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AN INVESTIGATION INTO THE PROSPECTIVE EFFECTS OF VITAMIN D SUPPLEMENTATION ON THE DEVELOPMENT & ATTENUATION OF NON-ALCOHOLIC FATTY LIVER DISEASE

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The purpose of this study is to shed light on the potential role of vitamin D in its hydroxylated form, 25-OH-Cholecalciferol(25OHD), in moderating the development and attenuation of non-alcoholic fatty liver disease (NAFLD) via an aged laying hen model. Specifically, thirty-month-old single-comb white Leghorn hens were assigned at random to one of three diets: a control diet (CON) consisting of a standard composition of feed and supplements, a vitamin-D-supplemented group (VID) consisting of the CON diet with an additional 69 μg/kg of 25OHD per day, and a whole flaxseed (WFX) supplemented group used as a comparative intervention standard against the VID group. The results were inconclusive as to the role of 25OHD in modifying the development and progression of NAFLD. Nonetheless, this study serves to emphasize the importance of future studies investigating this association. Discussion of the various limitations for this study can also inform the design of future studies of this nature.

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CHAPTER 1

INTRODUCTION

Introduction. In order to develop a comprehensive understanding of non-alcoholic fatty liver disease (NAFLD), it is first imperative to elaborate the various – and alarmingly prevalent - comorbidities associated with NAFLD as it develops in the United States. While a multitude of factors are known, primary among them are obesity and metabolic dysregulation. Less appreciated in its potential causal role in NAFLD is vitamin D deficiency. Understanding each of these in context will shed light on the rise in NAFLD in America.

Obesity. Obesity, defined by the CDC as a BMI in excess of 30 in adults or a BMI greater than or equal to the 95th percentile in children, has steadily risen in the United States over the past several decades.[1,2] According to the CDC's National Center for Health Statistics (NCHS), as of 2015 approximately 39.8% of Americans are obese. Based on the National Health and Nutrition Examination Survey (NHANES) data collected by NCHS from 2000-2015, the rise in obesity has accelerated in recent years. In 2000, 30.5% of American adults were obese. This means there has been a 9.3% absolute rise in obesity among American adults in the last 15 years. This translates to 21 million more obese American adults today than there were 15 years ago. Perhaps more alarmingly, 13.9% of American children were obese in 2000 compared to 18.5% today. In real numbers there are 4.3 million more obese American children today than there were a decade and a half ago.[3]

Obesity and NAFLD. Obesity is strongly correlated with NAFLD. This is because excessive hepatic metabolism of free fatty acids (FFAs) causes increased stress on the liver and can result in fatty acid retention in the liver. If sufficiently severe, this fatty acid retention will result in intrahepatocellular steatosis, or hepatic accumulation of fat deposits. Fatty acids are

transported to the liver primarily as FFAs from subcutaneous fat with minor transport occurring from visceral fat. The breakdown of FFAs from subcutaneous versus visceral fat is about 95% and 5% in lean individuals compared to 80% and 20% in obese individuals. However, this proportional shift is less important than the total FFA transport to the liver, which is generally much higher in obese individuals. This excessive hepatic FFA transport in obese individuals can result in steatosis.[4]

Diabetes. Diabetes, especially type two (T2DM), is another concerning co-morbidity that is pertinent to the development and prognosis of NAFLD. More specifically, between 60-70% of patients with T2DM also exhibit some degree of liver steatosis, which is the defining symptom of fatty liver disease generally.[5] According to a CDC report released in 2017, more than 30 million Americans have diabetes, and another 84 million have prediabetes. If left untreated, the average individual with prediabetes will develop type two diabetes within five years. Concerningly, 89% of individuals with prediabetes are undiagnosed, and nearly 24% of individuals with full-blown type two diabetes remain undiagnosed.[6]

Diabetes and NAFLD. Type two diabetes is strongly related with NAFLD. Impaired insulin sensitivity affects metabolic function throughout the body, and the liver is no exception. As mentioned previously, the primary source of fat in the liver (about 60%) is from FFAs. The remaining 40% comes from de novo lipogenesis (the process of making new fat from non-fat sources, predominantly carbohydrate) and diet, contributing 26% and 14%, respectively. De novo lipogenesis is directly increased by T2DM via insulin resistance.[7] This increased de novo lipogenesis, in turn, contributes to steatosis. Furthermore, those with T2DM typically have poor diets which further increases FFA in the liver, further compounding this problem.[8]

Defining NAFLD. Non-alcoholic fatty liver disease is a condition in which, as the name

would suggest, lipids accumulate in hepatocytes. More specifically, NAFLD is defined by the American Liver Foundation as an excess of ten percent of the liver's weight as fat.[9] Other standards for diagnosing NAFLD are also common, such as the presence of macrovesicular steatosis in greater than five percent of hepatocytes as measured by liver histology, or a proton density fat fraction exceeding 5.6% when measured via proton magnetic resonance spectroscopy.[5]

