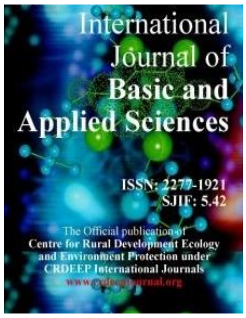
Vol. 8. No. 4. 2019

Contents available at:

www.crdeepjournal.org

International Journal of Basic and Applied Sciences (ISSN: 2277-1921)



Full Length Research Paper

Role of Vitamin D on the liver of Non-Alcoholic Fatty Liver Disease: An Experimental Study

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ARTICLE INFORMATION

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Article history:
Received: 05-11-2019
Accepted: 15-11-2019
Revised: 27-11-2019
Published: 03-12-2019

Key words:
Vitamin D3; Nonalcoholic fatty liver disease; Steatohepatitis; Cholecalciferol

ABSTRACT

Background: *Nonalcoholic fatty liver disease is the commonest chronic liver disease worldwide. Its prevalence steadily increased. The development of preventive measures is crucial.* ***Aim of the work:*** *to investigate the role of vitamin D in non-alcoholic fatty liver disease.* ***Methodology:*** *Thirty Male Wistar rats were randomly assigned to three equal groups; the control group. The high fructose group (for induction of NAFLD), and the high fructose group plus vitamin D3, where rats fed fructose in drinking water and had intraperitoneal injection of vitamin D3 (5µg/kg; each two days for 8 weeks). Blood and liver samples were collected for laboratory and histopathological examination. Then liver lipid contents and hepatic glutathione were determined and scoring of fibrosis and steatosis was calculated.* ***Results:*** *Liver weight and index were significantly increased in groups II and III when compared to control group. Blood glucose was significantly increased in group II and III when compared to control group. However, it was significantly lower in group III than group II (190.60±11.06 vs 206.20±12.80 mg/dl, respectively). Serum insulin significantly reduced in groups II and III than control group. HOMA significantly increased in group II than control group (1.88±0.17 vs 1.53±0.15, respectively) and group III. Liver total cholesterol was significantly increased in group II than control and the third groups (5.39±0.67 vs 4.43±0.32 and 4.28±0.31 µmol/g tissue, respectively). Histopathological examination of the liver sections revealed steatosis and hepatocyte ballooning in high fructose group, but there was no fibrosis. Vitamin D supplementation significantly decreased the steatosis and hepatocyte ballooning scores in high fructose group.* ***Conclusion:*** *Vitamin D3 supplementation could alleviate the hepatic lesions associated NAFLD induced by high fructose diet. This could be exerted by many mechanisms, chiefly anti-oxidative stress actions. Other potential mechanisms should be investigated.*

Introduction

Nonalcoholic fatty liver disease (NAFLD) is one of the commonest chronic diseases of the liver. NAFLD started by a condition called steatosis, which is a reversible pathology. Steatosis increased susceptibility of the liver to damage by oxygen radicals (reactive oxygen species), with subsequent advancement of steatosis to more advanced pathology of the liver (e.g, nonalcoholic steatohepatitis (NASH), fibrosis and even cirrhosis) (1). Thus, prevention of steatosis or its transformation to advanced lesions of the liver represents the base of different therapeutic strategies (2). Vitamin D is usually present in one of two forms: Vitamin D2 (ergocalciferol) and Vitamin D3 (cholecalciferol). Ergocalciferol (Vitamin D2) present in different food and can be obtained through proper nutrition. However, ergocalciferol (Vitamin D3) is synthesized in vivo in presence of ultraviolet light on the skin. Both forms are metabolized with hydroxylation reactions, leading to production of 25-hydroxyvitamin D [25(OH) D], which used as an indicator of vitamin D stores. The first hydroxylation reaction takes place in the liver, while the second hydroxylation process occurs in the kidneys, resulting in the production of 1,25 dihydroxyvitamin D [1,25(OH)2D] (the active form of Vitamin D) (3,4). Active form of vitamin D exerts its functions by binding to Vitamin D receptor (VDR) in the nucleus. The main and primary target of vitamin D is the bone, kidney, and intestines. but, VDRs are available in others tissues (e.g., endocrine system, immune system, muscles, liver and brain).

