Association between Vitamin D Status and Non-Alcoholic Fatty Liver Disease: A Population-Based Study

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Summary The relationship between vitamin D levels and non-alcoholic fatty liver disease (NAFLD) remains unestablished. In this study, we aimed to explore the relationship between vitamin D levels and NAFLD based on population survey data. This cross-sectional study was conducted based on data from the National Health and Nutrition Examination Survey. Liver steatosis was diagnosed by ultrasonography. Binary logistic regression analyses were performed to determine the relationship between vitamin D status and NAFLD. A total of 9,782 participants were identified in this analysis, with 46.8% male and an average age of 44.41 ± 0.16 y old. Among them, 6,047 (61.8%) cases were without NAFLD, 1,357 (13.9%) had mild NAFLD, 1,594 (16.3%) had moderate and 784 (8.0%) had severe NAFLD. Compared to those with non-NAFLD or mild NAFLD, patients in the moderate to severe NAFLD group had higher vitamin D deficiency or insufficiency rates (12.4% vs 11.5% and 36.8% vs 33.2%, respectively). After adjustment for male gender, older age, race, BMI, history of diabetes and vitamin D intake, vitamin D levels were independently associated with the severity of NAFLD (vitamin D deficiency group OR: 1.314, 95% CI: 1.129 to 1.529, vitamin D insufficiency group OR: 1.203, 95% CI: 1.090 to 1.328). Besides that, cold season was also found to be an independent factor for NAFLD (OR: 0.896, 95% CI: 0.820 to 0.979). Lower vitamin D level is an independent risk factor for NAFLD. Vitamin D levels are inversely associated with the severity of NAFLD. Cold season increases the risk of NAFLD independently.

Key Words vitamin D, non-alcoholic fatty liver disease, ultrasonography, NHANES, cold season

Non-alcoholic fatty liver disease (NAFLD) is one of the major causes of chronic liver disease and is increasing in prevalence (1). Currently, NAFLD has been shown to be associated with insulin resistance, dyslipidemia, obesity, cardiovascular disease and metabolic syndrome (2, 3) and is considered to be a risk factor for them (4). Despite this disease burden, there are currently few therapeutic methods that can efficiently treat NAFLD.

Vitamin D is a fat-soluble vitamin and plays key regulatory roles in calcium uptake, bone metabolism and immune function (5, 6). It is an important constituent of many tissues, organs and metabolic processes, and levels are often found to be abnormal in metabolic syndrome (5, 7, 8). Vitamin D deficiency, characterized by low levels of serum 25(OH)D, may result from limited exposure to sunlight, inadequate intake or malabsorption of vitamin D. Vitamin D deficiency is common in adults and children among the general population and has been demonstrated to be associated with wide-ranging health problems (5, 8).

Previous studies have explored the relationship between vitamin D deficiency and NAFLD but have not drawn consistent conclusions. It has been reported that there is a high concentration of vitamin D receptors in the liver and that the distribution of receptors may be associated with the severity of hepatitis, suggesting that vitamin D may affect the onset and progression of liver diseases (9). Several clinical studies have also revealed that vitamin D deficiency is an important risk factor for NAFLD (10, 11). However, these conclusions have not been consistently reproduced by other research groups, some of which produced contradictory results (12, 13) and even argued that vitamin D deficiency contributed to accumulation of liver fat (14). The relationship between vitamin D and the severity of liver steatosis remains unclear.

Therefore, the aim of this study was to demonstrate the association between vitamin D status and severity of NAFLD, based on the Third National Health and Nutrition Examination Surveys (NHANES III) data.

METHODS

Participants. In order to establish the relationship

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Fig. 1. Flow diagram of subjects included in this study.

between NAFLD and vitamin D levels, we downloaded free-access data from the website of the Third National Health and Nutrition Examination Survey (NHANES III). NHANES III was a nationwide study designed to assess the health and nutritional status of adults and children in the United States in 1988–1994. It was conducted by the National Center for Health Statistics of the Centers for Disease Control and Prevention (CDC) of the United States. The data of this survey contained questionnaires, laboratory, and examination components and was available on the Internet of CDC (<http://wwwn.cdc.gov/nchs.htm>).