As of 2018, it is estimated that as many as 100 million Americans suffer from NAFLD; it is the most prevalent form of liver disease in children, with an increase of more than 100% over the last two decades.[9] More worrying still, as recently as the 1990s, non-alcoholic models of fatty liver disease were not readily acknowledged as feasible. This makes the ubiquity of NAFLD in America extremely concerning, as it represents a silent epidemic. This epidemic is also reflective of a rise in deleterious lifestyle factors contributing to the development and progression of NAFLD. In other words, it is patently obvious that this astronomical shift is not secondary to a change in the human genome, but a change in the environmental effects on the genome.[10]

As NAFLD progresses, it evolves into a more acute form called non-alcoholic steatohepatitis (NASH). NAFLD is simply steatosis (lipid accumulation in the liver) associated with a threshold percentage of the liver mass as fat. When NAFLD progresses to NASH, the liver develops inflammation and, more consequentially, may also develop a second physiological symptom termed fibrosis, which is a thickening of adipose tissue. There are multiple progressive stages of NASH, ultimately leading to necrosis of liver cells and fibrosis affecting a portal vein from the liver. Once a portal vein is affected, hepatic function begins to decline, thus marking the beginning of cirrhosis.[11]

While many potential variables have been proposed as potentially driving the

development of NAFLD, it is not entirely clear which factors, whether alone or in combination, lead to the full onset of non-alcoholic fatty liver. Furthermore, most studies investigating correlations among various metrics and NAFLD have been cross sectional in nature, and therefore they cannot shed light on any potentially causal relationships. Determining causal factors of NAFLD is obviously paramount in establishing reliable preventative measures.[12]

Vitamin D Deficiency. Hypovitaminosis D (vitamin D deficiency), as clinically verified by serum measures of 25-hydroxyvitamin D below 30ng/mL, is one factor which is strongly correlated with NAFLD. Simultaneously, the bioavailability of Vitamin D3 is greatly diminished in obesity, which is likely a result of its fat solubility leading to excess storage in adipose tissue.[13] According to an NCHS report from 2011, 8% of Americans have hypovitaminosis D and a further 24% have vitamin D insufficiency (30-49nmol/L) putting them at greater risk for developing hypovitaminosis D in the future.[14]

Justification. Many of the hypothetical mechanisms driving the development of NAFLD are in some way correlated with low serum vitamin D.[12] As such, insufficient serum vitamin D is a strong metric of interest in both the development and prognosis of NAFLD. Therefore, this present study attempts to shed light on the thoroughly intertwined relationship between vitamin D deficiency and NAFLD.

CHAPTER 2

REVIEW OF LITERATURE

Overview. Excess accumulation of visceral adipose tissue secondarily leads to elevations in circulating free fatty acids. These fatty acids can, in turn, drive steatosis and the onset of NAFLD.[12] By definition, steatosis increases intrahepatocellular lipids, and these lipids cause insulin resistance in the liver.[15] While it is widely acknowledged that metabolic issues such as obesity and insulin resistance are co-morbid with NAFLD, impaired vitamin D utilization is much less appreciated as a secondary effect of metabolic maladaptation. A 2013 meta-analysis published in Alimentary Pharmacology and Therapeutics noted that individuals with NAFLD have a 26% greater chance of being vitamin D deficient than those without NAFLD.[16] A further study published in 2007 reported significantly lower circulating serum 25-hydroxyvitamin D3 in NAFLD patients as compared to their control counterparts.[12]

Vitamin D Metabolism. Vitamin D is either converted to vitamin D3 by 7-dehydrocholestrol after unprotected skin exposure to ultraviolet light (typically from the sun), or it is consumed directly as vitamin D3 in the diet. Vitamin D3, however, is not bioactive. Vitamin D3 is therefore transported to the liver where it is hydroxylated to become 25OHD. This is the predominant bioactive form of vitamin D in circulation.[17] As such, when liver function is impaired, it is vital to supplement with pre-hydroxylated vitamin D (25OHD) because hepatic hydroxylation is not reliable. So impaired vitamin D status is generally prevalent in the industrialized world, and it is strongly correlated with obesity, which can predispose one to NAFLD. Once one has NAFLD, if liver function is sufficiently impaired, hydroxylation of vitamin D to 25OHD may also be impaired.[13]

