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Predictors of Non-Alcoholic Fatty Liver Disease in Children and Adults: Findings from the Continuous National Health and Nutrition Examination Survey NHANES 2001-2006

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Abstract

Introduction: Non-Alcoholic Fatty Liver Disease (NAFLD) is the most common chronic liver disease in children and adults, outpacing alcoholic and viral liver disease in recent years. NAFLD has been linked to obesity, diabetes and the metabolic syndrome (MetS). Adverse health outcomes include cardiovascular disease, liver cirrhosis, and liver cancer. Prevalence of NAFLD is increasing in all ages but reasons remain unclear.

Aim: To determine which individual MetS components and related disturbances (e.g., elevated uric acid and insulin resistance) are linked to NAFLD, and if these relationships varied by age (adolescents vs adults).

Methods: Data from the National Health and Nutrition Examination Survey (NHANES) collected in 2001-2006 (n=12,248) were analyzed. Outcome variable was >30 u/L of the liver enzyme aminotransferase (ALT), a surrogate marker of NAFLD *in lieu* of the Gold Standard liver biopsy or imaging. Independent variables were: five MetS components and other NAFLD-related risk factors. We conducted multivariate Binary Logistic Regression of these study variables adjusted for education level, race, age, sex, and physical activity.

Results: In adults (20+ years), high uric acid was associated with a three-fold likelihood of having NAFLD (OR=3.64, 95% CI 2.89-4.59). Compared to participants with a college degree, those who completed some college or some high school had higher risks of NAFLD (OR=1.32 95% CI 1.03-1.69; OR=1.33, 95% CI 1.01-1.68; respectively). In adolescents (12-19 years), high uric acid also was associated with NAFLD (OR=3.46, 95% CI 2.48-4.83) as were black

adolescents (OR=1.20, 95% CI 1.003-1.44). For all ages, MetS individual components were not significantly associated with NAFLD nor was physical inactivity, or obesity.

Conclusion: Our study adds novel population-based evidence that social factors were associated with NAFLD. Specifically, NAFLD was relatively more prominent in black adolescents and, in adults, with less than a college degree. The three-fold greater prevalence of elevated uric acid in both age groups warrants further research and might have translational value for screening for liver disease.

INTRODUCTION

Overview. Nonalcoholic fatty liver disease is considered the hepatic component of the metabolic syndrome even though it is not yet part of the clinical definition. The concept of this form of liver disease was first introduced in 1980 when a group of physicians in Mayo clinic described biopsy findings of 20 patients that mimic alcoholic hepatitis, however, the cause was unknown. Histological findings ranged from fatty infiltrates in liver cells, to inflammation and necrosis in the lobules, to frank cirrhosis in a few patients. Most of these patients were obese and many had diabetes or gall bladder stones (Ludwig, 1980).

Nonalcoholic fatty liver disease (NAFLD) is divided into two histological forms; the simple form is nonalcoholic fatty liver (NAFL) in which fat accumulates in liver cells without evidence of liver cell injury and inflammation, and the more severe form is nonalcoholic steatohepatitis (NASH) where fat accumulation is accompanied by liver cell injury and inflammation in the form of cell ballooning with or without fibrosis. Gold standard diagnosis of NAFLD is by histology, liver imaging is also a good diagnostic tool, both need the exclusion of other causes of liver steatosis, including significant alcohol consumption, viral hepatitis, hereditary disorders and certain medications. NAFLD has been linked to metabolic risk factors, including diabetes, obesity, lipid disorders and metabolic syndrome. The most common cause of death in patients with NAFLD is cardiovascular disease, patients with NASH particularly have an increased risk of liver-related mortality due to cirrhosis and liver cancer (Chalasani, 2012).

Liver enzymes Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) could be elevated in people with NAFLD. Even though they could be normal, they are still the most common cause of referral to the gastroenterologists and NAFLD remains the most common cause of elevated liver enzymes. Evidence showed that elevated liver enzymes in the presence of NAFLD is a risk factor for developing end-stage liver disease (Ekstedt, 2006). Liver enzymes are also correlated with metabolic syndrome and its components in people with NAFLD, a study in China found that persons with ultrasound-diagnosed NAFLD were more likely to have metabolic syndrome if they have high levels of ALT (Chen, 2007).

While NAFLD is associated with metabolic syndrome in adults, adolescents, and children, population-based evidence is lacking on the relationship between elevated liver enzymes and the specific metabolic syndrome components, which could have implications for possible differences in causal etiology in adults versus younger patients. Similarly, also lacking are high-quality data on other potential correlates of NAFLD such as systemic inflammation (e.g., C-Reactive Protein), smoking status, physical inactivity, and other possible risk factors, and, if the relationships also differ by age group. The following sections describe these issues in more detail as the foundation of this research project.

Epidemiology. Since imaging doesn't recognize NAFL from NASH, and given the invasiveness of biopsy as a procedure, that renders the prevalence of NAFLD hard to determine. Back in 1990, an autopsy study of 351 nonalcoholic patients found

NAFLD changes in 18.5% of the obese and 2.7% of the lean patients (Wanless and Lentz, 1990).

In the general population worldwide, and with using different criteria for determining people with NAFLD, research showed that the condition is prevalent and is associated with obesity and insulin resistance (Lazo, 2008). In the participants of the Dallas Heart Study in the United States the prevalence was 31% by measuring fat content of the liver (Browning, 2004). Lazo and colleagues found the prevalence of NAFLD to be around 20% using liver ultrasonography in participants 20-74 years old of NHANES III (Lazo, 2013). A study used 70 living liver donors found steatosis in 38.5% of the participants, which was the most common histological finding (Tram, 2005). The prevalence was found to be higher in high risk groups, it reached 90% in obese patients (Machado, 2006) and 69% in diabetics (Leite, 2008).

For epidemiological research, it is neither ethical nor practical to perform a liver biopsy for each suspected case of NAFLD, liver enzymes have been used as surrogate markers (Clark, 2003). Several studies aimed to assess the prevalence and extent of NAFLD using liver enzymes as surrogates. Clark and colleagues studied liver enzymes in participants 17 and older in the third NHANES 1988-1994, the cutoff points for elevated liver enzymes were AST > 37 U/L or ALT >40 U/L in men, and AST or ALT >31 U/L in women. The prevalence of elevated aminotransferases (AST, ALT, or both) was 7.9%, 69% of these elevations were not explained by viral hepatitis or significant alcohol consumption. They also found that men and women with unexplained elevations were more likely to have higher

BMI, waist circumference, triglycerides (TG) and insulin, they also were more likely to have type 2 diabetes and lower high density lipoprotein (HDL) (Clark, Frederick, Brancati & Diehl, 2003).