A total of 39,695 participants were initially screened. Among them, 29,597 participants were excluded for following reasons: no ultrasonography results ($n=25,839$), without serum 25(OH)D data ($n=580$), no body mass index (BMI) ($n=22$), alcohol consumption ($n=2,804$), no data on diabetes history ($n=10$), no data on hypertension history ($n=102$), no serum alanine aminotransferase (ALT) data ($n=219$), no serum cholesterol data ($n=20$), no serum creatinine (Cr) data ($n=1$) and with chronic viral hepatitis B or C ($n=316$). The final study sample consisted of 9,782 subjects (Fig. 1). This study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Ethics Review Board of the First Affiliated Hospital of Fujian Medical University.

Laboratory measurements. Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine (Cr), blood urea nitrogen (BUN) and cholesterol were downloaded from the original database. Blood specimens were stored under appropriate refrigerated ($4-8^{\circ}\text{C}$) or frozen (-20°C) conditions until they were shipped to analytical laboratories for testing. All the specimens were tested by the same method.

Measurements and stratification of serum vitamin D. Vitamin D has no biological activity itself and must be hydroxylated into 25(OH)D in the liver; therefore, serum 25(OH)D is widely used for the evaluation of the vitamin D status in the human body (15). Serum 25(OH)D concentrations of participants were measured at the National Center for Environmental Health, CDC, Atlanta, GA, using the DiaSorin RIA kit (Stillwater, MN). The participants were divided into three groups: (1) the vitamin D sufficient group (≥ 20 ng/mL (50 nmol/L));

(2) the vitamin D insufficient group (≥ 12 ng/mL (30 nmol/L) and < 20 ng/mL (50 nmol/L)); and (3) the vitamin D deficient group (< 12 ng/mL (30 nmol/L)) according to the Food and Nutrition Board, Institute of Medicine (IOM) report (16).

Definition of NAFLD. NAFLD was assessed by re-reviewing the archived hepatic/gallbladder ultrasound video images by three ultrasound readers who were trained by a board-certified radiologist specializing in hepatic imaging. The original data contained the following information: 1) the presence of liver-to-kidney contrast, 2) the degree of the brightness of the liver parenchyma, 3) the presence of deep beam attenuation, 4) the presence of echogenic walls in the small intra-hepatic vessels, and 5) the definition of the gall bladder walls. Finally, an overall primary finding was given based on the presence or absence of each of the above parameters. The liver was graded as normal, or exhibiting mild, moderate, or severe hepatic steatosis. If the ultrasound images did not contain all those five parameters, the NAFLD could not be assessed and were treated as missing data (Details in <http://www.cdc.gov/nchs/nhanes/nhanes3/DataFiles.aspx>).

Statistical analysis. Continuous variables are shown as means $\pm$ standard deviation and categorical variables are shown as frequencies and percentages (17). The relationship between vitamin D levels and the severity of NAFLD was analyzed by the chi-squared test and the trend was assessed by the Jonckheere-Terpstra trend test. We used binary logistic regression to adjust the confounding factors of NAFLD (gender, age, race, body mass index (BMI), season of blood draw (cold season was from Nov. 1 to Apr. 30, warm season was from May 1 to Oct. 31)), history of diabetes and hypertension, serum ALT, AST, Cr, BUN and cholesterol level.

All reported p values were two-sided, and p values of < 0.05 were considered statistically significant. Statistical analyses were conducted using SPSS, version 13.0.

