*Research Article*

# Study of the Role of Vitamin D in Non-Alcoholic Fatty Liver Disease in Male Albino Rats

**Mariam Y. Ibrahim, Merhan M. Ragi, Heba A. Abd El-Hamid and Sally W. Ayed.**
Department of Physiology, El-Minia Faculty of Medicine

## Abstract
**Background:** Non-alcoholic fatty liver disease (NAFLD) is a very common cause of chronic liver disease. High fat diet (HFD) has been widely used to induce NAFLD in rats as obesity is a major cause of this disease. Vitamin D receptor was found in most tissues and cells of the body. It has been estimated that VDR regulates over 200 genes involved in glucose and lipid metabolism, inflammation, cellular proliferation, differentiation and apoptosis besides the classical skeletal functions of vitamin D. **Objective:** This work aimed to study the possible role of Vitamin D on the progression of nonalcoholic liver disease (NAFLD) in male albino rats and its possible mechanisms of action. **Materials and methods:** thirty adult male albino rats of local strain were used. They were divided into three groups; control group, high fat diet group (HFD) and high fat diet treated with vitamin D group (HFD+V.D). Serum liver enzymes, tumor necrosis factor-α (TNF-α) and B Cell Leukemia protein-2 (BCL2) level in liver tissue were measured in addition to histo-pathological examination of liver tissue. **Results:** HFD diet fed rats showed vacuolated hepatocytes with necro-inflammatory foci in histopathological examination along with elevated liver enzymes, TNF-α level in their serum and reduced BCL2 level in their liver tissue. Meanwhile rats treated with vitamin D showed reduction in fat vacuoles in liver sections and decreased inflammation along with reduced levels of liver enzymes and TNF-α in the serum and elevated levels of BCL2 in liver tissue**. Conclusion:** vitamin D has anti-inflammatory and anti-apoptotic effects on HFD induced NAFLD.
**Key words:** NAFLD, vitamin D, HFD, anti-inflammatory, anti-apoptotic.

## Introduction
Non-alcoholic fatty liver disease (NAFLD) is the most common cause of elevated liver enzymes, but only non-alcoholic steatohepatitis (NASH) progresses to cirrhosis and hepatocellular carcinoma. With the growing epidemic of obesity, the prevalence and impact of NAFLD continues to increase, making NASH potentially the most common cause of advanced liver disease in coming decades (Vernon et al., 2011). The presence of vitamin D receptors in organs other than bone, intestine and kidneys has led to new discoveries of the possible functions of vitamin D in these organs. It has been estimated that VDR regulates over 200 genes involved in glucose and lipid metabolism, inflammation, cellular proliferation, differentiation and apoptosis besides the classical skeletal functions of vitamin D (Eliades and Spyrou, 2015).

## Material and methods
### I. Animals:
Thirty adult male albino rats of local strain were used. They were housed at room temperature with normal day/night cycles. The rats were fed a standard commercial rat chow and tap water ad libitum and left to acclimatize prior to inclusion in the experiment. Principles of laboratory animal care were followed.

### II. Experimental groups:
The experiment lasted for 12 weeks for all the groups. The rats were randomly divided into three groups, ten rats each as follows:

1- Control group (C group): rats were fed the standard commercial rat chow ad libitum without treatment.
2- High fat diet group (HFD group): the rats were fed high fat diet (40% of total content of the diet was fat) according to

(Mashmoul et al., 2016) for induction of non-alcoholic fatty liver disease (NAFLD) for 12 weeks (Spagnuolo et al., 2015).

3- HFD group treated with Vitamin D (HFD+V.D group): the rats were fed high fat diet and simultaneously received intra-peritoneal injection of vitamin D at a dose of 5 µg/kg body weight /each two days according to (Han et al., 2015) .

**III. Experimental diet composition:**

1) Standard commercial rat chow diet: it was composed of 24% protein, 3.85% fat, 45% carbohydrates and 27.15% other constituents including fibers, vitamins and minerals. It provided about 3100 Kcal/kg diet (information provided by the manufacturer). It was purchased from El-Qahera Company (El-Minia, Egypt).
2) High fat diet (HFD): it was made by mixing 48% of the standard rat chow with 38% butter, 6% milk, 6% casein and 2% vitamins and mineral mix. It contained 40% fat, 30% carbohydrates, 20% protein and 10% fibers, vitamins and minerals. It provided 5600 Kcal/kg diet (Mashmoul et al., 2016).