Thus, it could play a role in gene regulation, immune function, cellular growth and differentiation, protein synthesis and inflammation, in addition, to its primary role in regulation of bone homeostasis. It was reported to play a crucial preventive role of many diseases like hypertension, diabetes, autoimmune diseases, cardiovascular diseases and even cancer (3-7).

It had been proposed that, vitamin D deficiency may stimulate insulin resistance, and play a pathogenetic role in the development of metabolic syndrome and NAFLD (8-12). The mechanism under the association between vitamin D deficiency and fatty liver is not fully understood. However, it was proposed to be due to increased oxidative stress, cytokine production or increased apoptosis (13-16).

High fructose diet is used to induce metabolic syndrome (17) and fatty liver in experimental models. However, the knowledge about role of vitamin D-supplementation in such models is limited (18).

Aim of the work

The current study aimed to investigate the role of vitamin D in non-alcoholic fatty liver disease

Materials and methods

Fructose (Fr), and other chemicals were purchased from Sigma-Aldrich (Saint-Louise, MI, USA). Vitamin D2 was obtained from (e.g., El- GOMHOURIA Company for drugs, pharmaceuticals and Medical Supplies (Egypt).

Animals: Thirty Male Wistar rats (145-165 g) were obtained from the Faculty of Veterinary animal house (Cairo University), Egypt. Animals were kept and housed in plastic cages with free access to food and water. Animals were kept at standardized conditions for one week for acclimatization.

After the end of the first week, the experiment was started and continued for 8 weeks. All animals were fed on a laboratory chow diet (Cairo Bio-Pharm CO, Nasr City, Cairo, Egypt). At the start, rats were randomly assigned to three groups (each 10 rats); the first is the control group, and rats in this group were given drinking water and injected with saline each two days (the vehicle) interperitoneally as the tested substances. The second group is the high fructose group (for induction of NAFLD), where rats received fructose (30%; w/v, in drinking water). The third group was high fructose group plus vitamin D3, where rats fed fructose in drinking water and had intraperitoneal injection of vitamin D3 (5µg/kg; each two days for 8 weeks). This dose and regiment were selected after Yin et al. (13), where they used different doses of vitamin D (1, 3, 5 µg/kg). Here were used the higher tested dose in their study.

Sampling: At the end of the 8^{th} week, animals had an overnight fasting, anesthetized, sacrificed and blood samples were collected in dry tubes, centrifuged and serum was obtained and kept at -80^{o}C till the time of analysis. In addition, the liver was removed, cleaned with 0.9% NaCl, weighed and kept in ice. The liver index was calculated from the equation [liver index = (liver weight/body weight)/100]. Liver tissues were homogenized in ice-cold, centrifuged at 600 g for 10 minutes and post-nuclear fraction (PNF) was obtained and kept at –80 °C until analysis.

The following parameters were measured in serum: fasting blood sugar, cholesterol, triglycerides, and liver enzymes (ALT and AST) using autoanalyzer (Automatic Biochemistry Analyzer, No. 7179A, Hitachi, Japan). In addition, serum insulin was measured using ELISA kits according to instructions provided by the manufacturer. The insulin resistance was determined through assessment of homeostasis model (HOMA), which was calculated from the equation [HOMA= fasting insulin concentration (pmol/L) x fasting glucose concentration (mmol/L)/135 [19]**.** High HOMA scores indicate IR (low insulin sensitivity).

Determination of liver total cholesterol (TC) and triglycerides ((TG): the method of hepatic lipid extraction was performed as previously described by Folch et al. (20). A solution of chloroform: methanol (2:1) was used and levels of TC and TG were assayed and results were expressed in µmol/g tissue.

Determination of hepatic glutathione (GSH): Hepatic glutathione levels were estimated by the use of 5,5-dithiobis-(2- nitrobenzoate) at 412 nm. Results were expressed as nmol/ mg protein as described previously by Beutler et al. (21).

