2017

Vitamin D to reduce liver fibrosis in non-alcoholic fatty liver disease

BOSTON UNIVERSITY

SCHOOL OF MEDICINE

Thesis

VITAMIN D TO REDUCE LIVER FIBROSIS IN NON-ALCOHOLIC FATTY LIVER DISEASE

by

RYAN FOX

B.S., Colorado State University, 2013

Submitted in partial fulfillment of the

requirements for the degree of

Master of Science

2017

Approved by

First Reader _____

Michelle T Long, M.D.
Assistant Professor of Medicine

Second Reader _____

Oren Berkowitz, Ph.D., PA-C
Assistant Professor of Medicine

ACKNOWLEDGMENTS

I would like to thank my advisor Dr. Michelle Long for her help in both guiding me toward and developing an exciting and interesting topic of research. I would also like to thank Dr. Oren Berkowitz for sharing his knowledge in the field of research with us in the classroom and personalized meetings. I would also thank my family for providing encouragement and support during my time spent on this endeavor.

VITAMIN D TO REDUCE LIVER FIBROSIS IN NON-ALCOHOLIC FATTY LIVER DISEASE

RYAN FOX

ABSTRACT

Background

As the prevalence of metabolic risk factors in the American population has increased over time, so too has the diagnoses of non-alcoholic fatty liver disease (NAFLD). Within this spectrum of disease lies the potential for silent progression towards cirrhosis, leaving the patient with few options for treatment. Currently, the standard of care remains counseling on diet and exercise with the goal of reversing disease progression by addressing the underlying risk factors.

Literature Review

Recent studies have shown that a correlation exists between low levels of serum 25-hydroxyvitamin D and hepatic injury from NAFLD. This has become an active area of research, due in part to the anti-inflammatory and immunoregulatory properties of vitamin D. The prospect of a simple and cost effective intervention that can exert its effects on the mechanisms behind the development of NAFLD is interesting and warrants further research.

Proposed Project

This proposal is for a double-blind, randomized, experimental study of vitamin D3 (cholecalciferol) versus placebo in a patient population of those with both clinically

proven NAFLD and concomitant vitamin D deficiency. Liver fibrosis will be measured and staged with the use of FibroScan elastography. The statistical analysis thereafter will determine if a clinically significant reduction in hepatic fibrosis exists, compared with the results of the placebo group.

Conclusions/Significance

Should vitamin D prove to be an effective treatment option in reversing the progression of NAFLD, clinicians would be equipped with a simple and safe tool to augment their management of the patient. For those that experience barriers (i.e. lower socioeconomic status, other comorbidities, etc.) preventing them from improving diet and exercise, vitamin D would serve as an alternative therapy to aid in reducing their disease burden. Easier methods to treat their disease now projects improved quality of life years later.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF ABBREVIATIONS

ALT……..…….Alanine aminotransferase

BU ..... Boston University

DHA……Docosahexanoic Acid

FFA……...Free Fatty Acid

NAFLD ..... Non-Alcoholic Fatty Liver Disease

NASH..... Non-Alcoholic Steatohepatitis

VDD……...Vitamin D Deficiency

INTRODUCTION

Background

Non-alcoholic fatty liver disease (NAFLD) is a term used to refer to a spectrum of liver disease that ranges from the relatively benign simple hepatic steatosis to the far more severe and life threatening cirrhosis. As the name suggests, this condition is not a result of alcohol consumption, but rather heavily associated with many of the same risk factors that comprise metabolic syndrome such as obesity, dyslipidemia, and elevated blood glucose. In fact, the literature commonly refers to NAFLD as the hepatic manifestation of metabolic syndrome. NAFLD is a major public health concern as it is the current leading cause of chronic liver disease in both adult and children in Western populations[1]. At its core, NAFLD is the accumulation of excess triglycerides within the hepatocyte. This occurs through various mechanisms such as lipolysis of dietary sources, adipose tissue, and lipogenesis within the body[2].

Recently, concomitant Vitamin D deficiency (VDD) in the setting of NAFLD has received more attention, with the association between the two being firmly established in various studies[3-5]. Recent studies have elucidated the expanded role Vitamin D plays in the human body outside of bone and calcium homeostasis, including exerting effects in inflammatory and immune pathways[6]. Such pathways have been shown to be involved in the pathogenesis of NAFLD and are currently a focus of research as a potential therapeutic target for the use of vitamin D supplementation.

Statement of the Problem

The existence of VDD in patients with NAFLD compounds their problems, predisposing them not only to issues surrounding bone and calcium homeostasis, but also exacerbated hepatic steatosis, necroinflammation, and fibrosis, all of which expedite the disease course towards cirrhosis[2]. While the association between VDD and NAFLD has been firmly established, the number of studies that have aimed to assess its therapeutic potential in humans is quite limited. According to the US Department of Health and Human Services, currently no pharmacologic treatment exists for NAFLD and the standard of care is to counsel the patient on leading a healthy lifestyle, including changes in diet and exercise[7]. Should the liver disease progress to the point of cirrhosis the only treatment option available is transplantation. Beyond the risks associated with transplant surgery, it is estimated that the list of adults awaiting a healthy liver has almost tripled since 2004, thus demand is a significant issue as well[8]. This sets the stage for research into alternative methods such as Vitamin D supplementation that could be used to delay or even reverse NAFLD progression.