RESULTS

Characteristics of participants

A total of 9,782 participants were involved in this analysis, with 46.8% males and an average age of 44.41 ± 0.16 y old. Among them, 6,047 (61.8%) cases did not have NAFLD, 1,357 (13.9%) had mild NAFLD, 1,594 (16.3%) had moderate and 784 (8.0%) had severe NAFLD. Patients with vitamin D deficiency, insufficiency and sufficiency totaled 1,158 (11.8%), 3,383 (34.6%) and 5,241 (53.6%) respectively. There were significant differences in gender, age, race, BMI, season of blood draw, history of diabetes, history of hypertension, vitamin D level, AST, ALT, serum cholesterol levels and history of vitamin D intake among the four groups, but no significant change in BUN or Cr levels. Male, non-black participants and patients with histories of diabetes or hypertension tended to have more severe NAFLD. Age, BMI, ALT, AST and serum cholesterol levels also increased with the severity of NAFLD. There was a higher detection rate of moderate and severe NAFLD in the cold season compared to the warm season.

Table 1. Characteristics of participants stratified by the severity of NAFLD, the third National Health and Nutrition Examination Surveys (1988 to 1994).

	Overall (n=9,782)	Severity of NAFLD				p value
		Non-NAFLD (n=6,047)	Mild (n=1,357)	Moderate (n=1,594)	Severe (n=784)	
Male n (%)	4,576 (46.8)	2,776 (45.9)	606 (44.7)	789 (49.5)	405 (51.7)	0.001
Age (y)	44.41±0.16	42.73±0.21	44.67±0.44	47.82±0.39	49.95±0.53	<0.001
Race, n (%)						<0.001
Black	2,821 (28.8)	1,921 (31.8)	384 (28.3)	334 (21.0)	182 (23.2)	
Non-Black	6,961 (71.2)	4,126 (68.2)	973 (71.7)	1,260 (79.0)	602 (76.8)	
BMI (kg/m ²)	27.60±0.06	26.31±0.07	28.14±0.18	30.53±0.17	30.68±0.22	<0.001
Season of blood draw, n (%)						<0.001
Cold season	5,201 (53.2)	3,118 (51.6)	730 (53.8)	902 (56.6)	451 (57.5)	
Warm season	4,581 (46.8)	2,929 (48.4)	627 (46.2)	692 (43.4)	333 (42.5)	
History of diabetes, n (%)	865 (8.8)	339 (5.6)	138 (10.2)	248 (15.6)	140 (17.9)	<0.001
History of hypertension, n (%)	2,576 (26.3)	1,372 (22.7)	377 (27.8)	527 (33.1)	300 (38.3)	<0.001
Vitamin D level (nmol/L)	54.16±0.21	55.18±0.27	53.54±0.57	51.99±0.49	51.77±0.68	<0.001*
Vitamin D status, n (%)						0.001
Deficiency	1,158 (11.8)	693 (11.5)	164 (12.1)	196 (12.3)	105 (13.4)	
Insufficiency	3,383 (34.6)	2,008 (33.2)	498 (36.7)	600 (37.6)	277 (35.3)	
Sufficiency	5,241 (53.6)	3,346 (55.3)	695 (51.2)	798 (50.1)	402 (51.3)	
AST (U/L)	21.81±0.14	20.35±0.13	21.69±0.47	24.76±0.43	27.31±0.76	<0.001
ALT (U/L)	18.16±0.16	15.75±0.15	18.11±0.43	23.15±0.53	26.73±0.77	<0.001
Cholesterol (mmol/L)	5.32±0.01	5.25±0.01	5.32±0.03	5.49±0.03	5.60±0.04	<0.001
BUN (mmol/L)	4.92±0.02	4.89±0.02	4.87±0.05	5.01±0.05	4.99±0.07	0.076
Cr (μmol/L)	94.04±0.28	94.22±0.36	93.73±0.93	93.53±0.57	94.27±0.72	0.797
History of vitamin D intake, n (%)	3,353 (34.3)	2,137 (35.3)	458 (33.8)	496 (31.1)	262 (33.4)	0.015

p value: chi-squared test for categorical data and analysis of variance (ANOVA) test for continuous variables among four NAFLD groups.