Histopathology, scoring of fibrosis and steatosis

Liver samples were immersed in formalin fixative (10% buffered formalin), embedded in paraffin, sectioned and stained with hematoxylin and eosin for histologic examinations. Masson's trichrome staining was also performed to show reticulin fibers of fibrotic areas. Steatosis, liver damage, and fibrosis scores were made according to the protocol proposed by Goodman (22). Briefly, steatosis was score as none (0- < 5.0%), mild (5-33.0%), moderate (34-66.0%) and severe (≥67.0%). Fibrosis was categorized by Ishak's staging system, where (0= no fibrosis, 1= fibrous expansion of some portal areas, with or without inclusion of short fibrous septa, 2 = expansion of fibrosis to include most portal areas, with or without inclusion of short fibrous septa, 3= 3=fibrous expansion included most of the portal areas with occasional portal to portal bridging; 4= expansion of fibrosis to include areas with marked bridging (portal to portal as well as portal to central); 5=marked bridging (portal to portal and/or portal to central) with occasional nodules (incomplete cirrhosis); and 6=cirrhosis, probable or definite).

Statistical analysis

Collected data were fed to Microsoft excel, coded and transferee to the statistical analysis software package (SPSS, version 16; SPSS Inc., Chicago, IL, USA). Normal distribution of quantitative data was carried out by Kolmogorov–Smirnov test. Quantitative variables were expressed as arithmetic mean (measure of central tendency) ± standard deviation (SD) (measure of dispersion). One way analysis of variance or Kruskal-Wallis tests were used to test significance between groups, and in presence of significant differences, the two-way, post hoc test or Mann-Whiteny (U) tests were used, according to normal distribution of data. P value < 0.05 was set as significant.

Results

Male Wistar rats were the animal model of this work to avoid the estrogenic effect. All rats reached the end of the study. Group I was assigned for control group, group II for high fructose diet and group III for high fructose diet with vitamin D3. The initial weight of included animals did not significantly differ between groups. However, food intake was significantly reduced in groups II and III when compared to control group (11.10±1.0 and 12.30±1.25 vs 24.20±2.10 g/day, respectively).

But water intake did not differ significantly between groups. The final weight significantly decreases in group III (high fructose+ vitamin D3) when compared to group II (286.70±10.01 vs 302.30±14.47 g, respectively). However, the difference was not significant between control group and each of group II or group III. Furthermore, both liver weight and liver index were significantly increased in groups II and III when compared to control group. However, the difference between groups II and III was non-significant (Table 1).

In the current experimental study, blood sugar was significantly increased in group II and III when compared to control group. However, it was significantly lower in group III than group II (190.60±11.06 vs 206.20±12.80 mg/dl, respectively). Serum insulin significantly reduced in groups II and III than control group. However, the difference between groups II and III was not significant. HOMA significantly increased in group II than control group (1.88±0.17 vs 1.53±0.15, respectively) and group III (1.64±0.15). however, the difference between control group and group III, was not significant. Total cholesterol was significantly reduced and triglycerides were significantly increased in groups II and III than control group. However, TG significantly lower in group IIII than group II. ALT and AST significantly increased in group II than control group. However, both were lower in group III than group II (Table 2).

In the current work, liver total cholesterol was significantly increased in group II than control and the third groups (5.39±0.67 vs 4.43±0.32 and 4.28±0.31 μmol/g tissue, respectively). The liver TC interestingly decreased in group III than control group. However, the difference as non-significant. Liver TG was significantly increased in groups II and III than control group and in group II than group III. However, hepatic GSH significantly decreased in groups II and III than control group (Table 3).

Histological examination

Histopathological examination of the liver sections revealed steatosis and hepatocyte ballooning in high fructose group, but there was no fibrosis was observed. 1,25(OH)2D3 treatment significantly decreased the steatosis and hepatocyte ballooning scores in high fructose group. Steatosis did not exceed 5% in any rat.