Hypothesis

Supplementation with Vitamin D3 (25(OH)D3) in patients with Non-Alcoholic Fatty Liver Disease and concomitant Vitamin D Deficiency will provide a significant reduction in hepatic fibrosis.

Objectives and specific aims

The aim of this study is to evaluate the efficacy of vitamin D supplementation as a treatment option that would aid in the reduction of hepatic fibrosis and steatosis in patients with NAFLD. Rigorous lifestyle changes involving diet and exercise are often a challenge for patients to maintain, and while this would not fully replace such interventions, it would provide both the healthcare provider and the patient another tool to aid in the fight against their disease progression. Vitamin D is cost-effective, simple, and safe, making it an attractive option should it prove efficacious.

Specific aims:

1. To quantify any potential reduction of hepatic fibrosis as a result of Vitamin D supplementation
2. To quantify any potential reduction of hepatic steatosis as a result of Vitamin D supplementation

REVIEW OF THE LITERATURE

Overview

An overwhelming proportion of today's society has grown increasingly comfortable with a sedentary lifestyle; the conveniences that new technology, economically friendly fast food, and the ever-growing sources of entertainment are difficult to deny. An unfortunate result of all this is seen in the large prevalence of metabolic syndrome. Metabolic syndrome is characterized by central obesity, insulin resistance, elevated fasting blood glucose, hypertension, dyslipidemia, and poor cholesterol[9]. Many of these features are also seen in non-alcoholic fatty liver disease (NAFLD) and thus it is often referred to as the hepatic manifestation of metabolic syndrome. It is reported that worldwide there is approximately a 20% prevalence of NAFLD in the general population; however, Western countries are at an increased risk due to the staggering 34% of United States citizens that are estimated to exhibit metabolic syndrome[9]. As a consequence, reports estimate that the incidence of NAFLD in the US ranges from 10% to 30%[10].

The term NAFLD does not refer to one particular disease itself, but rather a spectrum of diseases that ranges from simple hepatic steatosis, to non-alcoholic steatohepatitis (NASH), to the most severe outcome known as cirrhosis. In simple steatosis, liver fat is present but there is no hepatocellular injury, while in NASH there is both liver fat and hepatocellular injury present and it may or may not include fibrosis[11]. Due to the close relationship between metabolic syndrome and NAFLD, many of the risk factors overlap. The most prevalent instigators are obesity, type 2 diabetes mellitus, and hypertriglyceridemia resulting in 60-95%, 28-55%, and 27-92% of NAFLD cases

respectively[12]. Fat distribution is often a greater predictor of morbidity than is body mass index (BMI), and NAFLD is no exception, with visceral fat being associated with a greater degree of inflammation and fibrosis[13]. That being said, 30% to 37% of obese individuals exhibit simple hepatic steatosis and exerts an additive effect when combined with other predisposing factors to NAFLD[14]. Type 2 diabetes is not only often seen in conjunction with NAFLD, but is also thought to increase the progression of liver disease[14]. In fact, a recent study showed that of 3,861 patients with Type 2 diabetes, 1,751 of them also had NAFLD[15].

In addition to metabolic risk factors, age, gender, and ethnicity have also been reported to increase the likelihood of developing NAFLD. Studies that divided the population by age ranges showed that with increasing age there is a greater prevalence of NAFLD and fibrosis, though this can partly be attributed to the increased comorbidities of the elderly that serve as risk factors, as well as the longer duration of disease[14]. Previously thought to be more common in women, NAFLD is found to be more common in men with one study showing a 31% and 16% prevalence on routine medical check-up in men and women respectively[16]. In terms of ethnicity, Hispanics exhibit the highest prevalence, followed by Caucasians, and with lowest prevalence in African Americans. The research behind these relationships is ongoing, but one study noted that the differing rates between Hispanic and African Americans could potentially be attributed to fructose malabsorption in the latter, providing a protective effect against liver steatosis[17].

Understanding of the pathogenesis of NAFLD has undergone multiple evolutions throughout the past and is still a subject of debate. This is largely due to the many

contributing factors that play a role in the disease progression, as opposed to one isolated mechanism. Originally the accepted idea was a "two hit" hypothesis in which the first hit was when the liver parenchyma became sensitized by hepatic steatosis. This then enabled a metabolic stressor to act as the second hit which triggered inflammation, steatonecrosis, and fibrosis that characterize the disease[18]. Currently it is believed that this theory is an oversimplification and as previously mentioned, many various mechanisms all play a role in what is now termed the "multiple-hit" hypothesis.

While many contributing mechanisms play a part in the complex pathophysiology of NAFLD, the development of insulin resistance and hepatic inflammation are the most pertinent for the scope of this paper in regards to the role Vitamin D plays. The core issue in NAFLD is hepatic steatosis which is believed to occur as a result of free fatty acids (FFAs) liberated via lipolysis of adipocytes, as well as lipids ingested in the diet, lipogenesis within the body, and lipid disposal impairment[4]. As a consequence of this excess buildup of FFAs, defects arise in insulin signaling as well as in glucose metabolism within cells. This in turn leads to elevated blood glucose levels which elicits an increase in lipogenesis via an increase in activation of both sterol regulatory element binding proteins (SREBPs) and carbohydrate response element binding proteins (CHREBPs)[4]. As this vicious circle continues, the degree of both hepatic and adipocyte insulin resistance as well as free fatty acid accumulation becomes more severe and thus propels the progression of NAFLD.