At the end of all experiments, all rats were sacrificed by decapitation after overnight fasting. The livers were rapidly extracted after rats' scarification. About half of each liver was fixed in 10% formalin for histopathology. The other half was homogenized and used for the measurement of tissue BCL2. Additionally; blood samples were obtained from jugular vein for measurement of serum ALT, AST and TNF-α. All serological tests were done according to manufacturer instructions.

## Results

Histopathological examination confirmed development of NAFLD in HFD fed rats compared to control group while vitamin D injection had protective effects on the livers of treated rats as illustrated in (figure 1) .

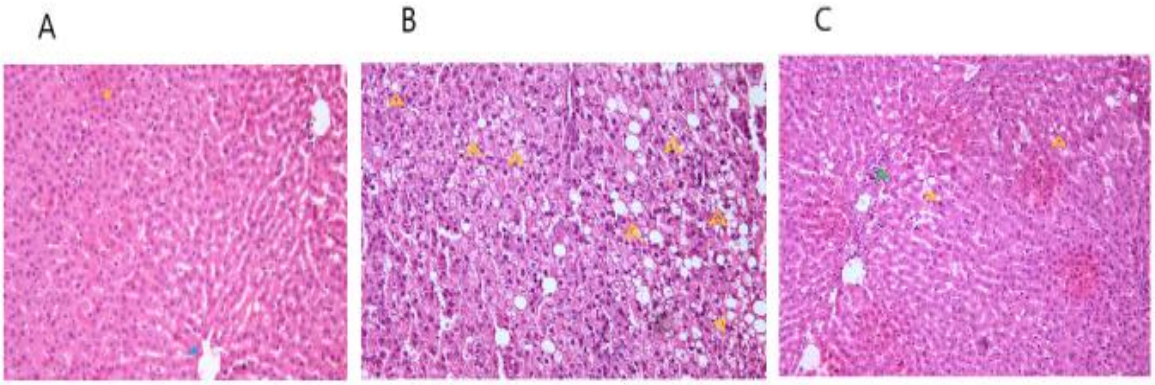


**Figure 1: Histopathological findings of the three groups**

**A:** control group showing normal hepatic lobule architecture with regular hepatocyte trabeulae.
**B:** HFD group showing many hepatocytes with moderate ballooning and severe macrovesicular steatosis.
**C:** HFD+ V.D showing showing few hepatocytes with mild ballooning and mild steatosis.

Chemical assays for the HFD group showed significantly elevated ALT, AST, TNF-α levels and reduced BCL2 level in liver tissue compared to control group while vitamin D treated group showed significantly reduced ALT, AST, TNF-α levels and elevated BCL2 level in liver tissue compared to HFD non treated group as illustrated by the data in **(table 1).**

**Table (1) :Effect of HFD and vitamin D injection on liver enzymes, inflammatory marker and anti-apoptotic marker.**

| | Control group | HFD group | HFD+V.D group |
|---|---|---|---|
| **ALT (IU/L)** | 13.5 ± 3.3 | 52.8[a] ± 11.3 | 20.5 [b] ± 6.7 |
| **AST (IU/L)** | 64 ± 22.8 | 159.3[a] ± 40.2 | 63.8 [b] ± 22.2 |

| | | | |
|---|---|---|---|
| **TNF-α (ng/L)** | 138 ± 18.6 | 383.6 [a] ± 189.2 | 262.4 [b] ± 72.7 |
| **BCL2 (ng/L)** | 72.3 ± 11.5 | 36.1 [a] ± 14.9 | 67.8 [b] ± 20.3 |

- Data are expressed as mean ± standard deviation. The mean difference between groups was considered statistically significant when P value < 0.05.
- [a] significant with the corresponding mean of the control group.
- [b] significant with the corresponding mean of the HFD group.

## Discussion

NAFLD is a very common cause of chronic liver disease (Davoodi et al., 2017). NAFLD model was successfully induced in rats using HFD. This was proved by histopathological findings in the form of ballooning of hepatocytes with vacuolation either micro or macro-vesicular that is probably the result of dissolved fat droplets during the tissue preparation along with inflammation, necrosis and some fibrosis which is consistent with Li et al., (2013)'s findings. Fat accumulation inside hepatocytes caused chronic liver injury which led to cells breakdown with release of their contents into the circulation causing the significantly elevated liver enzymes levels in the serum of HFD group as reported by Ma et al., (2019).