Table (1): Comparison between studied groups regarding body weight, liver weight, food and water intake and liver index

	Control group (I)	**Group II**	**Group III**	**F**	**p**
Initial weight (g)	154.10±6.10	155.00±6.73	156.50±5.26	0.400	0.674
Food intake (g/day)	24.20±2.10	11.10±1.00#	12.30±1.25#	***226.19***	***<0.001****
Water intake (ml/day)	34.90±2.23	38.30±6.36	39.70±6.45	2.10	0.142
Final weight (g)	296.40±14.63	302.30±14.47	286.70±10.01@	***3.55***	***0.043****
Liver weight (g)	7.90±0.74	9.50±1.35#	9.40±1.51#	***5.18***	***0.012****
Liver index	2.67±0.24	3.14±0.38#	3.28±0.48#	***7.12***	***0.003****

** Indicate significant variance; # indicate significant difference when compared to control group; @ indicate significant difference when compared to NAFLD group*

Table (2): Serum levels of blood sugar, insulin, lipids and lever enzymes among studied groups

	Control group (I)	**Group II**	**Group III**	**F**	**p**
Blood sugar (mg/dl)	125.10±6.72	206.20±12.80#	190.60±11.06#@	167.618	<0.001*
Serum insulin (pmol/L)	29.80±2.90	22.20±2.10#	20.90±1.91#	42.132	<0.001*
HOMA-IR	1.53±0.15	1.88±0.17#	1.64±0.15@	13.063	<0.001*
Total cholesterol (mg/dl)	151.0±5.89	140.0±5.33#	137.10±3.84#	20.713	<0.001*
Triglycerides (mg/dl)	43.40±2.91	100.50±5.64#	70.50±4.95#@	377.557	<0.001*
ALT (IU/dl)	4.20±1.40	6.60±0.97#	5.20±0.92@	11.679	<0.001*
AST (IU/Dl)	10.40±1.43	14.60±1.43#	9.10±0.99#@	48.821	<0.001*

** Indicate significant variance; # indicate significant difference when compared to control group; @ indicate significant difference when compared to NAFLD group*

Table (3): Liver TC, TG, GSH and steatosis scores among studied groups

	Control group (I)	**Group II**	**Group III**	**F**	**p**
Liver TC (μmol/g tissue)	4.43±0.32	5.93±0.67#	4.28±0.31@	***39.03***	***<0.001****
Liver TG (μmol/g tissue)	15.70±1.34	30.60±2.67#	17.60±1.51#@	***175.99***	***<0.001****
Hepatic GSH (mmol/g tissue)	22.60±2.91	18.50±2.12#	18.0±3.13#	***8.39***	***0.001****
Steatosis score (units)	0.0	3.30±0.67	1.30±0.48@	***120.33***	***<0.001****
Fibrosis score (units)	0.0	0.0	0.0	-	-

** Indicate significant variance; # indicate significant difference when compared to control group; @ indicate significant difference when compared to NAFLD group*

Discussion

NAFLD represented by an excessive fat accumulation in the liver of individuals with no history of alcohol intake and no other causes of hepatic steatosis. It actually a spectrum of disorders ranging from simple steatosis to NASH, which may be progressed to fibrosis, cirrhosis and hepatic carcinoma (23). It affects 20-30% of populations (in healthy individuals, the incidence is about 15%, that dramatically increased in high-risk patients (e.g. increased to 16% in diabetics and to 90.0% in hyperlipemia and 91.0% in obese individuals)) (24-26).

Fructose is a highly lipogenic substance, and mainly metabolized in the liver. High fructose diet induces hepatic steatosis and led to insulin resistance and non-alcoholic fatty liver disease (NAFLD). Oxidative stress and advanced glycation end products (AGEs) could play a role in the pathogenesis of high fructose-induced toxicity (27).

Results of the current work revealed significant elevation of liver enzymes in high fructose group. In addition, there was microvascular steatosis and hepatocyte ballooning, which was reduced by concomitant administration of vitamin D3. In addition, there was hyperglycemia, insulin resistance and oxidative stress. In line with results of the current work, Elseweidy et al. (18) reported that, vitamin D3 supplementation was associated with improvement of some metabolic disturbances such as insulin resistance, hyperglycemia and dyslipidemia. In addition, Maia-Ceciliano et al. (28) also reported improvement of hepatic lesions (e.g., steatosis) and reduced insulin resistance and lipogenesis gene expression and inflammatory changes of the liver of high fructose fed-rats. These effects were proposed to be due antioxidant and anti-inflammatory actions of vitamin D3 (29). The antioxidant properties of vitamin D3 was ascribed to its structural similarity to cholesterol and induction of the expression of different molecules include in oxidant-antioxidant system (e.g., GSH, glutathione peroxidase and superoxide dismutase) (30).