One study found that subjects with NAFLD compounded the excess FFAs with a decreased ability to inhibit their oxidation, thus increasing their susceptibility to further

hepatic steatosis[19]. Utzschnider and Kahn, 2006 summed up this complex physiology concisely by stating that "diminished insulin responsiveness at the level of the adipocyte may contribute to hepatic steatosis by excess FFA flux to the liver"[19]. It is worth mentioning that this mechanism of insulin resistance occurs as one aspect of broader factors such as dietary habits, genetic, and environmental factors.

For the individuals in which hepatic steatosis progresses towards NASH, chronic inflammation, due in part to insulin resistance, is the primary driving force behind it. Fortunately this occurs in only 10% to 20% of those with fatty liver, but the dire consequences that become apparent as the inflammation progresses warrants a thorough understanding of what causes it. The reason behind why some patients' steatosis progresses and others do not is not known, and it is worth mentioning that inflammation does not always occur after liver fat deposition.

Adipose tissue has come to be regarded as a significant source of metabolic and immune related activity in the body, producing various cytokines that act on other organs. One such example that is thought to be highly involved in NAFLD is adiponectin. This adipocytokine plays a protective role against inflammation and is found to be reduced in obese individuals and thus in a large portion of those with NAFLD[20]. The deletion of Sirtuin 1, one of the two receptors in the liver which it acts through, has been shown to lead to both hepatic steatosis and inflammation[21]. A study done with obese mice in which the isoform for adiponectin was overexpressed showed a reversal of the diabetic phenotype and normalization of blood glucose and insulin levels, leading to a reduction

in both insulin resistance and liver fat[20]. In addition to adiponectin, leptin, resistin, and TNF-α have also been implicated in NAFLD development.

Vitamin D is a fat soluble secosteroid hormone that is well known for its involvement in skeletal and calcium homeostasis. Though humans can derive it from dietary sources such as oily fish (tuna, salmon, mackerel) or egg yolks, there exist few foods that naturally contain it. To combat this, many foods in the US are fortified with Vitamin D to supplement the American diet. For example, in the 1930s rickets was a major public health concern so in response, a milk fortification program was established and is still in place today[22]. Barring supplements, humans get the majority of their Vitamin D through sunlight exposure, though various factors impact to what degree this occurs such as time of day, season, and melanin content in the skin.

Recently, new light has been shed upon the role Vitamin D plays in the human body, particularly concerning the relationship between its serum levels and diabetes and cardiovascular disease. Due to the close relationship NAFLD has with such conditions, researchers were prompted to investigate if Vitamin D levels had any contribution in its pathophysiology. Inflammation and oxidative stress serve as the common ground between Vitamin D deficiency (VDD) and NAFLD.

The liver is one of the primary sites for vitamin D metabolism from both the skin and diet, responsible for the hydroxylation of its inactive form into 25-hydroxyvitamin D [25(OH)D] via the enzyme 25-hydroxylase. From the liver, 25(OH)D travels to the kidneys where 1α-hydroxylase converts it to the biologically active form 1α,25-dihydroxyvitamin D [1,25(OH)2D][23]. Vitamin D receptors (VDRs) are expressed

abundantly throughout the body and they are the means by which Vitamin D exerts its biological effects. Examples of where VDR can be found include immune cells, adipocytes, non-parenchymal hepatocytes, β cells of the pancreas, and skin tissue[6]. While the exact mechanisms are not yet fully understood, it is thought that through the VDR constitutively expressed on macrophages and lipopolysaccharide-stimulated macrophages that Vitamin D is able to function as an anti-inflammatory agent[24]. 1,25(OH)2D3 upregulates the inhibitor of nuclear factor NF-κB, a key player in the pro-inflammatory signaling pathway, through increasing the stability of mRNA and reducing phosphorylation of NF-κB[24].

Vitamin D deficiency (VDD) has long been thought to almost exclusively affect the elderly and hospitalized patients, but its prevalence outside such demographics has slowly been increasing over the past years. Regarding NAFLD, researchers have shown that there exists an association with VDD. One study found that of 6,800 patients from the National Health and Nutrition Examination Survey (NHANES III) database, 308 exhibited an elevation in alanine aminotransferase (ALT) that could not be explained. When the Vitamin D levels of these patients were compared with those of the 979 matched controls, it was found that the NHANES III patients had lower levels, despite metabolic syndrome, insulin resistance (IR), and triglycerides being controlled for[25]. Another study further illustrated this when they found that the association between VDD and NAFLD maintained despite age, gender, IR, and triglycerides[26]. Finally, in a study done by Targher et al., NAFLD patients were again shown to have markedly lower levels

of serum 25(OH)D, but in addition they evaluated liver histopathologic features and found there to be a linear relationship[27].