* Jonckheere-Terpstra trend test for the trend of vitamin D level in severity of NAFLD.

Table 2. Vitamin D status in non-NAFLD to mild NAFLD group versus moderate to severe NAFLD group.

Vitamin D status	Overall (n=9,782)	Non-NAFLD to mild NAFLD (n=7,658)	Moderate to severe NAFLD (n=2,440)	p value
Deficiency	1,158 (11.8)	693 (11.5)	465 (12.4%)	<0.001
Insufficiency	3,383 (34.6)	2,008 (33.2)	1,375 (36.8%)	
Sufficiency	5,241 (53.6)	3,346 (55.3)	1,895 (50.7%)	

Relationship between serum vitamin D level and the severity of NAFLD

Vitamin D levels decrease with the severity of NAFLD, as shown in Table 1. The Jonckheere-Terpstra trend test showed that vitamin D status was negatively associated with the severity of NAFLD (Table 1). According to the severity of NAFLD, patients were divided into two groups: the non-NAFLD to mild NAFLD group and the moderate NAFLD to severe NAFLD group. Compared to those with non-NAFLD to mild NAFLD, patients with moderate to severe NAFLD had higher vitamin D deficiency or insufficiency rates (12.4% vs 11.5% and 36.8% vs 33.2%, respectively) (Table 2 and Fig. 2).

The effect of season on BMI, vitamin D levels and the prevalence of NAFLD

Interestingly, as shown in Table 1, the cold season

Fig. 2. Vitamin D level between the non-NAFLD to mild NAFLD group and the moderate to severe NAFLD group.

Table 3. Characteristics of participants by the season.

	Season		<i>p</i> value
	Cold season (5,375)	Warm season (4,723)	
BMI	27.69±0.08	27.50±0.09	0.132
Vitamin D level (nmol/L)	50.70±0.27	58.08±0.31	<0.001
Vitamin D status, <i>n</i> (%)			<0.001
Deficiency	771 (14.8)	387 (8.4)	
Insufficiency	1,977 (38.0)	1,406 (30.7)	
Sufficiency	2,453 (47.2)	2,788 (60.9)	
Severity of NAFLD, <i>n</i> (%)			<0.001
Non-NAFLD	3,118 (60.0)	2,929 (63.9)	
Mild	730 (14.0)	627 (13.7)	
Moderate	902 (17.3)	692 (15.1)	
Severe	451 (8.7)	333 (7.3)	

p value: chi-squared test for categorical data and analysis of variance (ANOVA) test for continuous variables.

Table 4. Binary logistic regression analysis of the risk factors for moderate to severe NAFLD.

	OR	95% CI	<i>p</i> value
Vitamin D levels			
Sufficiency	reference		
Deficiency	1.314	1.129 to 1.529	<0.001
Insufficiency	1.203	1.090 to 1.328	<0.001
Age	1.011	1.008 to 1.014	<0.001
Male gender	1.287	1.177 to 1.407	<0.001
Black race	0.574	0.517 to 0.638	<0.001
Cold season	0.896	0.820 to 0.979	0.015
History of diabetes	1.962	1.681 to 2.290	<0.001
BMI	1.103	1.095 to 1.112	<0.001
Vitamin D intake	0.913	0.832 to 1.003	0.057

seems to see higher detection rates of NAFLD. Approximately 56.9% of the cases of moderate to severe NAFLD were identified during the cold season. Since the cold season has been shown to be associated with lower levels of vitamin D, we compared the BMI and vitamin D level between the two seasons. The levels of vitamin D were relatively lower in the cold season than in the warm season and the severity of NAFLD is positively correlated with this. However, the BMI levels were comparable between the two seasons (Table 3).