Results of animal studies demonstrated that, the administration of vitamin D3 in diabetic mice reduce the formation of reactive oxygen species by the suppression of Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase gene expression (31). The activation of NADPH oxidase was considered as a positive indicator for oxidative stress (32). Vitamin D3 reduces the lipid peroxidation and improves activity of superoxide dismutase (SOD), the first line of cellular defense against antioxidants (33).

Furthermore, Foroozanfard et al. (34) suggested that vitamin D plays an antioxidant role as a result of associated increase of hepatic GSH in rats that have administered cholecalciferol. The combination of calcium with vitamin D supplementations were associated with an enhanced antioxidant effects than separate administration of calcium and vitamin D.

In the current work, body weight, liver weight and liver index were significantly increased in high fructose diet and slightly reduced by administration of vitamin D. Zhu et al. (35) reported that, body weights, liver enzymes, hepatic triglycerides were significantly elevated in high fat-diet group and the liver pathology demonstrated tissue changes remarkable of non-alcoholic liver disease.

The effects of vitamin D3 supplementation on the liver weight may be due to lipid metabolism gene- expression regulation of in the liver as reported by Yin et al. (13). Zhu et al. (35) reported that, the body weight expresses a trend like liver weight and the biochemical indicators with no significant difference between high fat diet group with and without vitamin D3 supplementation. This may be due to different responses of different organs to vitamin D. also, body weight is affected by many factors.

Additionally, vitamin D deficiency was prevalent among cases with non-alcoholic steatohepatitis (36, 37).

Nakano *et al*. (38) also revealed that, the sunlight exposure increased serum D3 levels and ameliorated the progression of NASH in a diet-induced NASH animal model.
Yin *et al*. (13) suggested that vitamin D3 protect against induced hepatic steatosis by prevention of fatty acid oxidation and restoring lipogenesis.

In conclusion, results of the current work revealed that, vitamin D3 supplementation could alleviate the hepatic lesions associated NAFLD induced by high fructose diet. This could be exerted by many mechanisms, chiefly anti-oxidative stress actions.

Conflict of interest
None

Financial disclosure
The research did not receive any fund by any organization. Authors are the only funders