The clinical course varies depending on the stage of NAFLD the patient has and is broadly divided into two categories: non-NASH and NASH. In simple hepatic steatosis (or non-NASH) the disease progression, if any, is normally very slow and mostly benign[11]. Conversely, in NASH there can be histological progression that may ultimately lead to cirrhosis[14]. 5% to 20% of patients with simple steatosis will progress to NASH, 10% to 20% of those will go on to develop more severe fibrosis, and <5% of those end up with cirrhosis[28]. In terms of mortality, a community based report of NAFLD patients in Minnesota looked at a mean follow-up of 7.6 years and observed 13% mortality, with higher mortality rates associated with NASH[29]. For patients that have progressed to the point of advanced fibrosis and cirrhosis, development of hepatocellular carcinoma (HCC) becomes a risk[11]. Fortunately, disease progression in NAFLD is reversible.

Treatment for NAFLD requires addressing not only the liver disease itself, but the other metabolic comorbidities that are often present as well, such as obesity, Type 2 Diabetes Mellitus, and hypertriglyceridemia. Fortunately in simple hepatic steatosis there is little, if any, liver disease present so the primary focus for treatment becomes lifestyle interventions. Currently, no pharmaceutical therapy exists that is used to specifically treat NAFLD and the most effective treatment involves adhering to strict lifestyle changes as well as maintained weight loss. The recommendations set forth by the American Gastroenterological Association state that a reduction in hepatic steatosis generally follows weight loss of at least 3% to 5% of total body weight; however, a

weight loss of up to 10% of body weight may be necessary in order to improve necroinflammation[11]. The effect of solely exercise in patients with NAFLD is not as well established but it is thought that an improvement can be seen in hepatic steatosis, though any effect on liver histology is unknown.

In addition to lifestyle interventions, various other therapies are being explored that revolve around treating the comorbidities. Vitamin E's anti-oxidant properties allow it to work against oxidative stress that contributes towards hepatocellular injury in patients with NASH[2]. However, high-dosage interventions have been linked to both increased mortality as well as hemorrhagic stroke and prostate cancer, though this is still a topic of debate[30]. Another treatment option for patients with biopsy-proven NASH is Pioglitazone; however, the long term safety of thiazolidinediones is still an active area of research. For this reason, Pioglitazone's use is heavily restricted in the United States and is no longer available in Europe[11]. With a large proportion of NAFLD patients being obese, bariatric surgery has become a viable option to help reverse their disease progression. Various studies have shown a decrease in hepatic steatosis as well as fibrosis in patients who underwent bariatric surgery[28]. One meta-analysis showed an improvement or complete resolution of steatosis, steatohepatitis, and fibrosis following bariatric surgery, but due to a lack of randomized clinical trials a definitive recommendation cannot be made[31].

Existing research

The concept that Vitamin D could have a potential therapeutic impact on the disease progression of NAFLD is fairly novel, but several studies have been done that have sought to explore the possibility. Evidence has been published involving both human and animal subjects and while there are some conflicting results, it remains to be seen if Vitamin D can ameliorate NAFLD over an extended time interval. The Farnesoid X Receptor Ligand Obeticholic Acid in NASH Treatment (FLINT) trial published in 2014 established 72 weeks as the time period to monitor the liver for changes in fibrosis[32].

One of the hallmark studies that helped lay the foundation for future research into this topic was a rat study performed by Nakano et al. in 2011[33]. The stimulus that prompted research into this area was the lack of both quality and availability of liver transplants, in addition to their increasing need as NASH grew in prevalence. Phototherapy as well as Vitamin D3 supplementation was used as a means of increasing serum Vitamin D3 levels in an attempt to slow down disease progression in NASH. Following a 12 week diet that resulted in 36 rats with livers that exhibited steatosis, NASH, and fibrosis, they were divided into four groups: 6 or 12 weeks of phototherapy and 6 or 12 weeks as control. Phototherapy was likely chosen as the means for supplementation as that is the way in which the vast majority of the world population obtains Vitamin D and VDD is very much a global health issue. However, in addition to sunlight exposure the rats were also administered oral 1α-hydroxy-cholecalciferol (1α(OH)D3) three times per week for six weeks in order to better examine effects of Vitamin D.

The significant finding that came out of this study was that phototherapy proved to be efficacious in delaying the progress of NASH, but it did not reduce hepatic steatosis. This showed that the anti-inflammatory and anti-fibrotic activity possessed by Vitamin D was able to ameliorate disease progression in NASH. They also referenced other beneficial effects of Vitamin D that may have contributed to this finding, including improvement in insulin sensitivity and its anti-apoptotic effect on hepatocytes, other key mechanisms involved in NAFLD pathophysiology.

The association of VDD with fibrosis that develops in NAFLD served as a foundation for a study that hypothesized vitamin D supplementation in addition to docosahexanoic acid (DHA) would be efficacious in treating a pediatric cohort. The rationale cited in the study discusses the role VDD in inflammation and oxidative stress, consequently predisposing the individual to hepatic fibrosis. DHA, a type of omega-3 fatty acid has been shown to aid in the reduction of hepatic steatosis in NAFLD patients[34].