Multivariate analysis of vitamin D level and presence of NAFLD

Binary logistic regression analysis was conducted to adjust confounding factors that could be contributing to the presence of moderate to severe NAFLD. As shown in Table 4, male gender, older age, black race, history of diabetes and history of vitamin D intake are all risk factors for NAFLD ($p<0.001$). The results showed that vitamin D level was independently associated with the presence of moderate to severe NAFLD (vitamin D deficiency group OR: 1.314, 95% CI: 1.129 to 1.529, vitamin D insufficiency group OR: 1.203, 95% CI: 1.090 to 1.328). Furthermore, the cold season was also inde-

pendently associated with the presence of moderate to severe NAFLD after adjusting for other confounders (OR: 0.896, 95% CI: 0.820 to 0.979).

DISCUSSION

In this national population-based, cross-sectional study, vitamin D levels were inversely associated with the severity of NAFLD in the general population.

The relationship between vitamin D and NAFLD remains unclear, but some studies find that vitamin D deficiency is associated with the presence and severity of NAFLD (10, 18–22). Low vitamin D levels are associated with the presence of NAFLD independently from metabolic syndrome among adults with normal serum liver enzymes (18). A Chinese community-based study with 2,960 Chinese participants indicated that vitamin D was inversely correlated with liver fat content in middle-aged and elderly males but not females (19). A study involving 232 patients referred to endocrinology and gastroenterology outpatient clinics showed that vitamin D levels were lower in patients with NAFLD than those without, while the differences were not significant between mild, moderate and severe NAFLD groups (21). Another cross-sectional study with 5,066 subjects suggested that vitamin D acted as an independent factor for NAFLD (22). Data from Korean studies also supported this result (10).

On the other hand, some researchers believe there is no correlation between vitamin D levels and NAFLD (12, 13). A study looking at the gene expression of vitamin D suggested that there is no relationship, finding that either vitamin D deficiency or hepatic expression of vitamin D-relative genes are not associated with the presence or histological severity of NAFLD (13). Another population-based study from Korea found that vitamin D deficiency was not associated with the presence of NAFLD. However, the definition of NAFLD in that particular study was assessed by a non-invasive fatty liver model, which compromises the reliability of those results (12). In this present study, larger popula-

tion-based samples were analyzed and the diagnosis of NAFLD was reliable; the results showed that the severity of NAFLD was inversely associated with serum levels of vitamin D. These results support the hypothesis that vitamin D plays a vital role in NAFLD.

The possible mechanisms underlying the correlation between vitamin D and NAFLD could be explained by the following findings. Vitamin D has effects on extra-skeletal metabolic organs and it can also indirectly influence hepatic lipid metabolism, decrease serum TC, TG, and LDL levels and cause lipid accumulation in the liver (23, 24). Vitamin D also has anti-inflammatory and immune-modulatory activity. A lower vitamin D level results in adipose tissue dysfunction and subsequent chronic inflammation, which may also contribute to the development of NAFLD (20, 25, 26). Furthermore, an animal study indicated that vitamin D deficiency is related to the development and progression of NAFLD through the activation of TLR2 and TLR4 via CD14/LBP, and stimulation of the downstream inflammatory signaling molecules involved with steatosis in the liver (27). Finally, in NAFLD patients, the increased body fat mass resulted in a greater volume of distribution of vitamin D in the adipose tissue compartment, and thereafter, a relatively lower plasma concentration (28, 29). These studies support the suggestion that vitamin D status is a risk factor for NAFLD.

Interestingly, we also found that the cold season was correlated with a higher detection rate of NAFLD compared to warmer seasons. During the cold season, lack of exercise usually increases fat accumulation in the body. However, there were no significant differences in patients' BMI between the cold season and the warm season, which indicates that the change in BMI might not account for the different incidences of NAFLD between the two seasons. In addition, the cold season was also correlated with a lower average vitamin D level in patients, as found in this study and previous research (30, 31). Vitamin D levels were negatively associated with the severity of NAFLD; therefore, lower vitamin D levels might play a more important role in the development of NAFLD in the cold season. However, in a multivariate analysis, cold seasons were found to be associated with the presence of NAFLD independently from BMI or vitamin D levels, suggesting a more complex disease mechanism.