Author contribution
Authors contribute equally

References
1. Ibrahim MA, Kelleni M, Geddawy A. Nonalcoholic fatty liver disease: current and potential therapies. Life Sci. 2013 Feb 7;92(2):114-8. doi: 10.1016/j.lfs.2012.11.004. Epub 2012 Nov 15. PMID: 23159641.
2. Takeuchi M, Takino JI, Sakasai-Sakai A, Takata T, Tsutsumi M. Toxic AGE (TAGE) Theory for the Pathophysiology of the Onset/Progression of NAFLD and ALD. Nutrients. 2017 Jun 20;9(6):634. doi: 10.3390/nu9060634. PMID: 28632197; PMCID: PMC5490613.
3. Wang H, Chen W, Li D, Yin X, Zhang X, Olsen N, Zheng SG. Vitamin D and Chronic Diseases. Aging Dis. 2017 May 2;8(3):346-353. doi: 10.14336/AD.2016.1021. PMID: 28580189; PMCID: PMC5440113.
4. Kwok RM, Torres DM, Harrison SA. Vitamin D and nonalcoholic fatty liver disease (NAFLD): is it more than just an association? Hepatology. 2013 Sep;58(3):1166-74. doi: 10.1002/hep.26390. Epub 2013 Jul 29. PMID: 23504808.
5. Rak K, Bronkowska M. Immunomodulatory Effect of Vitamin D and Its Potential Role in the Prevention and Treatment of Type 1 Diabetes Mellitus-A Narrative Review. Molecules. 2018 Dec 24;24(1):53. doi: 10.3390/molecules24010053. PMID: 30586887; PMCID: PMC6337255.
6. Farhangi MA, Mesgari-Abbasi M, Hajiluian G, Nameni G, Shahabi P. Adipose Tissue Inflammation and Oxidative Stress: The Ameliorative Effects of Vitamin D. Inflammation. 2017 Oct;40(5):1688-1697. doi: 10.1007/s10753-017-0610-9. PMID: 28674792.
7. Liu W, Zhang L, Xu HJ, Li Y, Hu CM, Yang JY, Sun MY. The Anti-Inflammatory Effects of Vitamin D in Tumorigenesis. Int J Mol Sci. 2018 Sep 13;19(9):2736. doi: 10.3390/ijms19092736. PMID: 30216977; PMCID: PMC6164284.
8. Shojaei Zarghani S, Soraya H, Alizadeh M. Calcium and vitamin D_3 combinations improve fatty liver disease through AMPK-independent mechanisms. Eur J Nutr. 2018 Mar;57(2):731-740. doi: 10.1007/s00394-016-1360-4. Epub 2016 Dec 17. PMID: 27988847.
9. Angellotti E, Pittas AG. The Role of Vitamin D in the Prevention of Type 2 Diabetes: To D or Not to D? Endocrinology. 2017 Jul 1;158(7):2013-2021. doi: 10.1210/en.2017-00265. PMID: 28486616; PMCID: PMC5505219.
10. Garbossa SG, Folli F. Vitamin D, sub-inflammation and

insulin resistance. A window on a potential role for the interaction between bone and glucose metabolism. Rev Endocr Metab Disord. 2017 Jun;18(2):243-258. doi: 10.1007/s11154-017-9423-2. PMID: 28409320.

11. Chen G, Ni Y, Nagata N, Xu L, Ota T. Micronutrient Antioxidants and Nonalcoholic Fatty Liver Disease. Int J Mol Sci. 2016 Aug 23;17(9):1379. doi: 10.3390/ijms17091379. PMID: 27563875; PMCID: PMC5037659.

12. Dakshinamurti K. Vitamins and their derivatives in the prevention and treatment of metabolic syndrome diseases (diabetes). Can J Physiol Pharmacol. 2015 May;93(5):355-62. doi: 10.1139/cjpp-2014-0479. PMID: 25929424.

13. Yin Y, Yu Z, Xia M, Luo X, Lu X, Ling W. Vitamin D attenuates high fat diet-induced hepatic steatosis in rats by modulating lipid metabolism. Eur J Clin Invest. 2012 Nov;42(11):1189-96. doi: 10.1111/j.1365-2362.2012.02706.x. Epub 2012 Sep 8. PMID: 22958216.

14. Eliades M, Spyrou E. Vitamin D: a new player in non-alcoholic fatty liver disease? World J Gastroenterol. 2015 Feb 14;21(6):1718-27. doi: 10.3748/wjg.v21.i6.1718. PMID: 25684936; PMCID: PMC4323447.

15. Mostafa DK, Nasra RA, Zahran N, Ghoneim MT. Pleiotropic protective effects of Vitamin D against high fat diet-induced metabolic syndrome in rats: One for all. Eur J Pharmacol. 2016 Dec 5; 792:38-47. doi: 10.1016/j.ejphar.2016.10.031. Epub 2016 Oct 24. Erratum in: Eur J Pharmacol. 2017 Jul 5; 806:110. PMID: 27789220.

16. Mazzone G, Morisco C, Lembo V, D'Argenio G, D'Armiento M, Rossi A, Giudice CD, Trimarco B, Caporaso N, Morisco F. Dietary supplementation of vitamin D prevents the development of western diet-induced metabolic, hepatic and cardiovascular abnormalities in rats. United European Gastroenterol J. 2018 Aug;6(7):1056-1064. doi: 10.1177/2050640618774140. Epub 2018 May 17. PMID: 30228894; PMCID: PMC6137584.