Ioannoua and colleagues conducted a similar study using the continuous NHANES 1999-2002 their cutoff values for elevated liver enzymes were AST >40 and ALT > 43 in men and women. The prevalence of elevated ALT, AST or both were 8.9%, 4.9% and 9.8%, high ALT in NHANES 1999-2002 was more than double that in NHANES III using the same cutoff values. Elevated ALT was associated with male gender, Mexican American ethnicity, higher BMI, hepatitis C virus (HCV) antibody and alcohol consumption. Even after excluding HCV and significant alcohol consumption, the prevalence of high ALT was still high (7.3%) and associated with risk factors for NAFLD (Ioannou, 2006).

Link with the metabolic syndrome. Ioannou also found in NHAES III that metabolic syndrome was associated with increased ALT (OR 3.3, CI, 1.4-8.0), as well as obesity and insulin resistance (Ioannou, 2005). In another NHANES III study he found that participants with elevated ALT without viral hepatitis or excessive alcohol consumption had a higher Framingham Risk Score (FRS) of CHD (Ioannou, Weiss, Boyko, Mozaffarin &Lee, 2006)

In a nested case control study using NHNAES III to assess the relationship between unexplained elevations in ALT and metabolic syndrome, researchers found that the prevalence of elevated ALT in people with metabolic syndrome was

double that in those without it (OR 2.07, CI, 1.78-2.41) (Liangpunsakul &Chalsani, 2005).

The metabolic syndrome is defined by the NCEP: ATPIII as having three or more of the following: central obesity: waist circumference of greater than 102 cm in males and greater than 88 cm in females, hypertriglyceridemia: triglycerides level of 150mg/dL or greater, low HDL: less than 40 mg/dL in males and less than 50 mg/dL in females, hypertension: blood pressure of 135/85 or higher and fasting blood glucose of 110 mg/dL or higher (Expert panel on detection, evaluation, and treatment of high blood cholesterol in adults, 2001). The International Diabetes Federation (IDF) and the American Heart Association/ National Heart, Lung, and Blood Association (AHA/NHLBI) agreed in the Joint Scientific Statement to harmonize the definition of metabolic syndrome in 2009. The criteria are: triglyceride level of 150mg/dL or greater or drug treatment for elevated triglycerides, HDL level of less than 40 mg/dL in men or less than 50 mg/dL in women or drug treatment for low HDL, systolic blood pressure greater than 135 mm Hg and/or diastolic blood pressure greater than 85 mm Hg or drug treatment for hypertension, fasting blood glucose of 100 mg/dL or higher or drug treatment for diabetes, waist circumference has population- and country- specific definitions (Alberti et al., 2015). Currently, the ATP III and The AHA are the two most widely used definitions.

Health outcomes. Liver steatosis alone without inflammation (NAFL) was thought to be non- progressive, the more severe form, NASH, has been linked to liver

cancer with or without cirrhosis (Fazel, 2016). Research attempted to better understand the natural history of NAFLD. A study in Sweden followed patients with biopsy-proven NAFLD for a mean of 13.7 years found that NASH rather than NAFL was more associated with reduced survival, the most common cause of death was cardiovascular events, followed by liver-related causes (Ekstedt, 2006). A more recent smaller study in France with a shorter duration showed that NAFL can progress to NASH, especially in the presence of metabolic risk factors (Pais, 2013). In the United States, a group of researchers conducted a cohort study using the Rochester Epidemiology Project in Minnesota, patients diagnosed with NAFLD by imaging or histology between 1980 and 2000 were identified and their survival was analyzed. Results showed increased mortality compared to general Minnesota population, the leading cause was liver-related mortality and associations were age, impaired fasting glucose and liver cirrhosis (Adams, 2005).

Hepatocellular carcinoma (HCC) is the most common primary liver cancer in the united states, it is the 5th most common cancer and the third leading cause of cancer death. risk factors include viral hepatitis and alcoholic cirrhosis (Mittal and El-Seraj, 2013). Research also showed that obesity (Marengo, 2016), diabetes (Davila, 2005) and metabolic syndrome (Welzel, 2011) are additional risk factors and are increasing in the United States (Flegal, 2010) and (Cowie, 2006). HCC in also increasing in the United States as was shown by a study using Surveillance, Epidemiology and End Results (SEER) registries from 1975-2005, the incidence tripled and the 1-year survival remains less than 50% (Altekruse, 2009). NAFLD

has been also linked to HCC (Ascha, 2010) and (Mittal et.al, 2015), even in the absence of cirrhosis (Perumpail, 2015) and (Pocha, 2015).

Intervention. Treatment of NAFLD includes treating the liver disease itself in addition to the associated obesity, hyperlipidemia and diabetes. The mainstay of treatment is lifestyle modification. A randomized controlled trial found that low calorie diet and exercise improved liver histology, even without weight loss (Eckard, 2013). Another study emphasized weight loss in diabetics to reduce liver fat content (Peterson, 2005). Bariatric surgery has been also found to improve histological features of NAFLD (Taitano, 2014). Other treatments include insulin sensitizers like pioglitazone and antioxidants like vitamin E. pioglitazone reduces insulin resistance, while Vitamin E improves histology of NAFLD and reduces liver enzymes levels (Sanyal, 2010).

Pediatric NAFLD. NAFLD is also prevalent in children and adolescents in the United States, it is the leading cause of liver disease in children. The disease in children resembles the adult form with differences in the details of fibrosis and inflammation in NASH patients. The diagnosis also needs liver imaging or histological examination. Pediatric NAFLD parallels the rise in obesity rates in children (Loomba, 2010).

A study that examined natural history of NAFLD in children showed association with metabolic syndrome components. It also showed disease progression to fibrosis and even cirrhosis, and decreased survival in comparison with the general

population (Feldstein, 2009). A liver transplant study showed that NASH in childhood can progress to end-stage liver disease and it can recur in the transplanted liver (Alkhouri, 2016). The associated morbidities are cardiovascular, metabolic syndrome components, type 2 diabetes and low bone mineral density which leads to increased risk of fractures (Nidhi, 2016).

To estimate the prevalence of NAFLD in children and adolescents, an autopsy study found it to be 9.6% in children between 2-19 year- old and reached up to 17.3% in 15-19-year-old, it was the most common liver abnormality and reached 38% in obese children (Schwimmer, 2006). Studies using the continuous NHANES used liver enzymes as surrogates of NAFLD, one (NHANES 1999-2004) found that 8% of adolescents 12-19 years old have NAFLD, the prevalence was higher in Mexican American (11.5%) and lower in black (6%) (Fraser, 2008). Another study found that the prevalence rose from 3.9% in NHANES III to 10.7% in the continuous NHANES 2007-2010 in adolescents 12-19 years old. The criteria used for NAFLD were overweight and high ALT, with the exclusion of hepatotoxic medications and chronic hepatitis (Welsh, 2013).