The study design was a randomized control trial with two arms, one administered 800 IU of vitamin D and 500 mg of DHA and the other a placebo. Intervention took place over the course of 24 weeks. The primary outcome for the authors' hypothesis was improvement in liver histology, determined by comparing a baseline biopsy for each of the 20 subjects in the VDD/DHA group to one at the conclusion of the study. Overall, the NAFLD Activity Score (NAS), a summation of liver histology marker scores, improved from 5.40 to 1.92. Significant improvements were observed in steatosis (2.25 to 1), ballooning (1.6 to 0.46), lobular inflammation (1.5 to 0.88), and portal

inflammation (1.6 to 1.0)[34]. However, despite the modest improvement in fibrosis (2.0 to 1.5), it failed to be statistically significant. Consistent with the research the study was founded upon, lower levels of 25-hydroxyvitamin D were associated with patients exhibiting greater levels of steatosis and fibrosis[34]. From their data, the researchers were able to determine that vitamin D aided in the reduction of hepatic stellate cell and myofibroblast activation, both of which contribute toward development of liver fibrosis[34].

Regarding lab values such as triglyceride and ALT related to metabolic syndrome that are so commonly associated with NAFLD, the authors came to an interesting conclusion. The treatment group exhibited a decline in these values, as well as insulin resistance, but the data was compared to a past trial they performed. Said trial examined the role of DHA in NAFLD when compared to vitamin D in a pediatric population in regards to their effect on the metabolic profile. It was revealed that no significant difference existed between the vitamin D and DHA group, thus in this trial, the improvement of triglycerides, ALT, and insulin resistance is attributed to only DHA with likely no contribution from vitamin D[34].

This study helps promote vitamin D as a therapeutic option to treat NAFLD but certain limitations exist that necessitate further research. The pediatric focus of the study helps establish a greater font of knowledge for the growing population of children with NAFLD, but unfortunately makes it difficult to translate the authors' findings to an adult population. For ethical reasons it is understandable that biopsies only were performed on the treatment group, though having samples from the control group would help better characterize the true value of vitamin D and DHA in NAFLD. Lastly, there were no

patients included in the study that had cirrhosis or bridging fibrosis, so the results can only be applied to those with mild to moderate NAFLD.

A recent cross-sectional analysis performed in 2016 was published in the American Journal of Gastroenterology that approached the relationship between Vitamin D and NAFLD from a slightly different angle. Through their research they explored if the Vitamin D levels in the subjects correlated with NAFLD severity, using degree of hepatic steatosis, lobular inflammation, ballooning hepatocytes, and stage of fibrosis as their primary outcomes. 244 adults with biopsy-proven NAFLD comprised one cohort while 39 controls made up the other, a respectable sample size for the former that lends credibility to the study. Consistent with previous studies, vitamin D levels were found to be significantly different based on the individual's weight, with lower serum levels correlated with increasing BMI[35]. However, the histological analysis failed to find any significant relationship between vitamin D levels and the primary outcomes[35]. Interestingly, vitamin D levels actually increased with stage of fibrosis, shown in Table 1.

Table 1: Fibrosis and VitD

Fibrosis Stage	25-OH Vitamin D level
0	24.4±4.4
1	26.5±8.9
2	29.1±12.5
3	30.7±4.1
4	20.2±20.2

While there was found to be a lower vitamin D level in patients with stage IV fibrosis, the number of patients with that level of disease severity was too small to be statistically significant[35].

One strength of this study was the attention paid to the control cohort, as the researchers ensured that the subjects included were similar to the NAFLD group in as many ways possible. This includes comparable gender, age, and BMI, as well as presence of vitamin D deficiency and risk factors for metabolic syndrome[35]. Combined with the large and diverse sample size, the results can be well generalized. Regarding weaknesses, vitamin D supplementation by subjects prior to the study was difficult to quantify due to the retrospective nature of patient selection.

METHODS

Study design

The proposed study will be a double-blind, placebo-controlled randomized experimental study comparing two treatment arms, vitamin D supplementation and a placebo.

Study population and sampling

The study population for this trial will consist of consenting patients age between 18-70 with a formal diagnosis of NAFLD as well as vitamin D deficiency. Initial NAFLD diagnosis will be defined according to the histological parameters established by the NASH Clinical Criteria Network, which includes hepatic steatosis >5%, presence of necroinflammation, ballooned hepatocytes, or hepatic fibrosis[36]. Diagnosis for Vitamin D deficiency will be defined as any serum value <30 ng/ml. Exclusion criteria will include consumption of alcohol that exceeds 14 or more servings per week for men or 7 or more servings per week for women, serological evidence of other forms of hepatic disease (i.e. Wilson's disease, hereditary hemochromatosis, chronic viral hepatitis, α1-antitrypsin deficiency), or any histological evidence of potential co-existing hepatic disease. In addition, patients who have reported consumption of vitamin D supplements or medications with hepatotoxic side effects within the last 6 months will also be excluded. There will be no exclusions for patients based on gender, ethnicity, or metabolic risk factors. Sample size appropriate for this study will be approximately 115 patients in each group for a total of 230 participants, using 80% power and an alpha of 0.05 to detect a decrease of 1 stage of fibrosis on FibroScan[37].