Fat accumulation in the liver could be secondary to increased transcription of lipogenesis gene, adeponutrin and decreased transcription of lipolysis gene, adeponectin causing increased fat synthesis in the liver as explained by Li et al., (2013) or due to insulin resistance in skeletal muscles, liver and adipose tissue as a result of high fat feeding and overweight (Samuel and Shulman, 2016).

Inflammation is the hallmark factor in the progression of NAFLD into non-alcoholic steatohepatitis (NASH). An inflammatory response was evident in our study by the histopathological finding along with the significantly higher serum levels of TNF-α in the NAFLD group when compared with the control groups. Similar results were shown by Li et al., (2013) and Lai et al., (2016).

Free fatty acids were found to trigger kupffer cells activation by enhancing Toll-like receptor (TLR) expression which in turn produce pro-inflammatory cytokines as TNF-α, IL-1β and IL-12 (Leroux et al., 2012).

This study showed that apoptosis is a step in the pathogenesis of NAFLD. This was indicated by the significantly lower levels of anti-apoptotic protein BCL2 that was found in the liver tissue of HFD group compared with the control group similar to what was found by Jiang et al., (2011). This implied that anti-apoptotic proteins expression is down-regulated during the development of NAFLD as explained by Xiao et al., (2013).

On the other hand, Vitamin D injection was proved to have protective effects on the progression of NAFLD as indicated from the histopathological findings which were milder than the diseased group with less steatosis and less infiltration with inflammatory cells and no fibrosis, along with the significantly reduced serum level of ALT and AST in the vitamin D treated group when compared with the NAFLD group. These data are in agreement with Drori et al., (2017)'s findings.

Vitamin D was found to decrease fat accumulation in liver by modulating insulin sensitivity. Its administration was found to up regulate glucose transporter 4 (GLUT4) translocation to cell membrane of the muscle as proposed by Benetti et al., (2018) with subsequent decrease in insulin resistance and enhanced glucose uptake and utilization by the muscles so that it wouldn't be used as a substrate for lipogenesis in liver (Cicero et al., 2018).

Active vitamin D also has an anti-inflammatory role in the protection from NAFLD progression. Vitamin D treated group showed significantly lower serum levels of TNF-α when compared to NAFLD non treated group which can indicate the anti- inflammatory role of vitamin D. Wang et al., (2015) stated that the anti-inflammatory role of vitamin D is secondary to its effect on TLR4 expression. It was found that TLR4 expression was down-regulated with vitamin D treatment.

Active vitamin D was found to enhance the expression of anti-apoptotic protein BCL2 as evident in our experiment by the significantly higher liver's BCL2 levels in the NAFLD vitamin D treated group compared with the NAFLD non treated group. This finding is consistent with what was reported by Zhang et al., (2007) that calcitriol inhibited apoptosis by increasing the level of BCL2 in liver cells. Similar findings were shown by Tabasi et al., (2015), their work demonstrated increased expression of BCL2 secondary to vitamin D treatment.

## References


1. 1-Benetti, E., Mastrocola, R., Chiazza, F., Nigro, D., D'Antona, G., Bordano, V., Fantozzi, R., Aragno, M., Collino, M., and Minetto, M. A. (2018). Effects of vitamin D on insulin resistance and myosteatosis in diet-induced obese mice. PloS one 13, e0189707-e0189707.
2. 2- Cicero, A. F. G., Colletti, A., and Bellentani, S. (2018). Nutraceutical Approach to Non-Alcoholic Fatty Liver Disease (NAFLD): The Available Clinical Evidence. Nutrients 10, 1153.

**4-** Davoodi, I., Rahimi, R., Abdollahi, M., Farzaei, F., Farzaei, M. H., Memariani, Z., and Najafi, F. (2017). Promising effect of Rosa damascena extract on high-fat diet-induced nonalcoholic fatty liver. Journal of Traditional and Complementary Medicine 7, 508-514.

**5-** Drori, A., Rotnemer-Golinkin, D., Avni, S., Drori, A., Danay, O., Levanon, D., Tam, J., Zolotarev, L., and Ilan, Y. (2017). Attenuating the rate of total body fat accumulation and alleviating liver damage by oral administration of vitamin D-enriched edible mushrooms in a diet-induced obesity murine model is mediated by an anti-inflammatory paradigm shift. BMC gastroenterology 17, 130-130.

**6-** Eliades, M., and Spyrou, E. (2015). Vitamin D: a new player in non-alcoholic fatty liver disease? World J Gastroenterol 21, 1718-27.