Treatment of pediatric NAFLD with increasing physical activity and tailoring diet to the patient's individual needs showed improvement in liver histology and liver enzymes in children 5-18 years old (Nobili, 2008). Antioxidants and metformin have not been found to be more effective than placebo (Lavine, 2010).

In children and adolescents, metabolic syndrome has a modified definition of the ATP III criteria; literature defined it as having three of the following five criteria: triglycerides of 110 mg/dL or more, HDL of 40 or less, waist circumference on the

90th or above percentile for age/gender, blood glucose of 100 mg/dL or more, and blood pressure on the 90th or above percentile for age, height and gender.

In NHANES 1999-2010, the prevalence of metabolic syndrome in 12-19-year-old was 10.6% in Hispanics, 8.4% in whites and 4.2% in blacks. The prevalence of high ALT >30 U/L parallels the prevalence of metabolic syndrome: 13.7% in Hispanics, 8.6% in Whites and 5.2% in blacks. adolescents with metabolic syndrome were 7.8 times more likely to have elevated ALT (DeBoer et al., 2013).

Specific aims of the study. While NAFLD is associated with metabolic syndrome in adults and children, this study wants to explore the relationship between metabolic syndrome components and elevated ALT in adolescents and adults, in addition to other risk factors, including C-Reactive protein (CRP), uric acid, lifestyle factors like smoking and exercise, and education as an indicator of socioeconomic status and their association with elevated liver enzymes. High uric acid levels (higher than 7) has been shown to be associated with elevated liver enzymes and NAFLD in adults. Insulin resistance can lead to reduced excretion of uric acid through the kidneys which causes elevated levels in the blood (shih, 2012). This association in adolescents will be examined in our study, it is expected that uric acid will be associated with liver enzymes. High ALT level is expected to be associated with metabolic syndrome components, lower levels of exercise and lower education. The degree of association in adults and children are compared together.

METHODS

Sample. The NHANES is a large, ongoing population-based surveillance program conducted by the National Center for Health Statistics (NCHC), a part of the Centers for Disease Control and Prevention (CDC) it started in early 1960s as a series of surveys and became a continuous study in 1999. The survey is a combination of questionnaire, interview, medical examination at home and in a mobile center and Laboratory tests. Data from NHANES provides information about nutrition, infection, and environmental and chronic health conditions in the United States. The survey uses a multistage probability sampling of the civilian non-institutionalized population of the United States of all ages. Detailed description of the sampling method and the survey could be found in the CDC website at the link: http://www.cdc.gov/nchc/nhanes/about_nhanes.htm. Our study sample (n=12,448) consists of males and non-pregnant females in NHANES 2001-2006 ages 12 and older with valid ALT results; n=2,257 with high ALT values and n=10,191 with normal ALT.

Variables. ALT was used as a surrogate marker for NAFLD (dependent variable). ALT is more closely related to liver fat than other liver enzymes (Schindhelm, 2005). In our study, it was dichotomized into normal (30 U/L and lower) and elevated (over 30 U/L) in both children and adults. The cutoff point was used previously (DeBoer, 2013) and was the 97th percentile in NHANES III (Strauss, 2000). Several independent variables were examined in the study. Demographic characteristics including age, sex, race, and education. Metabolic syndrome components based on the definition by the Joint Scientific statement in adults 20

and older and the modified definition in 12-19-year-old participants. Body Mass Index (BMI) which is weight in kilograms divided by the squared height in meters. In adults, BMI was categorized as normal if it was between 18.5 and 24.9, overweight between 25-29.9, and obese if it was 30 or greater. In 12-19 age group, BMI was categorized as normal if it was under the 85th percentile for age, overweight is between the 85th and the 95th percentiles and obese above the 95th percentile. Smoking status was classified into three categories: never, previous and current in adults 20 and older.

C-reactive protein (CRP) is an inflammatory marker and has been linked to colon cancer and cardiovascular disease. The CDC/ American Heart Association workshop classified CRP as low risk for cardiovascular disease if it is less than1 mg/L, average risk between 1 and 3, and high risk above 3 mg/L (Roberts, 2004). In our study, we classified it as normal (3 or less) and high (higher than 3). Homeostasis Model Assessment method (HOMA) was calculated using the formula: (Fasting Insulin(uU/ml) *Fasting glucose(mmol/L)/22.5), HOMA-IR with a score of 3.6 (the 75th percentile in our study) or higher indicates insulin resistance. Uric acid has been also linked with coronary heart disease. Hyperuricemia, or high uric acid, was categorized into normal (7mg/dL or less) or high (greater than 7 mg/dL). Physical activity was classified based on meeting the American College of Sports Medicine (ACSM) recommendations for daily exercise as no exercise, below, or meeting ACSM recommendations. Physical activity was assessed by self-report in the last 30 days (Garber, 2011).

Metabolic syndrome components were classified into normal or high based on the Joint Scientific Statement definition. In 12-19 age group, waist circumference was divided by height in centimeters to get a waist circumference/height ratio (WHR), a variable that has been found to be associated with high ALT in Japanese Children (Ochiai, 2015) and with higher blood pressure in Chinese children (Zhang, 2017). WHR has increased over time in United States children 2-19 years old (Li, 2006). In our study, it was categorized into normal and elevated with the cut point of (0.66) which was the 90^{th} percentile.

Statistical analysis was done using Statistical package or Social Sciences (SPSS) software version 24. Chi square test was used to examine the prevalence of independent variables and compare them between the two groups of high versus normal ALT. Logistic regression was used to find variables that predict high ALT. statistical significance was defined as P value of 0.05 or less.

RESULTS

Demographic characteristics of our study population are shown in Table 1. The overall prevalence of elevated ALT (i.e., surrogate for liver disease) was 18.1% (2257/12,448). The prevalence of high ALT varied somewhat across age groups and approached significance (16.3% to 19%, p=0.055). Prevalence also differed by race/ethnic group (p=0.063): 18.2% for Mexican American/other Hispanics, 17.3% in Non-Hispanic Whites, and 19.4% in Non-Hispanic Blacks. Prevalence did not differ by sex (males, 18.3%; females, 17.7%; p=n.s). Elevated ALT rate varied somewhat by education group but no clear pattern emerged (p= 0.052).

Tables 2a through 2e stratify our study population by age category to compare prevalence of elevated ALT. Uric acid was elevated among all those with NAFLD in each age group, ranging from 19.8% (12-19 yrs) to 24.2% (80 yrs and older) in a stepwise manner. Insulin resistance, as measured by the Homeostatic Model Assessment (HOMA-IR), was observed to be present in proportionally lower rates among participants with NAFLD compared to healthy participants in age groups 40-59 (18.3% vs 27.8%, p=0.003), and 60-79 (20.7% vs 32.7%, p=0.007). The same pattern was observed in participants 80 yrs old and higher but this difference did not reach statistical significance.