Treatment

The intervention in this study will consist of treatment with either vitamin D supplementation or a placebo. Subjects in the Vitamin D supplementation treatment arm will be administered 10,000 IU of vitamin D3 (cholecalciferol) daily, while subjects in the placebo arm will be administered a pill that is similar in size, color, shape, and package. These interventions will take place over the course of 16 months, as per the standard set forth in the FLINT trial for accurately assessing change in hepatic fibrosis[32]. Subjects will be both randomized and blinded into which intervention they receive and they will receive instruction as to how to take the pill each day. Researchers will be blinded to which medication they are administering and will assess compliance based on number of pills remaining every 2 months. Researchers will also inquire about any side effects at that time.

Study variables and measures

The independent variable of this study will be the administered medication, whether it be vitamin D or placebo. The dependent variable that will be analyzed is the stage of fibrosis determined by FibroScan. FibroScan is an imaging modality used to perform ultrasound-based elastography, an FDA approved non-invasive technique that serves as a tool to assess for hepatic fibrosis. Stage of fibrosis will be gauged using the assigned values of F≤1=mild, F≥2=moderate, F≥3=severe, and F4=cirrhosis[38]. Scans will be performed at intervals throughout the study: once prior to the start of the study to establish a baseline for each patient, followed by one every 4 months until the conclusion of study. Comparisons will be made between the patient's baseline score and the

subsequent scans. The primary end point of the study will be to determine if supplementation with vitamin D helped to ameliorate the degree of fibrosis from the baseline after a 16 month intervention period.

Recruitment

Subjects will be referred to this study by the liver specialists at Boston Medical Center who deem them to be within the outlined parameters for entry. This includes a formal diagnosis (i.e. liver biopsy, elastography, etc.) of NAFLD that exhibits mild to moderate fibrosis, as well as concomitant vitamin deficiency. Following referral, potential subjects will then be assessed by clinical members of the study to confirm that they fall within the designated criteria.

Data collection

Prior to the start of the study, subjects who agree to participate will undergo an initial FibroScan test and blood draw in order to determine baseline hepatic fibrosis and serum 25-hydroxyvitamin D level respectively. Following the initial tests, subjects admitted to the study will be randomized into either the vitamin D or placebo treatment arms and begin receiving their assigned medication protocol. Subjects will be instructed to take their medication once daily in the morning. This will last for a 16 month period, in which repeat FibroScans will be obtained at 4 months intervals throughout the study. In addition, subjects will be asked about any side effects or worsening symptoms at each interval to ensure there is no evidence of severe liver disease progression, hypercalcemia, or vitamin D toxicity.

Data analysis

To determine if and to what extent there was a decrease in the patient's stage of fibrosis following the treatment regimen, their baseline FibroScan will be compared to subsequent scans performed at 4 month intervals. To adjust for potential confounders, data will be stratified into categories based on the subject's age, gender, BMI, skin tone, and grade of hepatic fibrosis. Both standard deviation and mean will be calculated for each treatment group. A Chi-Square test will be used to compare the vitamin D supplementation group with the placebo group. To assess for variance, ANOVA will be used.

Timeline and resources

The timeline for this study will be a 16 month interventional period. Prior to beginning the study, time will need to be allowed for recruiting potential subjects, researchers, planning and approval by the IRB. Considering these requirements, it is reasonable to approximate the start of this study to be in April of 2017. Accounting for the number of subjects involved and the relatively limited access to FibroScan machines, start date for subjects will likely need to be staggered. Conclusion of the study will consequently be August of 2018. FibroScans will be performed at 4 month intervals through the conclusion of the study, which necessitates access to multiple FibroScan machines at such times in order to efficiently handle the patient load.

Research staff will consist of one primary investigator whose job it will be to lead and coordinate the study, while 4-5 clinicians will be responsible for oversight of the patients in the time leading up to and throughout the 16 month period. The latter will

also be in charge of assessing the referrals from Boston Medical Center providers for potential admittance to the study. Representatives from FibroScan will be requested to be made available for any teaching necessary for the clinicians to feel comfortable with operating the machine if they are not already.

Institutional Review Board

The proposed study will require use of human subjects, consequently an application to the Institutional Review Board of Boston University Medical Center will be submitted. Upon acquisition of approval by the IRB, patients will then be recruited for the study.

CONCLUSION

Discussion

The potential for Vitamin D, a simple, affordable, easy to manage medication to aid in reducing fibrosis in those affected by NAFLD is certainly an exciting prospect. While this study will strive to fully elucidate any therapeutic benefit it may have, it does not come without its own weaknesses. One of Boston Medical Center's primary roles in the community is to serve as a "safety net" hospital, meaning that a vast majority of the patient population is of the lower socioeconomic demographic, insured by government programs such as MassHealth or Medicaid. Furthermore, such a population unfortunately does not always have access to healthcare and medications that others may take for granted. This culminates in a cohort that may not accurately reflect the exceedingly large population of individuals in the United States that have a diagnosis of NAFLD. However, what said population lacks in generalizability related to socioeconomic status, it gains in racial background. Boston Medical Center is in a unique position to have the privilege to provide care for a wide range of ethnicities, and consequently, outcomes of this study can be better generalized to those populations.

A weakness of this study can also be found in the technique used to assess stage of hepatic fibrosis. Neither patients nor the IRB would find multiple liver biopsies, the current gold standard for evaluating fibrosis, to be an acceptable method of assessment due to the myriad risks as well as the pain that accompany it. Thus, a non-invasive modality such as FibroScan is used alternatively. While ultrasound is a quick, easy, and

cost-effective method, it is limited by factors such as morbid obesity and hepatic inflammation[39].