This study had several advantages: (1) It was based on a national population, providing a large sample size, (2) We used ultrasonography, a widely available, non-invasive and recommended method for the diagnosis of liver steatosis (32), to diagnose NAFLD, especially in large population-based studies (33–35), while some early population-based studies used non-invasive fatty liver models (12, 36). The major limitation of this study is that it is only a cross-sectional study. The results only suggest the correlation between vitamin D and NAFLD rather than delineating a causal relationship. Further studies are needed to demonstrate the mechanism of this association and the effect of vitamin D supplementation on NAFLD.

In conclusion, our study revealed that vitamin D deficiency is an independent risk factor for NAFLD and is associated with higher severities of liver steatosis.

Disclosure of State of COI

The authors have no conflict of interest to declare.

Author Contributions Statement

(i) Guarantor of the article: Yueyong Zhu; (ii) Specific author contributions: Shiyong Liu and Bo Wan wrote the paper, Shiyong Liu, Haoyang Zhang, Yuxiu Liu, Sumei Wu and Zheng Zhu collected and analyzed the data, Su Lin and Yueyong Zhu contributed to the design of the study; (iii) all authors reviewed the manuscript.

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REFERENCES

- 1) Loomba R, Sanyal AJ. 2013. The global NAFLD epidemic. *Nat Rev Gastroenterol Hepatol* **10**(11): 686–690.
- 2) Mendez-Sanchez N, Arrese M, Zamora-Valdes D, Uribe M. 2007. Current concepts in the pathogenesis of non-alcoholic fatty liver disease. *Liver Int* **27**(4): 423–433.
- 3) Chalasani N, Younossi Z, Lavine JE, Diehl AM, Brunt EM, Cusi K, Charlton M, Sanyal AJ. 2012. The diagnosis and management of non-alcoholic fatty liver disease: practice Guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association. *Hepatology* **55**(6): 2005–2023.
- 4) Targher G, Day CP, Bonora E. 2010. Risk of cardiovascular disease in patients with nonalcoholic fatty liver disease. *N Engl J Med* **363**: 1341–1350.
- 5) Hewison M. 2012. An update on vitamin D and human immunity. *Clin Endocrinol* **76**(3): 315–325.
- 6) Wagner CL, Greer FR, American Academy of Pediatrics Section on Breastfeeding, American Academy of Pediatrics Committee on Nutrition. 2008. Prevention of rickets and vitamin D deficiency in infants, children, and adolescents. *Pediatrics* **122**(5): 1142–1152.
- 7) Kim DH, Sabour S, Sagar UN, Adams S, Whellan DJ. 2008. Prevalence of hypovitaminosis D in cardiovascular diseases (from the National Health and Nutrition Examination Survey 2001 to 2004). *Am J Cardiol* **102**(11): 1540–1544.
- 8) Theodoratou E, Tzoulaki I, Zgaga L, Ioannidis JP. 2014. Vitamin D and multiple health outcomes: umbrella review of systematic reviews and meta-analyses of observational studies and randomised trials. *BMJ* **348**: g2035.
- 9) Barchetta I, Carotti S, Labbadia G, Gentilucci UV, Muda AO, Angelico F, Silecchia G, Leonetti F, Fraioli A, Picardi A, Morini S, Cavallo MG. 2012. Liver vitamin D receptor, CYP2R1, and CYP27A1 expression: relationship with liver histology and vitamin D3 levels in patients with nonalcoholic steatohepatitis or hepatitis C virus. *Hepatology* **56**(6): 2180–2187.
- 10) Chung GE, Kim D, Kwak MS, Yang JI, Yim JY, Lim SH,