17. Takahashi Y, Soejima Y, Fukusato T. Animal models of nonalcoholic fatty liver disease/nonalcoholic steatohepatitis. World J Gastroenterol. 2012 May 21;18(19):2300-8. doi: 10.3748/wjg. v18.i19.2300. PMID: 22654421; PMCID: PMC3353364.

18. Elseweidy MM, Amin RS, Atteia HH, Ali MA. Vitamin D3 intake as regulator of insulin degrading enzyme and insulin receptor phosphorylation in diabetic rats. Biomed Pharmacother. 2017 Jan;85:155-159. doi: 10.1016/j.biopha.2016.11.116. Epub 2016 Dec 5. PMID: 27930980.

19. Giriş M, Doğru-Abbasoğlu S, Soluk-Tekkeşin M, Olgaç V, Uysal M. Effect of betaine treatment on the regression of existing hepatic triglyceride accumulation and oxidative stress in rats fed on high fructose diet. Gen Physiol Biophys. 2018 Sep;37(5):563-570. doi: 10.4149/gpb_2018005. Epub 2018 Jul 26. PMID: 30047921.

20. Folch J, Lees M, Sloane Stanley GH. A simple method for the isolation and purification of total lipides from animal tissues. J Biol Chem. 1957 May;226(1):497-509. PMID: 13428781.

21. Beutler E, Duron O, Kelly BM. Improved method for the determination of blood glutathione. J Lab Clin Med. 1963 May; 61:882-8. PMID: 13967893.

22. Goodman ZD. Grading and staging systems for inflammation and fibrosis in chronic liver diseases. J Hepatol. 2007 Oct;47(4):598-607. doi: 10.1016/j.jhep.2007.07.006. Epub 2007 Jul 30. PMID: 17692984.

23. Bhala N, Angulo P, van der Poorten D, Lee E, Hui JM, Saracco G, Adams LA, Charatcharoenwitthaya P, Topping JH, Bugianesi E, Day CP, George J. The natural history of nonalcoholic fatty liver disease with advanced fibrosis or cirrhosis: an international collaborative study. Hepatology. 2011 Oct;54(4):1208-16. doi: 10.1002/hep.24491. Epub 2011 Aug 9. PMID: 21688282; PMCID: PMC3238674.

24. Vernon G, Baranova A, Younossi ZM. Systematic review: the epidemiology and natural history of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis in adults. Aliment Pharmacol Ther. 2011 Aug;34(3):274-85. doi: 10.1111/j.1365-2036.2011.04724.x. Epub 2011 May 30. PMID: 21623852.

25. Williamson RM, Price JF, Hayes PC, Glancy S, Frier BM, Johnston GI, Reynolds RM, Strachan MW; Edinburgh Type 2 Diabetes Study Investigators. Prevalence and markers of advanced liver disease in type 2 diabetes. QJM. 2012 May;105(5):425-32. doi: 10.1093/qjmed/hcr233. Epub 2011 Dec 7. PMID: 22156706.

26. Gaggini M, Morelli M, Buzzigoli E, DeFronzo RA, Bugianesi E, Gastaldelli A. Non-alcoholic fatty liver disease (NAFLD) and its connection with insulin resistance, dyslipidemia, atherosclerosis and coronary heart disease. Nutrients. 2013 May 10;5(5):1544-60. doi: 10.3390/nu5051544. PMID: 23666091; PMCID: PMC3708335.

27. Jegatheesan P, De Bandt JP. Fructose and NAFLD: The Multifaceted Aspects of Fructose Metabolism. Nutrients. 2017 Mar 3;9(3):230. doi: 10.3390/nu9030230. PMID: 28273805; PMCID: PMC5372893.

28. Maia-Ceciliano TC, Dutra RR, Aguila MB, Mandarim-De-Lacerda CA. The deficiency and the supplementation of vitamin D and liver: Lessons of chronic fructose-rich diet in mice. J Steroid Biochem Mol Biol. 2019; xxx-xxx [Article in Press] doi: 10.1016/j.jsbmb.2019.105399. Epub 2019 Jun 5. PMID: 31175967.