Summary

NAFLD is a highly prevalent disease in the United States and around the globe that predisposes affected individuals to potentially severe health consequences. As NAFLD refers to a spectrum of disease burden that spans from benign hepatic steatosis to cirrhosis requiring liver transplantation, early intervention becomes paramount. There remains to be seen a treatment option targeted directly at reversing the disease process beyond the current standard of care: diet and exercise. Several studies performed in recent years have evolved from merely establishing a correlation between low serum 25-hydroxyvitamin D and NAFLD, to evaluating the efficacy of its anti-inflammatory properties when elevated in animals.

Much debate still exists over the relationship between decreased serum vitamin D and NAFLD severity. Should the hypothesis prove to be accurate, this study would add to the evidence supporting the use of vitamin D as a therapeutic option to reverse disease progression in patients with NAFLD. This study would also provide an opportunity to learn more about the efficacy of FibroScan in regards to the assessment of disease progression specifically in NAFLD. Currently, it is gaining traction in the United States since earning FDA approval. Should it prove as a reliable and accurate imaging modality, its use may grow around the country, offering a fast and inexpensive test that can better characterize the state of a patient's liver.

Clinical and/or public health significance

The current state of healthcare in the United States has the potential to render treatment relatively inaccessible to a large number of people, often due to costly insurance premiums or medications. Vitamin D is a safe, affordable, and easy to use supplement that, if proven to aid in reversing hepatic fibrosis in patients with NAFLD, could be an excellent tool in the belt of healthcare providers responsible for their treatment. Sedentary lifestyle and unhealthy food options remain highly prevalent in the US, and as consequence a vast number of new NAFLD diagnoses is maintained each year. The patient may not notice the benefits of reduced fibrosis immediately, but the reversal of disease progression would spare them potentially severe health complications in later years. The goal of this study is not to establish vitamin D as the sole treatment for NAFLD, but rather as a therapy aimed at augmenting the progress achieved through lifestyle and dietary changes.

REFERENCES

1. Nelson JE, Roth CL, Wilson LA, et al. 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? *American Journal of Gastroenterology*. 2016;111(6):852-863.

2. Dongiovanni P, Lanti C, Riso P, Valenti L. Nutritional therapy for nonalcoholic fatty liver disease. *Journal of Nutritional Biochemistry*. 2016;29:1-11.

3. Seo JA, Eun CR, Cho H, et al. Low vitamin D status is associated with nonalcoholic Fatty liver disease independent of visceral obesity in Korean adults. *PLOS ONE*. 2013;8(10):e75197.

4. Kwok RM, Torres DM, Harrison SA. Vitamin D and nonalcoholic fatty liver disease (NAFLD): is it more than just an association? *Hepatology*. 2013;58(3):1166-1174.

5. Black LJ, Jacoby P, She Ping-Delfos WC, et al. Low serum 25-hydroxyvitamin D concentrations associate with non-alcoholic fatty liver disease in adolescents independent of adiposity. *Journal of Gastroenterology and Hepatology*. 2014;29(6):1215-1222.

6. Sharifi N, Amani R, Hajiani E, Cheraghian B. Does vitamin D improve liver enzymes, oxidative stress, and inflammatory biomarkers in adults with non-alcoholic fatty liver disease? A randomized clinical trial. *Endocrine*. 2014;47(1):70-80.

7. Naga Chalasani M, FACG; Zobair Younossi, MD, FACG; Joel E. Lavine, MD, PhD; Anna Mae Diehl, MD; Elizabeth M. Brunt, MD; Kenneth Cusi, MD; Michael Charlton, MD; Arun J. Sanyal, MD. 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. 2012; 7/16/2016. Available at: https://www.guideline.gov/summaries/summary/37629?

8. Satapathy SK, Sanyal AJ. Epidemiology and Natural History of Nonalcoholic Fatty Liver Disease. *Seminar in Liver Disease.* 2015;35(3):221-235.

9. Wainwright P, Byrne CD. Bidirectional Relationships and Disconnects between NAFLD and Features of the Metabolic Syndrome. *International Journal of Molecular Sciences.* 2016;17(3):367.

10. Younossi ZM, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. Global epidemiology of nonalcoholic fatty liver disease-Meta-analytic assessment of prevalence, incidence, and outcomes. *Hepatology.* 2016;64(1):73-84.

11. Chalasani N, Younossi Z, Lavine JE, et al. 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. *American Journal of Gastroenterology.* 2012;107(6):811-826.

12. Bhala N, Jouness RI, Bugianesi E. Epidemiology and natural history of patients with NAFLD. *Current Pharmaceutical Design.* 2013;19(29):5169-5176.

13. van der Poorten D, Milner KL, Hui J, et al. Visceral fat: a key mediator of steatohepatitis in metabolic liver disease. *Hepatology.* 2008;48(2):449-457.

14. 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. *Alimentary Pharmacology and Therapeutics.* 2011;34(3):274-285.