Table 3a describes metabolic syndrome components in 12-19 yr age group participants using the modified ATP III definition, and measuring waist circumference/height ratio instead of waist circumference alone. Persons with high ALT did not significantly differ from those with normal ALT with respect to the presence or absence of individual components. Tables 3b and 3c describe

metabolic syndrome components in males and females, respectively, in the age group of 20 years and older in relation to ALT level. The only statistically significant difference (p=0.007) was, unexpectedly, that 49.3% of healthy males had elevated fasting blood glucose compared to 41.9% in males with elevated ALT level.

Table 4 shows results of a logistic regression model where age, sex, race/ethnicity, physical activity and BMI were fit as predictors of high ALT for 12-19-year-olds. Black participants were 1.2 times more likely to have high ALT than Mexican/Other Hispanic (95% CI 1.003-1.437) controlling for sex, physical activity or BMI. Age was also inversely associated with high ALT level with odds ratio of 0.964 (CI 0.932-0.998) per one year of age. We also performed logistic regression in this age group to determine which components of Metabolic Syndrome as well uric acid and the HOMA-IR might predict elevated ALT. children with elevated uric acid level (i.e., > 7 mg/dL) had 3.46 times the risk (95% CI 2.48-4.83) of having high ALT compared to children with normal levels.

For adults ages 20 and older, a regression model was fit to examine predictors of high ALT (Table 6), which showed that, compared to people with college education, those with less than ninth grade education were 1.316 times more likely to have high ALT (95% CI 1.026-1.689). Likewise, people who finished high school were 1.33 times (CI 1.005-1.675) more likely to have high ALT than those with college degrees. As with the younger age group, elevated uric acid was a significant predictor of elevated ALT (OR=3.8 95% CI 2.97-4.87) in the 20 yrs and older group (Table 7). Insulin resistance was significant with p value 0.014, people

with HOMA of less than 3.6 were less likely to have high ALT than those with HOMA 3.6 or greater (OR 0.71, CI: 0.54-0.93).

DISCUSSION

Prevalence of NAFLD. The prevalence of elevated ALT in our study was 18.1% in the total population, which, surprisingly, did not vary substantially by age group, race/ethnicity or sex. Prior studies using the continuous NHANES found the prevalence to be lower than our study in adults and children but these studies of adults used higher cut points. (Clark, Frederick, Brancati & Diehl, 2003; & Ioannou, 2006). The prevalence rate in our study, however, is consistent with prevalence of NAFLD using better diagnostic tools like imaging (Lazo, 2013), and autopsy studies in children (Schwimmer, 2006).

Metabolic Syndrome and NAFLD. We did not find any significant association between specific metabolic syndrome components and high ALT, while prior studies using NHANES III found that the risk of having high ALT in people with metabolic syndrome was double that risk in people who did not have metabolic syndrome (Liangpunsakul &Chalsani, 2005). We did not use metabolic syndrome as a whole entity, however, as we wanted to explore individual components as predictors of NAFLD. In multivariate logistic regression, our study did not produce evidence of a relationship between elevated HOMA-IR (i.e., insulin resistance) with elevated ALT in either age group. It is possible that the age group of ≥20 years is a broad of a risk group, and we will analyze narrower age groups prior to submission of this study for publication.

On the other hand, metabolic syndrome has been shown to have low sensitivity to detect insulin resistance in adolescents (Deboer and Gurka, 2014) which might explain the lack of association of metabolic syndrome individual components with high ALT in our study in the young age group. The lack of an association between insulin resistance (i.e., elevated HOMA-IR) and ALT in those older than age 20 is puzzling for biologic reasons. Insulin resistance explains NAFLD physiologically in basic science by the release of free fatty acids from the insulin resistant fat tissue and because of imbalance between lipid excretion and uptake by the liver cells, this leads to fat accumulation in the liver cells and, when inflammation is present, to NAFLD and NASH (Dietrich & Hellerbrand, 2014).

Another diagnostic marker of metabolic syndrome is elevated waist circumference which reflects central obesity, but we did not see associations with this condition and elevated ALT in either age group. Central obesity is characterized by accumulation of both subcutaneous and visceral fat. While in adults central adiposity is part of the definition of the metabolic syndrome, in children, the modified definition of metabolic syndrome includes waist circumference that is at the 90th percentile for age and sex (Deboer, 2013), Waist/height ratio is believed to be a better measure of central obesity in children and adolescents since it considers growth in both height and waist circumference, it is also valid for males and females without the need for separating values for each sex (Li, 2006). Waist/height ratio in our study was not significant in predicting ALT in the age group 12-19 using more than one cut point.

Elevated Uric Acid and NAFLD. High uric acid was significantly associated with high ALT in all age groups, independent of metabolic syndrome components. A study of NHANES III participants ages 20-74 found that ultrasound -diagnosed NAFLD was significantly higher in people with higher uric acid levels, also, people with hyperuricemia were 1.4 times more likely to have NAFLD. Hyperuricemia was also significantly associated with elevated ALT. It is not known if the high uric acid is a cause or a consequence of NAFLD, however, a prospective Chinese study found after 3 year-follow up that people with higher basic levels of uric acid were more likely to develop NAFLD (Xu, 2010). An article hypothesized that fructose plays a role in NAFLD and metabolic syndrome (Lim, 2010). Our study extends the association between uric acid and ALT to children 12-19.

Demographic characteristics and NAFLD. We did observe, however, that African-Americans were at higher risk for elevated ALT among those under 20 years but, that in the older age group, education was inversely associated with elevated ALT. To the best of our knowledge, our study is the first to find an increased risk of high ALT as a marker of NAFLD in black adolescents. Prior reports have shown a lower prevalence of reported lower prevalence of NAFLD (and metabolic syndrome) in Blacks of all ages. (Beltran-Sanchez, 2011; Ioannou, 2006; Deboer, 2013; & welsh, 2013). With respect to education, a Chinese study found a relationship between SES and NAFLD in patients hospitalized with type 2 diabetes mellitus (Jia, 2015), patients in the lower SES were more likely to develop

NAFLD, had higher BMI, higher insulin resistance, and poorer general, emotional and mental health.

Strengths and Limitations. Our study was conducted on a large, nationally representative sample of civil non-institutionalized men, nonpregnant women and children 12-19 in the United States consisting of validated data. However, there are several limitations, most notably, the cross-sectional nature of the study shows does not permit causal inference. Causality, of course, helps direct prevention and targeted screening.