Cytokines. Cytokine is an umbrella term for several distinct classes of small proteins

produced by the autoimmune system which serve important roles in cell signaling. More specifically, a production cell receives a signal and produces cytokines into the interstitium whereby the cytokines act on a target cell to produce a desired effect (**see figure 1**).[18] Two autoimmune cells of particular importance in the production of cytokines are T-lymphocytes and macrophages.[12] Regarding T-lymphocytes, a specific subtype, referred to as CD8 cells, requires enzymatic activation. Upon activation, CD8 cells exhibit a very high concentration of vitamin D3 receptors. As such, CD8 cells are highly dependent on serum D3 for proper regulation. Because of this CD8 dependence on vitamin D, CD8 cells serve as a primary mediator of immune-regulated inflammatory responses as correlated with vitamin D.[19] Macrophages, on the other hand, are known to convert 25-hydroxyvitamin D3 to 1α,25-$(OH)_2$D3 when activated. In turn, 1α,25-$(OH)_2$D3 may serve as an important immunosuppressant. Most notably, 1α,25-$(OH)_2$D3 downregulates interleukin-6 (IL-6), a distinct serum marker of inflammation.[20] Limiting inflammation may be an important factor in NAFLD prevention.[12]

Adipokines. Adipokines are cytokines secreted by white adipose tissue. While hundreds of adipokines exist, a select few are notable for their contribution to inflammation and potential role in the development of NAFLD. Specifically, when excess weight is retained, the ratio of different adipokines produced is altered. This alteration drives up inflammation (**see figure 2**). Simultaneously, anti-inflammatory proteins exhibit diminished expression.[21,22,23]

Vitamin D and Inflammation. Various serum markers of inflammation show strong inverse correlations with serum vitamin D concentration. The most direct among these correlations is that between vitamin D and MMP9. MMP9 is one of several matrix metalloproteinases (MMPs), which primarily serve to degrade vascular walls.[24] Elevated MMPs have been observed in virtually all inflamed human tissues, and they directly alter inflammatory

cytokine activity.[25] Vitamin D is considered the single driver of MMP9 in the blood. In turn, TIMP-1, itself a serum marker of inflammation, is an inhibitor of MMP9. Therefore, when Vitamin D3 is high and MMP9 is low, TIMP-1 is also low, as less is needed to inhibit the lower concentration of MMP9. C-reactive protein (CRP), perhaps the most prominent circulating marker of inflammation, also shows an independent, inverse correlation to serum D3. Furthermore, supplementation with vitamin D3 results in a marked reduction in serum concentrations of MMP9, TIMP-1, CRP, and various other inflammatory markers.[24]

Inflammatory adipokines. IL-6 is notable for its pivotal role in hepatic injury prevention. However, this injury-prevention role also promotes steatosis in the liver.[26] IL-10, on the other hand, has shown both anti-inflammatory and anti-steatotic properties. Nonetheless, IL-10 may contribute to increased hepatic insulin resistance.[27] This means IL-10 may indirectly increase steatosis in the liver by increasing insulin resistance and de novo lipogenesis, as discussed in the introduction. Finally, tumor necrosis factor alpha (TNFa) is an inflammatory adipokine that is selectively produced in higher quantities by white adipocytes. Furthermore, TNFa is produced in even higher quantities by the adipocytes of obese insulin-resistant patients compared to their lean counterparts. Further still, positive correlations show that as fibrosis severity increases in fatty liver so too do TNFa levels. Certain polymorphisms of TNFa are even shown to worsen NAFLD risk specifically.[28]

PTH and NASH Development. Parathyroid hormone (PTH), notable for its role in the dynamic equilibrium of bone density, is another marker of concern for NAFLD. Due to PTH's involvement in bone resorption and rebuilding, when vitamin D is low, PTH is overproduced by the parathyroid glands in an attempt to compensate for the lack of vitamin D. This results in a condition known as secondary hyperparathyroidism.[29] One role of PTH is the induction of IL-6

secretion by osteoblasts, which are cells responsible for bone formation. IL-6, in turn, drives the creation of acute phase reactants (APRs) by the liver. Perhaps the most concerning of these APRs are CRP, as discussed previously, and fibrinogen.[29] Fibrinogen, when activated by the enzyme thrombin, becomes fibrin. Fibrin forms web-like filaments that inhibit blood flow. This is the process that, when unregulated, leads to fibrogenesis in the liver.[12,30,31] As mentioned previously, fibrosis of the liver differentiates NAFLD from its later stage counterparts.[11]

Summary. Virtually all proposed mechanisms in the development of NAFLD are closely related to hypovitaminosis D. Specifically, cytokines (particularly adipokines) drive inflammation (**Figure 2**). Inflammation drives NAFLD. Impaired vitamin D status is associated with both adipokine production (via IL-6) and inflammation through various serum markers. Vitamin D's relationship with PTH also shows potential roles in the progression of NAFLD to NASH via increased IL-6 production and hepatic inflammation (**Figure 3**).