15. Yi M, Chen RP, Yang R, Chen H. Increased prevalence and risk of non-alcoholic fatty liver disease in overweight and obese patients with Type 2 diabetes in South China. *Diabetic Medicine.* 2016.

16. Chen ZW, Chen LY, Dai HL, Chen JH, Fang LZ. Relationship between alanine aminotransferase levels and metabolic syndrome in nonalcoholic fatty liver disease. *Journal of Zhejiang University Science B.* 2008;9(8):616-622.

17. Walker RW, Le KA, Davis J, et al. High rates of fructose malabsorption are associated with reduced liver fat in obese African Americans. *Journal of the American College of Nutrition.* 2012;31(5):369-374.

18. Peverill W, Powell LW, Skoien R. Evolving concepts in the pathogenesis of NASH: beyond steatosis and inflammation. *International Journal of Molecular Sciences.* 2014;15(5):8591-8638.

19. Utzschneider KM, Kahn SE. Review: The role of insulin resistance in nonalcoholic fatty liver disease. *Journal of Clinical Endocrinology and Metabolism.* 2006;91(12):4753-4761.

20. Tilg H, Moschen AR. Evolution of inflammation in nonalcoholic fatty liver disease: the multiple parallel hits hypothesis. *Hepatology.* 2010;52(5):1836-1846.

21. Purushotham A, Schug TT, Xu Q, Surapureddi S, Guo X, Li X. Hepatocyte-specific deletion of SIRT1 alters fatty acid metabolism and results in hepatic steatosis and inflammation. *Cell Metabolism.* 2009;9(4):327-338.

22. Vitamin D Fact Sheet for Health Professionals. https://ods.od.nih.gov/factsheets/VitaminD-HealthProfessional/#h3.

23. Putz-Bankuti C, Pilz S, Stojakovic T, et al. Association of 25-hydroxyvitamin D levels with liver dysfunction and mortality in chronic liver disease. *Liver International.* 2012;32(5):845-851.

24. Rhee EJ, Kim MK, Park SE, et al. High serum vitamin D levels reduce the risk for nonalcoholic fatty liver disease in healthy men independent of metabolic syndrome. *Endocrine Journal.* 2013;60(6):743-752.

25. Liangpunsakul S, Chalasani N. Serum vitamin D concentrations and unexplained elevation in ALT among US adults. *Digestive Diseases and Sciences.* 2011;56(7):2124-2129.

26. Barchetta I, Angelico F, Del Ben M, et al. 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 Medicine.* 2011;9:85.

27. 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. *Nutrition, Metabolsim, and Cardiovascular Diseases.* 2007;17(7):517-524.

28. Weiss J, Rau M, Geier A. Non-alcoholic fatty liver disease: epidemiology, clinical course, investigation, and treatment. *Deutsches Arzteblatt International.* 2014;111(26):447-452.

29. Adams LA, Lymp JF, St Sauver J, et al. The natural history of nonalcoholic fatty liver disease: a population-based cohort study. *Gastroenterology.* 2005;129(1):113-121.

30. Albanes D, Till C, Klein EA, et al. Plasma tocopherols and risk of prostate cancer in the Selenium and Vitamin E Cancer Prevention Trial (SELECT). *Cancer Prevention Research (Phila).* 2014;7(9):886-895.

31. Mummadi RR, Kasturi KS, Chennareddygari S, Sood GK. Effect of bariatric surgery on nonalcoholic fatty liver disease: systematic review and meta-analysis. *Clinical Gastroenterology and Hepatology.* 2008;6(12):1396-1402.

32. Neuschwander-Tetri BA, Loomba R, Sanyal AJ, et al. Farnesoid X nuclear receptor ligand obeticholic acid for non-cirrhotic, non-alcoholic steatohepatitis (FLINT): a multicentre, randomised, placebo-controlled trial. *Lancet.* 2015;385(9972):956-965.

33. Nakano T, Cheng YF, Lai CY, et al. Impact of artificial sunlight therapy on the progress of non-alcoholic fatty liver disease in rats. *Journal of Hepatology.* 2011;55(2):415-425.

34. Della Corte C, Carpino G, De Vito R, et al. Docosahexanoic Acid Plus Vitamin D Treatment Improves Features of NAFLD in Children with Serum Vitamin D Deficiency: Results from a Single Centre Trial. *PLOS ONE.* 2016;11(12):e0168216.

35. Patel YA, Henao R, Moylan CA, et al. Vitamin D is Not Associated With Severity in NAFLD: Results of a Paired Clinical and Gene Expression Profile Analysis. *American Journal of Gastroenterology.* 2016;111(11):1591-1598.

36. Kleiner DE, Brunt EM, Van Natta M, et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. *Hepatology.* 2005;41(6):1313-1321.

37. Sample Size Calculator. 2012; https://www.surveysystem.com/sscalc.htm. Accessed 12/29/16.

38. Steadman R, Myers RP, Leggett L, et al. A health technology assessment of transient elastography in adult liver disease. *Canadian Journal of Gastroenterology.* 2013;27(3):149-158.

39. Evaluation and Staging of Liver Fibrosis. 2017; http://cdn.hepatitisc.uw.edu/pdf/evaluation-staging-monitoring/evaluation-staging/core-concept/all. Accessed 01/03/2017.

CURRICULUM VITAE