29. Sepidarkish M, Farsi F, Akbari-Fakhrabadi M, Namazi N, Almasi-Hashiani A, Maleki Hagiagha A, Heshmati J. The effect of vitamin D supplementation on oxidative stress parameters: A systematic review and meta-analysis of clinical trials. Pharmacol Res. 2019; xx-xxx. doi: 10.1016/j.phrs.2018.11.011. Epub 2018 Nov 15. PMID: 30447293.

30. Mokhtari Z, Hekmatdoost A, Nourian M. Antioxidant efficacy of vitamin D. Journal of Parathyroid Disease 2017; 5 (1): 11-16.

31. Labudzynskyi DO, Zaitseva OV, Latyshko NV, Gudkova OO, Veliky MM. Vitamin D3 contribution to the regulation of oxidative metabolism in the liver of diabetic mice. Ukr Biochem J. 2015 May-Jun;87(3):75-90. PMID: 26502702.

32. Görlach A, Brandes RP, Nguyen K, Amidi M, Dehghani F, Busse R. A gp91phox containing NADPH oxidase selectively expressed in endothelial cells is a major source of oxygen radical generation in the arterial wall. Circ Res. 2000 Jul 7;87(1):26-32. doi: 10.1161/01.res.87.1.26. PMID: 10884368.

33. Zhong W, Gu B, Gu Y, Groome LJ, Sun J, Wang Y. Activation of vitamin D receptor promotes VEGF and CuZn-SOD expression in endothelial cells. J Steroid Biochem Mol Biol. 2014 Mar; 140:56-62. doi: 10.1016/j.jsbmb.2013.11.017. Epub 2013 Dec 5. PMID: 24316428; PMCID: PMC3915503.

34. Foroozanfard F, Jamilian M, Bahmani F, Talaee R, Talaee N, Hashemi T, Nasri K, Asemi Z, Esmaillzadeh A. Calcium plus vitamin D supplementation influences biomarkers of inflammation and oxidative stress in overweight and vitamin D-deficient women with polycystic ovary syndrome: a randomized double-blind placebo-controlled clinical trial. Clin Endocrinol (Oxf). 2015 Dec;83(6):888-94. doi:

10.1111/cen.12840. Epub 2015 Jul 23. PMID: 26119844.
35. Zhu CG, Liu YX, Wang H, Wang BP, Qu HQ, Wang BL, Zhu M. Active form of vitamin D ameliorates non-alcoholic fatty liver disease by alleviating oxidative stress in a high-fat diet rat model. Endocr J. 2017 Jul 28;64(7):663-673. doi: 10.1507/endocrj. EJ16-0542. Epub 2017 May 23. PMID: 28539530.
36. Zhai HL, Wang NJ, Han B, Li Q, Chen Y, Zhu CF, Chen YC, Xia FZ, Cang Z, Zhu CX, Lu M, Lu YL. Low vitamin D levels and non-alcoholic fatty liver disease, evidence for their independent association in men in East China: a cross-sectional study (Survey on Prevalence in East China for Metabolic Diseases and Risk Factors (SPECT-China)). Br J Nutr. 2016 Apr;115(8):1352-9. doi: 10.1017/S0007114516000386. Epub 2016 Feb 18. PMID: 26888280.
37. Chung GE, Kim D, Kwak MS, Yang JI, Yim JY, Lim SH, Itani M. The serum vitamin D level is inversely correlated with nonalcoholic fatty liver disease. Clin Mol Hepatol. 2016 Mar;22(1):146-51. doi: 10.3350/cmh.2016.22.1.146. Epub 2016 Mar 28. PMID: 27044765; PMCID: PMC4825160.
38. Nakano T, Cheng YF, Lai CY, Hsu LW, Chang YC, Deng JY, Huang YZ, Honda H, Chen KD, Wang CC, Chiu KW, Jawan B, Eng HL, Goto S, Chen CL. Impact of artificial sunlight therapy on the progress of non-alcoholic fatty liver disease in rats. J Hepatol. 2011 Aug;55(2):415-25. doi: 10.1016/j.jhep.2010.11.028. Epub 2010 Dec 22. PMID: 21184788.