Given the prevalence and severity of NAFLD as a recent and meteoric epidemic in the United States, further investigation is pivotal in determining any potential causal links among insufficient serum 25-hydroxyvitamin D, non-alcoholic fatty liver disease, and non-alcoholic steatohepatitis. Many of the biochemical mechanisms associated with low Vitamin D are also closely related to one another, suggesting a robust relevance with NAFLD.[12] Delineating these various relationships and determining causality, or lack thereof, are crucial steps towards fully understanding the development of non-alcoholic fatty liver. To that end, the purpose of this research is to utilize a laying hen model to better evaluate the efficacy of Vitamin D supplementation in the prevention and attenuation of non-alcoholic fatty liver disease.

CHAPTER 3

METHODS

Note. Methods for this study were closely modeled on those utilized by J.E. Davis, J. Cain, C. Small, and D.B. Hales in *Therapeutic effect of flax-based diets on fatty liver in aged laying hens* as published in Poultry Science in May 2016.[32]

Nutrient Sourcing. Whole flaxseed and Hy-D® (25-hydroxy-cholecalciferol) were obtained from Omega Nutrition USA (Bellingham, WA) and DSM Nutritional Products (Parsippany, NJ), respectively. Qual fat was obtained from DarPro (Irving, TX). Corn gluten meal and solka floc (cellulose) were purchased from International Fiber Corporation (North Tonawanda, NY).[32] Complete composition of all experimental diets is detailed in full in **Table 1**.

Animals and Diets. Thirty-month-old single-comb white Leghorn hens (n = 247) were housed 10 hens/cage under 17-h light and 7-h dark cycle on the University of Illinois campus in Urbana-Champaign. Birds were randomly assigned to control (CON)(n=69), whole flaxseed (WFX)(n=99), or vitamin D (VID)(n=79) supplemented diet for 18 months. All diets were isocaloric and isonitrogenous and total diets were provided at 100g per day (308 kcal/day/hen) **(Table 1).** At termination, fasted hens were euthanized by CO2 asphyxiation followed by cervical dislocation. Animal management and procedures were reviewed and approved by the Institutional Animal Care and Use Committees at the University of Illinois at Urbana-Champaign and Southern Illinois University at Carbondale.[32]

Tissue Preparation. Tissue samples were immediately removed and snap frozen in liquid nitrogen and stored at -80°C for biochemical analysis. Liver compositions (i.e., lean, fat, and water) were determined using an EchoMRI-900™ Bioanalyzer (Echo Medical Systems LLC., Houston, TX)[32]. Liver MRIs were conducted in batches of 30-60 livers. Each MRI sample was

cut from the whole liver and bagged separately for testing. Prior to each batch run in the MRI, a test vial of pure vegetable oil consisting of 100% fat was scanned to ensure proper calibration of the machine. All test vials returned results of between 99.02% and 100.11% fat mass. As such, all vials tested within an acceptable 1% margin of error. Water stage was included in the MRI for every liver specimen tested to assess water weight and subtract it from total liver mass.

Statistical analyses. All data were tested for normality and subsequently analyzed using one-way ANOVA (SPSS Software, IBM Corporation, Armonk, NY). All values are presented means ± standard error of mean (SEM). Post hoc comparisons between groups were made using Bonferroni adjustments. Mean differences were considered significant in post-hoc analysis at $P<0.05$. This standard analysis was performed for both treatment groups and the control.

CHAPTER 4

RESULTS

Body Composition. There were no statistically significant findings. All three groups exhibited mean liver fat mass percentage above 23% (**Table 2**). One-way ANOVA for liver fat mass percentage across all three groups returned a p-value of 0.36 (not tabled). Bonferroni post-hoc analyses of liver fat mass percentage calculated separately between all experimental group pairings resulted in p values between 0.54 and 1.00 (**Table 3**).

Table 1. Composition of Experimental Diets

Ingredients (g/kg)	**CON**	**WFX**	**VID**
Corn Meal	67.4	47.6	67.4
Soybean Meal	18.3	18.3	18.3
Corn Gluten Meal	3.0		3.0
Whole Flaxseed		15.0	
Qual Fat		2.5	
Solka Floc (cellulose)	0.3	5.6	0.3
Limestone	8.75	8.75	8.75
Dicalcium Phosphate	1.50	1.50	1.50
Salt	0.30	0.30	0.30
Vitamin Premix	0.20	0.20	0.20
Trace Mineral Premix	0.15	0.15	0.15
DL-Methionine	0.10	0.10	0.10
Additional Analysis			
Total Metabolizable Energy (kcal/day)	308	308	308
Crude Protein (% kcal)	16.5	16.5	16.5
Secoisolariciresinol Diglycoside (mg/g)	—	2.0	—
Cholecalciferol (µg/kg)	25.0	25.0	25.0
25-OH-Cholecalciferol (µg/kg)	—	—	69.0