Also, we did not exclude participants with excessive alcohol use, a prominent risk factor of liver disease, due to a high level of missing data. Scientifically, we can justify this lack of exclusion based on findings from a large, longitudinal study that followed middle aged men and women without metabolic syndrome for average of 6.4 years, at the end of the follow up, people with high ALT were 2.25 times more likely to develop metabolic syndrome regardless of age, sex, or alcohol intake (Schindhelm, 2007). Further, NAFLD now reflects the vast majority of chronic liver diseases, rising from 46.8% of all cases in 1988 to 75.1% in 2008 (Younossi, 2011).

Conclusion

In a nationally representative cross-sectional study of US adolescents (12-19 yrs) and adults (≥20 yrs), we found that elevated uric acid was associated with a three-fold likelihood of NAFLD in both age groups controlling for individual components of the metabolic syndrome. With respect to demographic characteristics, we observed that, in adolescents, African Americans had a significantly higher risk of NAFLD, but, for adults, education was inversely related to NAFLD. Unexpectedly, insulin resistance, as measured by elevated HOMA-IR, was inversely linked to NAFLD in both age groups whereas no other components of the metabolic syndrome were associated with liver disease.

Our finding concerning high uric acid is consistent with an imaging-based national study with proven NAFLD in NAHNES III participants (Sirota et al., 2013). Since having high liver enzymes is the most common cause of referral to the gastroenterologists, these risk factors could help guide clinicians to develop screening protocols for their patients. NAFLD can lead to advanced liver disease and other sequelae like diabetes and cardiovascular disease in all ages. It is for these reasons that NAFLD has been described in the literature as a component of metabolic syndrome although it has not yet been officially included in the clinical definition. Of note, metabolic syndrome, when introduced by a Swedish physician over 80 years ago, included high uric acid as one of the characteristics (Pais, 2009). Based on the significant association between uric acid and high liver enzymes in our study, and the significant association between insulin resistance

and NAFLD, obesity and metabolic syndrome in many other studies to date, the value of high uric acid triggering screening for NAFLD should continue to be discussed. A recommendation for future research stemming from our work would be to study nutritional factors that contribute to high uric acid in the US population. High intake of sugar-sweetened soft drinks, for example, has been associated with high uric acid levels in adults (Choi, 2008; Gao, 2007). Lastly, links between higher dietary fructose and biopsy-proven NAFLD patients have been reported (Ouyang et al., 2008). Fructose should be a concern in all ages, especially in children, given the increased consumption of products containing high fructose corn syrup. Further study of added sugars in the diet of US children and adults is needed to elucidate the relationship between fructose intake and high levels of uric acid, which, ultimately, can help increase awareness among people, provide evidence-based guidelines for health care providers, and provide a solid ground for change in food policy.

Table 1: Demographic characteristics of the study population by NAFLD status in NHANES 2001-2006 (n=12448)

	NAFLD n=2257 (18.1%)	Healthy n=10191 (81.9%)	P value
Age Group (n, %)			
12-19	867 (18.3%)	3847 (81.7%)	0.055
20-39	582 (19.0%)	2479 (81.0%)	
40-59	494 (16.5%)	2494 (83.5%)	
60-79	252 (19.3%)	1050 (80.6%)	
>=80	62 (16.2%)	321 (83.8%)	
Sex			
Male (n=7195)	1317 (18.3%)	5878 (81.7%)	0.558
Female (n=5281)	940 (17.8%)	4341 (82.2%)	
Race/ethnicity (n, %)			
Mexican-Am./Other Hisp.	643 (18.2%)	2881 (81.8%)	0.063
Non-Hispanic white	913 (17.3%)	4343 (82.7%)	
Non-Hispanic Black	617 (19.4%)	2563 (80.6%)	
Education (n, %)			
College and above	293 (18.9%)	1256 (81.1 %)	0.052
Some College	357 (16.6%)	1796 (83.4%)	
High school	366 (19.7%)	1491 (80.3%)	
9-11 grade	210 (17.9%)	962 (82.1%)	
< 9 grade	162 (16.3%)	831 (83.7%)	

Table 2 a: NAFLD-related risk factors in NHANES 2001-2006, age 12-19

	NAFLD (n=867)	Healthy (n=3847)	P value
CRP			
Normal	795 (99.6%)	3491 (99.6%)	0.831
Elevated	3 (.04%)	15 (0.4%)	
Uric acid			
Normal (n, %)	695 (80.2%)	3536 (91.9%)	< 0.001
Elevated (n, %)	172 (19.8%)	311 (81%)	
BMI			
Normal	559 (65.9%)	2445 (65.5%)	0.948
Overweight	136 (16%)	594 (15.9%)	
Obese	153 (18%)	691 (18.5)	
Smoking			
Never			
Former	NA	NA	NA
Current			
Physical activity			
None	144 (16.6%)	613 (15.9%)	0.746
Below ACSM guidelines	402 (46.4%)	1759 (38.3%)	
Met ACSM guidelines	321 (37.0%)	1475 (38.3%)	
HOMA-IR			
Normal	302 (80.3%)	1338 (79.6%)	0.752
Elevated	74 (19.7%)	343 (20.4%)	

Table 2 b: NAFLD-related risk factors in NHANES 2001-2006, age 20-39

	NAFLD n= 582	Healthy n= 2479	P value
CRP			
Normal	534 (98.9%)	2291 (98.5%)	0.531
Elevated	6 (1.1%)	34 (1.5%)	
Uric Acid			
Normal	460 (79%)	2291 (92.4%)	< 0.001
Elevated	122 (21%)	188 (7.6%)	
BMI			
Normal	218 (38.7%)	1003 (41.4%)	0.241
Overweight	172 (30.5%)	755 (31.2%)	
Obese	174 (30.9%)	644 (27.4%)	
Smoking			
Never	311 (53.5%)	1442 (58.2%)	
Former	68 (11.7%)	303 (12.2%)	0.051
Current	202 (34.8)	734 (29.6%)	
Physical activity			
None	181 (31.1%)	822 (33.2%)	
Below ACSM guidelines	302 (51.9%)	1217 (491%)	0.473
Meeting ACSM guidelines	99 (17%)	440 (17.7%)	
HOMA-IR			
Normal	211 (81.2%)	914 (82.6%)	
Elevated	49 (18.8%)	193 (17.4 %)	0.592