- Itani M. 2016. The serum vitamin D level is inversely correlated with nonalcoholic fatty liver disease. *Clin Mol Hepatol* **22**(1): 146–151.
- 11) Giblin RJ, Bennett EJ, Zosky GR, Dwyer RM. 2017. The impact of sex and 25(OH)D deficiency on metabolic function in mice. *Nutrients* **9**(9): pii: E985.
  - 12) Ha Y, Hwang SG, Rim KS. 2017. The association between vitamin D insufficiency and nonalcoholic fatty liver disease: A population-based study. *Nutrients* **9**(8): pii: E806.
  - 13) Patel YA, Henao R, Moylan CA, Guy CD, Piercy DL, Diehl AM, Abdelmalek MF. 2016. Vitamin D is not associated with severity in NAFLD: Results of a paired clinical and gene expression profile analysis. *Am J Gastroenterol* **111**(11): 1591–1598.
  - 14) Liu XJ, Wang BW, Zhang C, Xia MZ, Chen YH, Hu CQ, Wang H, Chen X, Xu DX. 2015. Vitamin D deficiency attenuates high-fat diet-induced hyperinsulinemia and hepatic lipid accumulation in male mice. *Endocrinology* **156**(6): 2103–2113.
  - 15) Holick MF. 2007. Vitamin D deficiency. *N Engl J Med* **357**: 266–281.
  - 16) Ross AC, Taylor CL, Yaktine AL, Del Valle HB, eds. 2011. Committee to Review Dietary Reference Intakes for Vitamin D and Calcium IoM: Implications and special concerns: Conclusions about vitamin D deficiency in the United States and Canada. In: *Dietary Reference Intakes for Calcium and Vitamin D*, p 487. National Academies Press, Washington, DC.
  - 17) Zhang Z. 2016. Univariate description and bivariate statistical inference: the first step delving into data. *Ann Transl Med* **4**(5): 91.
  - 18) Barchetta I, Angelico F, Del Ben M, Baroni MG, Pozzilli P, Morini S, Cavallo MG. 2011. Strong association between non alcoholic fatty liver disease (NAFLD) and low 25(OH) vitamin D levels in an adult population with normal serum liver enzymes. *BMC Med* **9**: 85.
  - 19) Wang D, Lin H, Xia M, Aleteng Q, Li X, Ma H, Pan B, Gao J, Gao X. 2016. Vitamin D levels are inversely associated with liver fat content and risk of non-alcoholic fatty liver disease in a Chinese middle-aged and elderly population: The Shanghai Changfeng Study. *PLoS One* **11**(6): e0157515.
  - 20) Nelson JE, Roth CL, Wilson LA, Yates KP, Aouizerat B, Morgan-Stevenson V, Whalen E, Hoofnagle A, Mason M, Gersuk V, Yeh MM, Kowdley KV. 2016. Vitamin D deficiency is associated with increased risk of non-alcoholic steatohepatitis in adults with non-alcoholic fatty liver disease: Possible role for MAPK and NF-kappaB? *Am J Gastroenterol* **111**(6): 852–863.
  - 21) Kucukazman M, Ata N, Dal K, Yeniova AO, Kefeli A, Basyigit S, Aktas B, Akin KO, Agladioglu K, Ure OS, Topal F, Nazligul Y, Beyan E, Ertugrul DT. 2014. The association of vitamin D deficiency with non-alcoholic fatty liver disease. *Clinics* **69**(8): 542–546.
  - 22) Zhai HL, Wang NJ, Han B, Li Q, Chen Y, Zhu CF, Chen YC, Xia FZ, Cang Z, Zhu CX, Lu M, Lu YL. 2016. 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* **115**(8): 1352–1359.
  - 23) Jafari T, Fallah AA, Barani A. 2016. Effects of vitamin D on serum lipid profile in patients with type 2 diabetes: A meta-analysis of randomized controlled trials. *Clin Nutr* **35**(6): 1259–1268.