Table 2. Composition of Livers

Treatment Group	n	Mean Fat Mass	Mean Lean Mass	Mean Water Mass	Mean Fat Mass Percentage
Control (CON)	69	5.46±0.47	17.42±1.13	1.00±0.07[a]	23.63±0.63[b]
Whole Flaxseed (WFX)	99	4.14±0.24	13.20±0.68	0.75±0.04[a]	24.55±0.37[b]
25-OH Cholecalciferol (VID)	79	4.90±0.36	15.13±0.80	0.86±0.05[a]	24.46±0.49[b]

[a] Water mass was calculated during the MRI to control for water content in frozen liver specimens.

[b] Mean fat mass percentage is calculated as mean fat mass divided by the sum of mean fat mass and mean lean mass.

Table 3. Bonferroni Post-hoc comparisons of liver fat mass percentage among treatment groups

Treatment Group (I)	Comparison Group (J)	Standard Error	Significance (P)
CON	VID	0.0072	0.75
	WFX	0.0069	0.54
WFX	CON	0.0072	0.75
	VID	0.0066	1.00
VID	CON	0.0069	0.54
	WFX	0.0066	1.00

CHAPTER 5

DISCUSSION AND CONCLUSION

Commercial laying hens serve as very reliable models for hepatic dysregulation generally, given the various metabolic stressors inherent to their environment emanating from the cage effect. The cage effect simply acknowledges that the combination of physical inactivity and access to calorically dense feed makes laying hens highly susceptible to hepatic fat accumulation.[33] However, the hepatic model of laying hens as it relates to fat accumulation and the development of NAFLD, and how that process compares to the same etiology in humans, is notably more suspect.

First, chickens are not mammals; they are fowls. This is why rodents are often preferred as mammalian animal models when comparison to humans is the ultimate goal. However, similar to humans, laying hens are virtually exclusively dependent on hepatic de novo lipogenesis.[34] Rodents, on the other hand, only perform approximately half of their de novo lipogenesis hepatically.[35] Second, while laying hens do accumulate considerable hepatic fat, they do not develop NAFLD. Laying hens develop fatty liver hemorrhagic syndrome (FLHS). FLHS is an entirely different disease with markedly different prognosis. End-stage FLHS results in liver rupture which leads to hemorrhage and likely results in sudden death of the laying hen. NAFLD's progression to NASH and ultimately cirrhosis is obviously a much different disease progression. Lastly, there are various causal factors the differ between FLHS and NAFLD. The development of FLHS is strongly temperature dependent, whereas NAFLD is in no way related to environmental temperature. Perhaps most importantly, the specific dietary causes of FLHS and NAFLD differ considerably. In laying hens, FLHS is caused by overconsumption of wheat

and other grains.[33] In humans, NAFLD is caused by overconsumption of high-fat foods and processed sugars. More importantly, grain consumption in humans is actually inversely correlated with NAFLD risk.[36]

Limitations. There were several limitations in this study that are best addressed in future studies of this kind. First, as mentioned in the methods section, all livers were sectioned for MRI tests rather than testing the entire liver. This is potentially a confounding factor because fat distribution throughout the liver is not necessarily consistent or evenly distributed, so sampling only a portion of the liver could give misleading composition results. In fact, by visual inspection alone it is clear that hepatic fat accumulation in laying hens occurs in pockets rather than evenly marbling. Second, our statistical analysis did not control for any confounding factors such as the cage effect. Finally, and most importantly, this study began at 30 months of age in the laying hens. Average productive life for a laying hen at the commercial level is typically 24-36 months.[33] So, 30 months is far too late in life to see meaningful impact from an intervention of this kind when hepatic fat accumulation is already so extensive. This is reflected by the mean fat mass percentage of over 24% across all livers tested. The range in fat percentage from individual specimens was between 13-44%. So, all of the 247 livers tested had considerable fat accumulation which started long before the experimental period ever began.

Conclusion. Despite the various limitations of this study, compelling data strongly suggests a correlation between vitamin D status in humans and its potential relationship with NAFLD. Impaired vitamin D status strongly correlates to increased inflammation, and increased inflammation strongly correlates with NAFLD. Vitamin D also serves to regulate PTH, which, when unregulated, ultimately leads to the formation of fibrinogen and fibrosis in the liver advancing NAFLD to later stages. As discussed in the lit review, the biochemical logic for these

relationships is sound. All of this evidence underscores the need for more studies investigating the role of vitamin D deficiency in developing and advancing NAFLD.