**7-** Han, H., Cui, M., You, X., Chen, M., Piao, X., and Jin, G. (2015). A role of 1,25 (OH)2D3 supplementation in rats with nonalcoholic steatohepatitis induced by choline-deficient diet. Nutr Metab Cardiovasc Dis 25, 556-61.

**8-** Jiang, Y., Zhao, M., and An, W. J. J. o. M. M. (2011). Increased hepatic apoptosis in high-fat diet-induced NASH in rats may be associated with downregulation of hepatic stimulator substance. 89, 1207-1217.

**9-** Lai, Y.-S., Lee, W.-C., Lin, Y.-E., Ho, C.-T., Lu, K.-H., Lin, S.-H., Panyod, S., Chu, Y.-L., and Sheen, L.-Y. (2016). Ginger Essential Oil Ameliorates Hepatic Injury and Lipid Accumulation in High Fat Diet-Induced Nonalcoholic Fatty Liver Disease. Journal of Agricultural and Food Chemistry 64, 2062-2071.

**10-** Leroux, A., Ferrere, G., Godie, V., Cailleux, F., Renoud, M.-L., Gaudin, F., Naveau, S., Prévot, S., Makhzami, S., and Perlemuter, G. J. J.o.h. (2012). Toxic lipids stored by Kupffer cells correlates with their pro-inflammatory phenotype at an early stage of steatohepatitis.57,141-149.

**11-** Li, Y., Hai, J., Li, L., Chen, X., Peng, H., Cao, M., and Zhang, Q. J. E. (2013). Administration of ghrelin improves inflammation, oxidative stress, and apoptosis during and after non-alcoholic fatty liver disease development.43,376-386.

**12-** Ma, M., Long, Q., Chen, F., Zhang, T., and Wang, W. (2019). Active vitamin D impedes the progression of non-alcoholic fatty liver disease by inhibiting cell senescence in a rat model. Clinics and Research in Hepatology and Gastro-enterology.

**13-** Mashmoul, M., Azlan, A., Mohtarrudin, N., Mohd Yusof, B. N., Khaza'ai, H., Khoo, H. E., Farzadnia, M., and Boroushaki, M. T. (2016). Protective effects of saffron extract and crocin supplementation on fatty liver tissue of high-fat diet-induced obese rats. BMC Complement Altern Med 16, 401.

**14-** Samuel, V. T., and Shulman, G. I. (2016). The pathogenesis of insulin resistance: integrating signaling pathways and substrate flux. The Journal of Clinical Investigation 126, 12-22.

**15-** Spagnuolo, M. S., Mollica, M. P., Maresca, B., Cavaliere, G., Cefaliello, C., Trinchese, G., Scudiero, R., Crispino, M., and Cigliano, L. (2015). High Fat Diet and Inflammation - Modulation of Haptoglobin Level in Rat Brain. Front Cell Neurosci 9, 479.

**16-** Tabasi, N., Rastin, M., Mahmoudi, M., Ghoryani, M., Mirfeizi, Z., Rabe, S. Z. T., and Reihani, H. (2015). Influence of vitamin D on cell cycle, apoptosis, and some apoptosis related molecules in systemic lupus erythematosus. Iranian journal of basic medical sciences 18, 1107-1111.

**17-** Vernon, G., Baranova, A., and Younossi, Z. M. (2011). Systematic review: the epidemiology and natural history of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis in adults. Alimentary Pharmacology & Therapeutics 34, 274-285.

**18-** Wang, H., Zhang, Q., Chai, Y., Liu, Y., Li, F., Wang, B., Zhu, C., Cui, J., Qu, H., and Zhu, M. (2015). 1,25(OH)2D3 down-regulates the Toll-like receptor 4-mediated inflammatory pathway and ameliorates liver injury in diabetic rats. Journal of endocrinological investigation 38, 1083-1091.

**19-** Xiao, J., Guo, R., Fung, M.-L., Liong, E. C., Chang, R. C. C., Ching, Y.-P., Tipoe, G. L. J. E.-B. C., and Medicine, A. (2013). Garlic-derived S-allylmercaptocysteine ameliorates nonalcoholic fatty liver disease in a rat model through inhibition of apoptosis and enhancing autophagy. 2013.

**20-** Zhang, A., Wang, Y., Xie, H., and Zheng, S. (2007). Calcitriol inhibits hepatocyte apoptosis in rat allograft by regulating apoptosis-associated genes. International immunopharmacology 7, 1122-1128.