Table 2 c: NAFLD-related risk factors in NHANES 2001-2006, age 40-59

	NAFLD n=494	**Healthy n= 2493**	**P value**
CRP			
Normal	456 (98.1%)	2356 (98.9%)	0.131
Elevated	9 (1.9%)	26(1.1%)	
Uric Acid			
Normal	383 (77.5%)	2280 (91.5%)	< 0.001
Elevated	111 (22.5%)	213 (8.5%)	
BMI			
Normal	133 (27.5%)	621 (25.6%)	0.512
Overweight	170 (35.1%)	915 (37.7%)	
Obese	181 (37.4%)	889 (36.7%)	
Smoking			
Never	224 (45.3%)	1175 (47.2%)	
Former	118 (23.9%)	303 (12.2%)	0.388
Current	152 (30.8)	734 (29.6%)	
Physical activity			
None	219 (44.3%)	1017 (40.8%)	0.339
Below ACSM guidelines	206 (41.7%)	1112 (44.6%)	
Meeting ACSM guidelines	69 (14%)	365 (14.6%)	
HOMA-IR			
Normal	187 (81.7%)	811 (72.2%)	0.003
Elevated	42 (18.3%)	312 (27.8%)	

Table 2 d: NAFLD-related risk factors in NHANES 2001-2006, age 60-79

	NAFLD n= 252	Healthy n= 1050	P value
CRP			
Normal	242 (97.6%)	964 (97.1%)	0.670
Elevated	6 (2.4%)	29 (2.9%)	
Uric Acid			
Normal	199 (79%)	978 (93.1%)	< 0.001
Elevated	53 (21%)	72 (6.9%)	
BMI			
Normal	66 (27.4%)	254 (25.3%)	
Overweight	101 (41.9%)	447 (44.5%)	0.725
Obese	74 (30.7%)	304 (30.2%)	
Smoking			
Never	80 (31.9%)	304 (29%)	
Former	123 (49%)	561 (53.5%)	0.443
Current	48 (19.1 %)	181 (17.5%)	
Physical activity			
None	121 (48%)	502 (47.8%)	
Below ACSM guidelines	28 (19%)	371 (35.3%)	0.275
Meeting ASCM guidelines	52 (20.6%)	177 (16.9%)	
HOMA-IR			
Normal	92 (79.3%)	125 (67.3%)	0.007
Elevated	24 (20.7%)	161 (32.7%)	

Table 2e: NAFLD-related risk factors in NHANES 2001-2006, age 80 and older

	NAFLD n= 62	Healthy n=321	P-Value
CRP			
Normal	53 (100%)	296 (98%)	0.598 *
Elevated	0 (0%)	6 (2%)	
Uric Acid			
Normal	47 (75.8%)	292 (91%)	0.001
Elevated	15 (24.2%)	29 (9%)	
BMI			
Normal	22 (44%)	106 (391%)	0.067
Overweight	16 (32%)	128 (47.2%)	
Obese	12 (24%)	37 (13.7%)	
Smoking			
Never	19 (30.6%)	121 (37.9%)	0.097
Former	36 (58.1%)	183 (57.4%)	
Current	7 (11.3%)	15 (4.7%)	
Physical activity			
None	39 (62.9%)	168 (52.3%)	0.295
Below ACSM	14 (22.6%)	99 (30.8%)	
Meeting ACSM	9 (14.5%)	54 (16.8%)	
HOMA-IR			
Normal	22 (91.7%)	125 (80.6%)	0.258 *
Elevated	2 (8.3%)	30 (19.4%)	

*Fisher's exact was used because there was 1 cell count expected less than 5.

Table 3a: Metabolic syndrome components in NHANES 2001-2006 by NAFLD Status, ages 12-19

MetS component	NAFLD n= 845	Healthy n= 3734	P Value
WC/height ratio			
Males			
Normal	416(96.1%)	1843 (95.5%)	0.898
Elevated	17 (3.9%)	78 (4.1%)	
Females			
Normal	393 (95.4%)	1697 (93.6%)	0.170
Elevated	19 (4.6%)	116 (6.4%)	
HDL			
Males			
Low	77 (21.6%)	380 (23.6%)	0.416
Normal	279 (62.5%)	1227 (76.4%)	
Females			
Low	42 (12.5%)	207 (13.7%)	0.569
Normal	294 (87.5%)	1307 (86.3%)	
Triglycerides			
Males			
Normal	152 (76.4%)	686 (77.3%)	0.792
Elevated	47 (23.6%)	202 (22.7%)	
Females			
Normal	154 (85.6%)	638 (80.2%)	0.094
Elevated	26 (14.4%)	158 (19.8%)	
Fasting Glucose			
Males			
Normal	167 (81.9%)	728 (81.1%)	0.793
Elevated	37 (18.1%)	170 (18.9%)	
Females			
Normal	174 (94.1%)	742 (91.7%)	0.286
Elevated	11 (5.9%)	67 (8.3%)	

Table 3b: metabolic Syndrome Components in NHANES 2001-2006, Males 20 years and older

MetS Components	NAFLD n=825	Healthy n= 4283	P value
Fasting Blood Glucose			
Normal <100 mg/dL	232 (58.1%)	928 (50.7%)	0.007
Elevated>=100 mg/dL	167 (41.9%)	904 (49.3%)	
Waist Circumference			
Normal	475 (57.6%)	2185 (59.3%)	0.335
Elevated	350 (42.4%)	1498 (40.7%)	
Triglycerides			
Normal	261 (66.2%)	1125 (61.7%)	0.090
Elevated	133 (33.8%)	699 (38.3%)	
HDL			
Low	23 (30.7)	100 (27.8%)	0.613
Normal	52 (69.3%)	260 (72.2 %)	
Blood pressure			
Normal	473 (62.9%)	2105 (63%)	0.951
Elevated	279 (37.1%)	1236 (37.0%)	

Table 3c: Metabolic Syndrome Components in NHANES 2001-2006, Females 20 years and older

MetS Component	NAFLD n= 493	Healthy n= 2335	P value
Fasting Blood Glucose			
Normal	181 (76.4%)	815 (75%)	0.652
Elevated	56 (23.6%)	272 (25%)	
Waist Circumference			
Normal	202 (41%)	970 (41.5%)	0.816
Elevated	291 (59%)	1365 (58.5%)	
Triglycerides			
Normal	170 (72.6%)	824 (77.3%)	0.129
Elevated	64 (27.4%)	242 (22.7%)	
HDL			
Low	16 (36.4%)	89 (34.8%)	0.837
Normal	28 (63.6%)	167 (65.2%)	
Blood pressure			
Normal	339 (77.9%)	1630 (80.9%)	0.153
Elevated	96 (22.1%)	384 (19.1%)	

Table 4: Logistic model of demographic predictors of ALT >30, age 12-19

predictor	OR	95% CI	P value
Sex			
Females (ref)			
Males	0.96	(0.823-1.12)	0.6
Age	0.96	(0.93-0.99)	0.036
Race/ethnicity			
Hispanic (ref)			
White	0.96	(0.8-1.16)	0.68
Black	1.2	(1.003-1.437)	0.047
BMI			
Normal (ref)			
Overweight	0.999	(0.808-1.235)	0.99
obese	0.953	(0.779- 1.167)	
Exercise			
Meeting ACSM (ref)			
None	0.98	(0.785-1.223)	0.86
Below ACSM	0.909	(0.722-1.145)	0.42