Future studies of this nature should include time series data analyzing liver composition at the beginning of the study as well as multiple times throughout the study and upon completion. Also, aside from liver composition data, it would be good to determine ALT and AST (the two major liver enzymes in both humans and chickens which are typically elevated in NAFLD) levels and circulating 25OHD levels at each stage of analysis. Finally, future studies should start at the beginning of life rather than towards the end. This allows for data on vitamin D as a preventative measure for NAFLD rather than a treatment.

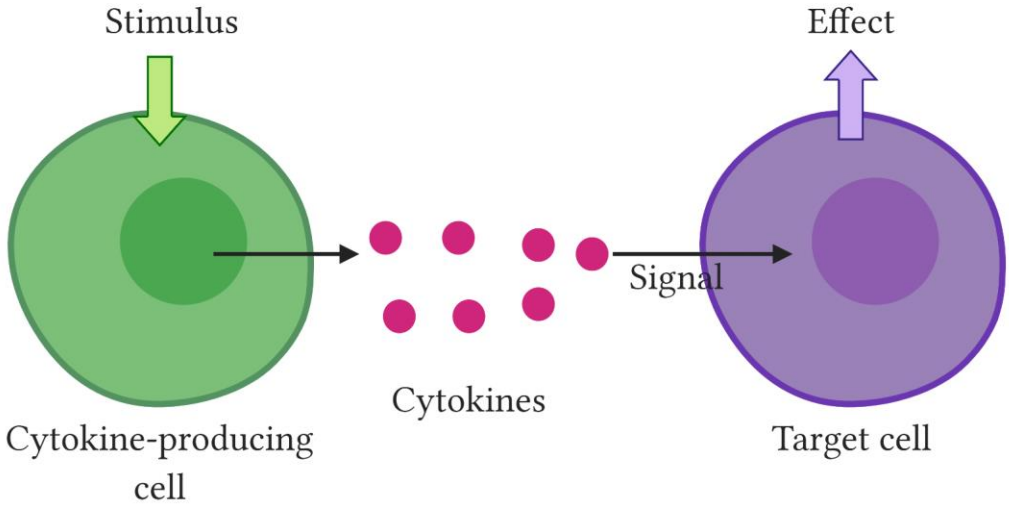

Figure 1 – Cytokine function. This illustration displays the role of cytokines as cell signaling molecules. A stimulus activates a cytokine-producing cell to release cytokines into the interstitium. Cytokines attach to a receptor site of a target cell to induce signal transduction in that cell. That signal is translated by the cell into an extra-cellular effect.

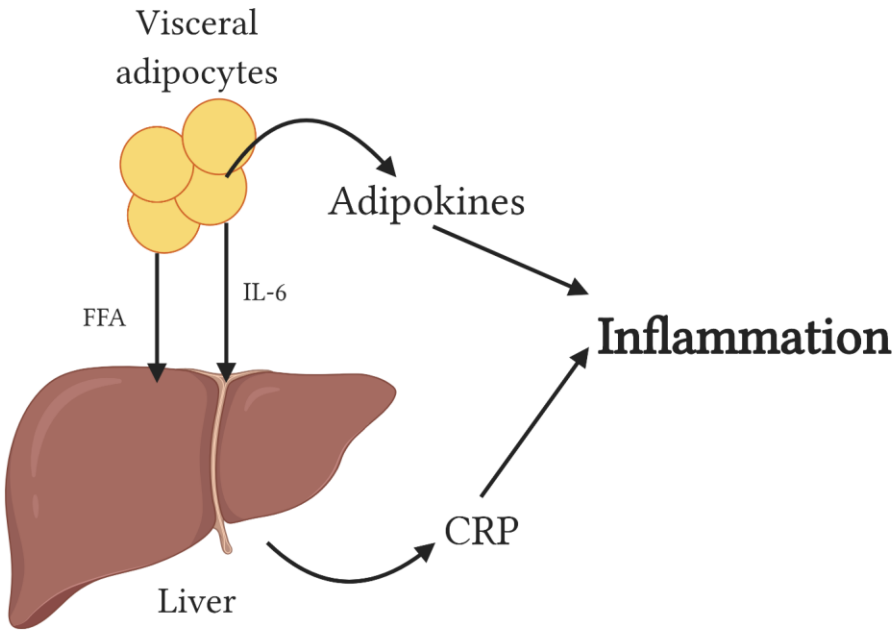

Figure 2 – Adipokines and CRP as drivers of inflammation. Visceral adipocytes release freely circulating fatty acids (FFA) and interleukin-6 (IL-6). FFAs and IL-6 travel to the liver. Excess hepatic metabolism of FFAs and IL-6 results in excessive production of C-reactive protein (CRP). Simultaneously, visceral adipocytes release adipose-tissue cytokines (adipokines) including, but not limited to, leptin, adiponectin, and IL-6. Adipokines and CRP both serve to over-activate the immune system and produce an inflammatory response. Note that vitamin D status is inversely correlated with IL-6, CRP, and visceral adiposity generally. Furthermore, NAFLD is positively correlated with inflammation.