  - 24) Norman AW. 2008. From vitamin D to hormone D: fundamentals of the vitamin D endocrine system essential for good health. *Am J Clin Nutr* **88**(Suppl): 491S–499S.
  - 25) Cimini FA, Carotti S, Bertocchini L, Baroni MG, Vespasiani-Gentilucci U, Cavallo MG, Morini S. 2017. Relationship between adipose tissue dysfunction, vitamin D deficiency and the pathogenesis of non-alcoholic fatty liver disease. *World J Gastroenterol* **23**(19): 3407–3417.
  - 26) Bril F, Maximos M, Portillo-Sanchez P, Biernacki D, Lomonaco R, Subbarayan S, Correa M, Lo M, Suman A, Cusi K. 2015. Relationship of vitamin D with insulin resistance and disease severity in non-alcoholic steatohepatitis. *J Hepatol* **62**(2): 405–411.
  - 27) Roth CL, Elfers CT, Figlewicz DP, Melhorn SJ, Morton GJ, Hoofnagle A, Yeh MM, Nelson JE, Kowdley KV. 2012. Vitamin D deficiency in obese rats exacerbates non-alcoholic fatty liver disease and increases hepatic resistin and Toll-like receptor activation. *Hepatology* **55**(4): 1103–1111.
  - 28) Lenders CM, Feldman HA, Von Scheven E, Merewood A, Sweeney C, Wilson DM, Lee PD, Abrams SH, Gitelman SE, Wertz MS, Klish WJ, Taylor GA, Chen TC, Holick MF, Elizabeth Glaser Pediatric Research Network Obesity Study Group. 2009. Relation of body fat indexes to vitamin D status and deficiency among obese adolescents. *Am J Clin Nutr* **90**(3): 459–467.
  - 29) Dasarathy J, Periyalwar P, Allampati S, Bhinder V, Hawkins C, Brandt P, Khiyami A, McCullough AJ, Dasarathy S. 2014. Hypovitaminosis D is associated with increased whole body fat mass and greater severity of non-alcoholic fatty liver disease. *Liver Int* **34**(6): e118–127.
  - 30) Cinar N, Harmanci A, Yildiz BO, Bayraktar M. 2014. Vitamin D status and seasonal changes in plasma concentrations of 25-hydroxyvitamin D in office workers in Ankara, Turkey. *Eur J Intern Med* **25**(2): 197–201.
  - 31) Choi R, Kim S, Yoo H, Cho YY, Kim SW, Chung JH, Oh SY, Lee SY. 2015. High prevalence of vitamin D deficiency in pregnant Korean women: the first trimester and the winter season as risk factors for vitamin D deficiency. *Nutrients* **7**(5): 3427–3448.
  - 32) Castera L, Vilgrain V, Angulo P. 2013. Noninvasive evaluation of NAFLD. *Nat Rev Gastroenterol Hepatol* **10**(11): 666–675.
  - 33) Chang Y, Ryu S, Sung K-C, Cho YK, Sung E, Kim H-N, Jung H-S, Yun KE, Ahn J, Shin H, Wild SH, Byrne CD. 2018. Alcoholic and non-alcoholic fatty liver disease and associations with coronary artery calcification: evidence from the Kangbuk Samsung Health Study. *Gut: gutjnl-2018-317666*.
  - 34) Huang J, Zhang H, Liu S, Wang M, Wan B, Velani B, Zhu Y, Lin S. 2018. Is *Toxoplasma gondii* infection correlated with nonalcoholic fatty liver disease?—a population-based study. *BMC Infect Dis* **18**(1): 629.
  - 35) Liu Y, Wan B, Lin S. 2018. Letter: significant burden of nonalcoholic fatty liver disease with advanced fibrosis in the USA: a cross-sectional analysis of 2011–2014 National Health and Nutrition Examination Survey. *Aliment Pharmacol Ther* **48**(5): 584–585.
  - 36) Putz-Bankuti C, Pilz S, Stojakovic T, Scharnagl H, Pieber TR, Trauner M, Obermayer-Pietsch B, Stauber RE. 2012. Association of 25-hydroxyvitamin D levels with liver dysfunction and mortality in chronic liver disease. *Liver Int* **32**(5): 845–851.