Table 5: Logistic model of metabolic syndrome components as predictors of ALT>30, age 12-19

Predictor	OR	95% CI	P value
HDL			
>=40 (ref)			
<40	1.114	(0.79-1.57)	0.53
TG			
<=110 (ref)			
>110	0.94	(0.673-1.3)	0.69
HOMA-IR			
< 3.6 (ref)			
>= 3.6	1.14	(0.82-1.61)	0.427
Uric Acid			
<7 (ref)			
>=7	3.46	(2.48-4.83)	<0.001
Waist/height ratio			
<0.66 (ref)	1.038	(0.57-1.89)	0.904
>=0.66			
Fasting glucose			
<100 (ref)			
>=100	0.838	(0.57-1.24)	0.377

Table 6: Logistic model of demographic predictors of ALT> 30, age 20 and older

Predictor	OR	95% CI	P value
Age	0.998	(0.994-1.002)	0.266
Sex			
Females (ref)			
Males	1.103	(0,996-1.259)	0.147
Physical activity			
Meeting ACSM guidelines (ref)			
None	0.943	(0.821-1.085)	0.413
Below guidelines	0.957	(0.797-1.15)	0.64
BMI			
Normal (ref)			
Overweight	0.935	(0.805-1.087)	0.382
Obese	1.069	(0.919-1.3)	0.384
Race/ethnicity			
Hispanic (ref)			
White	0.922	(0.787-1.080)	0.254
Black	1.053	0.876-1.265)	0.584
Education			
College or higher (ref)			
Some college	1.32	(1.026-1.689)	0.031
High school	1.068	(0.845-1.351)	0.58
9-11 grade	1.33	(1.055-1.675)	0.016
Less than 9th grade	1.106	(0.86-1.413)	0.418

Table 7: metabolic syndrome components and other variables as predictors of ALT>30, age 20 and older

Predictor	OR	95% CI	P value
Blood pressure			
Normal (ref)			
At risk (>130/90)	1.008	(0.812-1.25)	0.943
Triglycerides			
Normal (ref)			
High >150	1.022	(0.822-1.269)	0.847
Glucose			
<100 (ref)			
>= 100	0.881	(0.708-1.094)	0.251
Waist circumference			
Normal (ref)			
High (>102 cm in men and >88 cm in women)	1.103	(0.898-1.354)	0.352
HOMA-IR			
< 3.6 (ref)			
>=3.6	0.709	(0.54-0.932)	0.014
Uric acid			
< 7 (ref)			
>= 7	3.8	(2.98-4.87)	<0.001

References

Adams, L. A., Lymp, J. F., St Sauver, J., Sanderson, S. O., Lindor, K. D., Feldstein, A., & Angulo, P. (2005). The natural history of nonalcoholic fatty liver disease: A population- based cohort study. *Gastroenterology, 129*(1), 113-121. doi:S0016508505006943 [pii]

Alberti, K. G., Eckel, R. H., Grundy, S. M., Zimmet, P. Z., Cleeman, J. I., Donato, K. A., . . . International Association for the Study of Obesity. (2009). Harmonizing the metabolic syndrome: A joint interim statement of the international diabetes federation task force on epidemiology and prevention; national heart, lung, and blood institute; American heart association; world heart federation; international atherosclerosis society; and international association for the study of obesity. *Circulation, 120*(16), 1640-1645. doi:10.1161/CIRCULATIONAHA.109.192644 [doi]

Alkhouri, N., Hanouneh, I. A., Zein, N. N., Lopez, R., Kelly, D., Eghtesad, B., & Fung, J. J. (2016). Liver transplantation for nonalcoholic steatohepatitis in young patients. *Transplant International: Official Journal of the European Society for Organ Transplantation, 29*(4), 418-424. doi:10.1111/tri.12694 [doi]

Altekruse, S. F., McGlynn, K. A., & Reichman, M. E. (2009). Hepatocellular carcinoma incidence, mortality, and survival trends in the united states from 1975 to 2005. *Journal of Clinical Oncology, 27*(9), 1485-1491. doi:10.1200/JCO.2008.20.7753

Ascha, M. S., Hanouneh, I. A., Lopez, R., Tamimi, T. A., Feldstein, A. F., & Zein, N. N. (2010). The incidence and risk factors of hepatocellular carcinoma in patients with nonalcoholic steatohepatitis. *Hepatology (Baltimore, Md.), 51*(6), 1972-1978. doi:10.1002/hep.23527 [doi]

Beltran-Sanchez, H., Harhay, M. O., Harhay, M. M., & McElligott, S. (2013). Prevalence and trends of metabolic syndrome in the adult U.S. population, 1999-2010. *Journal of the American College of Cardiology, 62*(8), 697-703. doi:10.1016/j.jacc.2013.05.064 [doi]

Browning, J. D., Szczepaniak, L. S., Dobbins, R., Nuremberg, P., Horton, J. D., Cohen, J. C., . . . Hobbs, H. H. (2004). Prevalence of hepatic steatosis in an urban population in the united states: Impact of ethnicity. *Hepatology (Baltimore, Md.), 40*(6), 1387-1395. doi:10.1002/hep.20466 [doi]

Chalasani, N., Younossi, Z., Lavine, J. E., Diehl, A. M., Brunt, E. M., Cusi, K., . . . American College of Gastroenterologyh. (2012). The diagnosis and management of non-alcoholic fatty liver disease: Practice guideline by the american gastroenterological association, american association for the study of liver diseases, and american college of gastroenterology. *Gastroenterology, 142*(7), 1592-1609. doi:10.1053/j.gastro.2012.04.001 [doi]

Chen, Z. W., Chen, L. Y., Dai, H. L., Chen, J. H., & Fang, L. Z. (2008). Relationship between alanine aminotransferase levels and metabolic syndrome in nonalcoholic fatty liver disease. *Journal of Zhejiang University.Science.B, 9*(8), 616-622. doi:10.1631/jzus.B0720016 [doi]

Choi, J. W., Ford, E. S., Gao, X., & Choi, H. K. (2008). Sugar-sweetened soft drinks, diet soft drinks, and serum uric acid level: The third national health and nutrition examination survey. *Arthritis and Rheumatism, 59*(1), 109-116. doi:10.1002/art.23245 [doi]

Clark, J. M., Brancati, F. L., & Diehl, A. M. (2003). The prevalence and etiology of elevated aminotransferase levels in the united states. *The American Journal of Gastroenterology, 98*(5), 960-967. doi:S000292700300265X [pii]