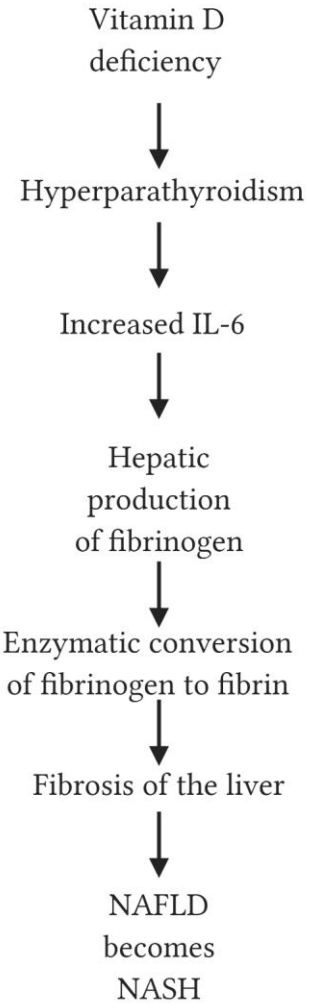

Figure 3 – Causal flow from Hypovitaminosis D to NASH. This outlines the logical flow of how vitamin D deficiency leads to elevated parathyroid hormone (PTH) production (secondary hyperparathyroidism) which can ultimately result in the progression of non-alcoholic fatty liver disease (NAFLD) into non-alcoholic steatohepatitis (NASH). Note that increased IL-6 is the pivotal point leading to hepatic fibrinogen formation and the causal cascade to NASH progression. IL-6 is also an adipokine as seen in **Figure 2**. This makes the relationships among vitamin D deficiency, adiposity, and NAFLD more robust.

REFERENCES

1. Defining Adult Overweight and Obesity. https://www.cdc.gov/obesity/adult/defining.html. Accessed November 3, 2019.
2. Defining Childhood Obesity. https://www.cdc.gov/obesity/childhood/defining.html. Accessed November 3, 2019.
3. Craig M. Hales MD, Margaret D. Carroll MSPH, Cheryl D. Fryar MSPH, Cynthia L. Ogden PD. NCHS Data Brief No. 288 - Prevalence of Obesity Among Adults and Youth: United States, 2015–2016. In: CDC, edOctober 2017.
4. Fabbrini E, Sullivan S, Klein S. Obesity and Nonalcoholic Fatty Liver Disease: Biochemical, Metabolic and Clinical Implications. *Hepatology.* 2010;51(2):679-689.
5. Dharmalingam M, Yamasandhi PG. Nonalcoholic Fatty Liver Disease and Type 2 Diabetes Mellitus. *Indian Journal of Endocrinology and Metabolism.* 2018;22(3):421-428.
6. National Diabetes Statistics Report, 2017 Estimates of Diabetes and Its Burden in the United States. In: Control CfD, ed2017.
7. Lipina C, Hundal HS. Sphingolipids: agents provocateurs in the pathogenesis of insulin resistance. *Diabetologia.* 2011;54:1596-1607.
8. Plessis Jd, Pelt Jv, Korf H, et al. Association of Adipose Tissue Inflammation With Histologic Severity of Nonalcoholic Fatty Liver Disease. *Gastroenterology.* 2015;149(3):635-648.
9. Nonalcoholic Fatty Liver Disease. https://liverfoundation.org/for-patients/about-the-liver/diseases-of-the-liver/non-alcoholic-fatty-liver-disease/. Accessed November 3, 2019.
10. #14 – Robert Lustig, M.D., M.S.L.: fructose, processed food, NAFLD, and changing the food system [Internet]; 2018 September 10, 2018. Podcast. Available from: https://peterattiamd.com/roblustig/
11. Calzadilla Bertot L, Adams LA. The Natural Course of Non-Alcoholic Fatty Liver Disease. *International journal of molecular sciences.* 2016;17(5).
12. Targher G, Bertolini L, Scala L, et al. Associations between serum 25-hydroxyvitamin D3 concentrations and liver histology in patients with non-alcoholic fatty liver disease. *Nutr Metab Cardiovasc Dis.* 2007;17(7):517-524.
13. Wartsman J, Matsuoka LY, Chen TC, Lu Z, Holick MF. Decreased bioavailability of vitamin D in obesity. *American Journal of Clinical Nutrition.* 2000;72:690-693.
14. Anne C. Looker PD, Clifford L. Johnson MPH, David A. Lacher MD, Christine M. Pfeiffer PD, Rosemary L. Schleicher PD, Christopher P. Sempos PD. Vitamin D Status: United States, 2001–2006. In: Statistics NCfH, ed2011.
15. Adams LA, Angulo P. Recent concepts in non-alcoholic fatty liver disease. *Diabetic Medicine.* 2005;22:1129-1133.
16. Eliades M, Spyrou E, Agrawal N, et al. Meta-analysis: vitamin D and non-alcoholic fatty liver disease. *Aliment Pharmacol Ther.* 2013;38(3):246-254.
17. Christakos S, Ajibade DV, Dhawan P, Fechner AJ, Mady LJ. Vitamin D: metabolism. *Endocrinology and metabolism clinics of North America.* 2010;39(2):243-253, table of contents.

