reduced functionality of thyroid gland and endoplasmic reticulum dilatation. Immuno histochemical staining showed decreased staining for thyroid transcription factor-1 and sodium iodide symporter (thyroid transcription factor-1 is a transcription factor which regulates expression of various genes involved in thyroid hormone synthesis, like thyroglobulin, thyroid peroxidase, sodium iodide symporter). When high fat diet was withdrawn, serum TG and LDL levels were decreased but no effect on thyroid hormones levels and thyroid gland morphology was seen. The contradiction in the levels of lipids in this study and our

study might be due to difference in the diet composition. Our high fat diet was containing cholesterol and lard, whereas, their high fat diet had lard only and no cholesterol which was less likely to induce hyperlipidemia.

Zhang et al[3] proved that endoplasmic reticulum stress induced by lipotoxicity decreased thyroid hormone synthesis by decreasing thyroglobulin output. They conducted four experiments on rats as well human thyrocytes in this study. They checked for radioactive iodine uptake, serum TSH and total thyroxine, histology of thyroid gland, transmission electron microscopy, mRNA analysis for various transcription factors. They proved that high fat diet in rats for 18 weeks increased body weight, decreased thyroid gland radioactive iodine uptake, increased TSH, decreased thyroxine levels, no difference in mRNA of thyroglobulin and sodium iodide symporter (NIS) between high fat diet and control group. There were decreased thyroglobulin levels in serum and thyroid gland as well, increased ER stress markers due to accelerated unfolded protein response induced by lipotoxicity. They also treated thyrocytes with ER stress inducer (palmitate) and then inhibitor (4-phenyl butyrate) for confirmation of their findings and they got positive results. Withdrawl of high fat diet caused decreased phosphorylation of PERK/elf2/ATF-4 (endoplasmic reticulum stress markers), increased thyroglobulin levels and increased radioactive iodine uptake. This was an important study which highlighted the mechanisms by which high fat diet reduced thyroid hormone levels.[3] We used vitamin D as an anti inflammatory agent to reduce ER stress. High fat diet plus vitamin D group had reduced serum lipids and improved thyroid hormone levels.

Vitamin D deficiency was associated with thyroid dysfunction, and vitamin D supplementation reversed that dysfunction. This was demonstrated by Abozaid et al.[16]They showed that vitamin D deficient rats had increased body weight, BMI, TSH, and decreased serum T_3, T_4, $25(OH)D_3$, $1,25(OH)_2D_3$ levels. Thyroid histology further strengthened the findings. Mallory trichrome staining showed increased collagen fibers in inter follicular spaces, immunohistochemistry showed increased thyrocytes proliferation. Vitamin D supplementation reversed all these changes showing that vitamin D not only improved thyroid hormone levels but also reduced fibrosis and proliferation in thyroid gland. They owed their findings of improved thyroid hormone levels by vitamin D to decreased hepatic deiodinase-3 activity, resulting in decreased inactivation of T_3 and T_4. They induced vitamin D deficiency and then gave vitamin D supplementation for 7 weeks to see the effects on thyroid gland but we gave HFD+ vitamin D together for 6 weeks to see the effects on total and free thyroxine levels.

CONCLUSION

Based on the results of the current study, it is concluded that vitamin D supplementation may have beneficial role by raising free and total thyroxine levels in male albino mice taking high fat diet.

REFERENCES

1. Logan IE, Bobe G, Miranda CL, Vasquez-Perez S, Choi J, Lowry MB, et al. Germ-Free Swiss Webster Mice on a High-Fat Diet Develop Obesity, Hyperglycemia, and Dyslipidemia. Microorganisms. 2020;8(4):520.2.
2. Shao SS, Zhao YF, Song YF, Xu C, Yang JM, Xuan SM, et al. Dietary high-fat lard intake induces thyroid dysfunction and abnormal morphology in rats. ActaPharmacol Sin. 2014;35(11):1411-20.
3. Zhang X, Shao S, Zhao L, Yang R, Zhao M, Fang L, et al. ER stress contributes to high-fat diet-induced decrease of thyroglobulin and hypothyroidism. Am J Physiol Endocrinol and Metab. 2019 ;316(3):E510-8.
4. Cawley K, Deegan S, Samali A, Gupta S. Assays for detecting the unfolded protein response. Methods enzymol. 2011; 490:31-51.
5. Sozen E, Ozer NK. Impact of high cholesterol and endoplasmic reticulum stress on metabolic diseases: An updated mini-review. Redox biology. 2017;12:456-61.
6. Christakos S, Dhawan P, Verstuyf A, Verlinden L, Carmeliet G. Vitamin D: Metabolism, Molecular Mechanism of Action, and Pleiotropic Effects. Physiol Rev. 2016;96(1):365–408.
7. Bikle D. Vitamin D: production, metabolism, and mechanisms of action. InEndotext [Internet] 2017 Aug 11. MDText. com, Inc.
8. Dankers W, Colin EM, van Hamburg JP, Lubberts E. Vitamin D in autoimmunity: molecular mechanisms and therapeutic potential. Front immunol. 2017;7:697.
9. Wen G, Eder K, Ringseis R. 1, 25-hydroxyvitamin D3 decreases endoplasmic reticulum stress-induced inflammatory response in mammary epithelial cells. Plos one. 2020;15(2):e0228945.
10. Serasanambati M, Chilakapati SR. Function of nuclear factor kappa B (NF-kB) in human diseases-a review. South Indian J Biol Sci. 2016;2(4):368-87.
11. Danik JS, Manson JE. Vitamin D and Cardiovascular Disease. Curr Treat Options Cardiovasc Med. 2012;14(4):414-24.
12. Alshishtawy MM. Vitamin D Deficiency: This clandestine endemic disease is veiled no more. Sultan Qaboos Univ Med J. 2012;12(2):140-52.
13. Body JJ, Bergmann P, Boonen S, Devogelaer JP, Gielen E, Goemaere S et al. Extraskeletal benefits and risks of calcium, vitamin D and anti-osteoporosis medications. Osteoporos Int.2012;23;1-23.
14. Welsh KJ, Soldin SJ. DIAGNOSIS OF ENDOCRINE DISEASE: How reliable are free thyroid and total T3 hormone assays?Eur J Endocrinol. 2016;175(6):255-63.
15. Shao SS, Zhao YF, Song YF, Xu C, Yang JM, Xuan SM, et al. Dietary high-fat lard intake induces thyroid dysfunction and abnormal morphology in rats. ActaPharmacol Sin. 2014;35(11):1411-20.
16. Abozaid ER, Khaled AA, Rahman MA. Thyroid Function Changes in a Rat Model of Vitamin D Deficiency and Effect of Vitamin D and Metformin Treatment. Medical J Cairo Univ. 2020;88:481-94.