18. Zhang JM, An J. Cytokines, inflammation, and pain. *Int Anesthesiol Clin.* 2007;45(2):27-37.
19. Veldman CM, Cantorna MT, DeLuca HF. Expression of 1,25-dihydroxyvitamin D(3) receptor in the immune system. *Arch Biochem Biophys.* 2000;374(2):334-338.
20. Willheim M, Thien R, Schrattbauer K, et al. Regulatory Effects of 1Alpha,25-Dihydroxyvitamin D3 on the Cytokine Production of Human Peripheral Blood Lymphocytes. *The Journal of Clinical Endocrinology & Metabolism.* 1999;84(10).
21. Hotamisligil GS, Arner P, Caro JF, Atkinson RL, Spiegelman BM. Increased adipose tissue expression of tumor necrosis factor-alpha in human obesity and insulin resistance. *The Journal of Clinical Investigation.* 1995;95(5):2409-2415.
22. Yang W-S, Lee W-J, Funahashi T, et al. Weight Reduction Increases Plasma Levels of an Adipose-Derived Anti-Inflammatory Protein, Adiponectin. *The Journal of Clinical Endocrinology & Metabolism.* 2001;86(8):3815-3819.
23. Jung SH, Park HS, Kim K-S, et al. Effect of weight loss on some serum cytokines in human obesity: increase in IL-10 after weight loss. *The Journal of Nutritional Biochemistry.* 2008;19(6):371-375.
24. Timms PM, Mannan N, Hitman GA, et al. Circulating MMP9, vitamin D and variation in the TIMP-1 response with VDR genotype: mechanisms for inflammatory damage in chronic disorders? *An International Journal of Medicine.* 2002;95:787-796.
25. Manicone AM, McGuire JK. Matrix metalloproteinases as modulators of inflammation. *Seminars in cell & developmental biology.* 2008;19(1):34-41.
26. Yamaguchi K, Itoh Y, Yokomizo C, et al. Blockade of interleukin-6 signaling enhances hepatic steatosis but improves liver injury in methionine choline-deficient diet-fed mice. *Nature.* 2010;90:1169-1178.
27. Asrih M, Jornayvaz FR. Inflammation as a potential link between nonalcoholic fatty liver disease and insulin resistance. *Journal of Endocrinology.* 2013;218(3):R25-R36.
28. Zhou Y-J, Li Y-Y, Nie Y-Q, et al. Influence of polygenetic polymorphisms on the susceptibility to non-alcoholic fatty liver disease ofChinese people. *Journal of Gastroenterology and Hepatology.* 2009;25(4):772-777.
29. McCarty MF. Secondary hyperparathyroidism promotes the acute phase response -- a rationale for supplemental vitamin D in prevention of vascular events in the elderly. *Med Hypotheses.* 2005;64(5):1022-1026.
30. McCullough AJ. The clinical features, diagnosis and natural history of nonalcoholic fatty liver disease. *Clin Liver Dis.* 2004;8(3):521-533, viii.
31. Angulo P. NONALCOHOLIC FATTY LIVER DISEASE. *New England Journal of Medicine.* 2002;36(16):1221-1231.
32. Davis JE, Cain J, Small C, Hales DB. Therapeutic effect of flax-based diets on fatty liver in aged laying hens. *Poult Sci.* 2016;95(11):2624-2632.
33. Zaefarian F, Abdollahi MR, Cowieson A, Ravindran V. Avian Liver: The Forgotten Organ. *Animals.* 2019;9(2):63.
34. Stevens L. *Avian Biochemistry and Molecular Biology.* 40 West 20th Street, New York NY 10011-4211: Cambridge University Press; 2004.
35. Takahashi Y, Soejima Y, Fukusato T. Animal models of nonalcoholic fatty liver disease/nonalcoholic steatohepatitis. *World J Gastroenterol.* 2012;18(19):2300-2308.

36. Mirmiran P, Amirhamidi Z, Ejtahed H-S, Bahadoran Z, Azizi F. Relationship between Diet and Non-alcoholic Fatty Liver Disease: A Review Article. *Iranian Journal of Public Health.* 2017;46(8):1007-1017.

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