Clark, J. M., & Diehl, A. M. (2003). Defining nonalcoholic fatty liver disease: Implications for epidemiologic studies. *Gastroenterology, 124*(1), 248-250. doi:10.1053/gast.2003.50032 [doi]

Cowie, C. C., Rust, K. F., Byrd-Holt, D. D., Eberhardt, M. S., Flegal, K. M., Engelgau, M. M., . . . Gregg, E. W. (2006). Prevalence of diabetes and impaired fasting glucose in adults in the U.S. population: National health and nutrition examination survey 1999-2002. *Diabetes Care, 29*(6), 1263-1268. doi:29/6/1263 [pii]

Davila, J. A., Morgan, R. O., Shaib, Y., McGlynn, K. A., & El-Serag, H. B. (2005). Diabetes increases the risk of hepatocellular carcinoma in the united states: A population based case control study. *Gut, 54*(4), 533-539. doi:10.1136/gut.2004.052167

DeBoer, M. D., & Gurka, M. J. (2014). Low sensitivity of the metabolic syndrome to identify adolescents with impaired glucose tolerance: An analysis of NHANES 1999-2010. *Cardiovascular Diabetology, 13*, 83-2840-13-83. doi:10.1186/1475-2840-13-83 [doi]

Deboer, M. D., Wiener, R. C., Barnes, B. H., & Gurka, M. J. (2013). Ethnic differences in the link between insulin resistance and elevated ALT. *Pediatrics, 132*(3), e718-26. doi:10.1542/peds.2012-3584 [doi]

Dietrich, P., & Hellerbrand, C. (2014). Non-alcoholic fatty liver disease, obesity and the metabolic syndrome. *Best Practice & Research.Clinical Gastroenterology, 28*(4), 637-653. doi:10.1016/j.bpg.2014.07.008 [doi]

Eckard, C., Cole, R., Lockwood, J., Torres, D. M., Williams, C. D., Shaw, J. C., & Harrison, S. A. (2013). Prospective histopathologic evaluation of lifestyle modification in nonalcoholic fatty liver disease: A randomized trial. *Therapeutic Advances in Gastroenterology, 6*(4), 249-259. doi:10.1177/1756283X13484078 [doi]

Ekstedt, M., Franzen, L. E., Mathiesen, U. L., Thorelius, L., Holmqvist, M., Bodemar, G., & Kechagias, S. (2006). Long-term follow-up of patients with NAFLD and elevated liver enzymes. *Hepatology (Baltimore, Md.), 44*(4), 865-873. doi:10.1002/hep.21327 [doi]

Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. (2001). Executive summary of the third report of the national cholesterol education program (NCEP) expert panel on detection, evaluation,

and treatment of high blood cholesterol in adults (adult treatment panel III). *Jama, 285*(19), 2486-2497. doi:jsc10094 [pii]

Fazel, Y., Koenig, A. B., Sayiner, M., Goodman, Z. D., & Younossi, Z. M. (2016). Epidemiology and natural history of non-alcoholic fatty liver disease. *Metabolism: Clinical and Experimental, 65*(8), 1017-1025. doi:10.1016/j.metabol.2016.01.012 [doi]

Feldstein, A. E., Charatcharoenwitthaya, P., Treeprasertsuk, S., Benson, J. T., Enders, F. B., & Angulo, P. (2009). The natural history of non-alcoholic fatty liver disease in children: A follow-up study for up to 20 years. *Gut, 58*(11), 1538-1544. doi:10.1136/gut.2008.171280 [doi]

Flegal, K. M., Carroll, M. D., Ogden, C. L., & Curtin, L. R. (2010). Prevalence and trends in obesity among US adults, 1999-2008. *Jama, 303*(3), 235-241. doi:10.1001/jama.2009.2014 [doi]

Fraser, A., Longnecker, M. P., & Lawlor, D. A. (2007). Prevalence of elevated alanine aminotransferase among US adolescents and associated factors: NHANES 1999-2004. *Gastroenterology, 133*(6), 1814-1820. doi:S0016-5085(07)01634-4 [pii]

Gao, X., Qi, L., Qiao, N., Choi, H. K., Curhan, G., Tucker, K. L., & Ascherio, A. (2007). Intake of added sugar and sugar-sweetened drink and serum uric acid concentration in US men and women. *Hypertension (Dallas, Tex.: 1979), 50*(2), 306-312. doi:HYPERTENSIONAHA.107.091041 [pii]

Garber, C. E., Blissmer, B., Deschenes, M. R., Franklin, B. A., Lamonte, M. J., Lee, I. M., . . . American College of Sports Medicine. (2011). American college of sports medicine position stand. quantity and quality of exercise for developing and maintaining cardiorespiratory, musculoskeletal, and neuromotor fitness in apparently healthy adults: Guidance for prescribing exercise. *Medicine and Science in Sports and Exercise, 43*(7), 1334-1359. doi:10.1249/MSS.0b013e318213fefb [doi]

Ioannou, G. N., Boyko, E. J., & Lee, S. P. (2006). The prevalence and predictors of elevated serum aminotransferase activity in the united states in 1999-2002. *The American Journal of Gastroenterology, 101*(1), 76-82. doi:AJG341 [pii]

Ioannou, G. N., Weiss, N. S., Boyko, E. J., Kahn, S. E., & Lee, S. P. (2005). Contribution of metabolic factors to alanine aminotransferase activity in persons with other causes of liver disease. *Gastroenterology, 128*(3), 627-635. doi:10.1053/j.gastro.2004.12.004

Ioannou, G. N., Weiss, N. S., Boyko, E. J., Mozaffarian, D., & Lee, S. P. (2006). Elevated serum alanine aminotransferase activity and calculated risk of coronary heart disease in the united states. *Hepatology (Baltimore, Md.), 43*(5), 1145-1151. doi:10.1002/hep.21171 [doi]

Jia, G., Li, X., Wang, L., Li, Q., Yang, L., Li, N., & Di, F. (2015). Relationship of socioeconomic status and non-alcoholic fatty liver disease in patients with type 2 diabetes mellitus. *Zhonghua Gan Zang Bing Za Zhi = Zhonghua Ganzangbing Zazhi = Chinese Journal of Hepatology, 23*(10), 760-764. doi:10.3760/cma.j.issn.1007-3418.2015.10.010 [doi]

Lavine, J. E., Schwimmer, J. B., Molleston, J. P., Scheimann, A. O., Murray, K. F., Abrams, S. H., . . . Nonalcoholic Steatohepatitis Clinical Research Network Research Group. (2010). Treatment of nonalcoholic fatty liver disease in children: TONIC trial design. *Contemporary Clinical Trials, 31*(1), 62-70. doi:10.1016/j.cct.2009.09.001 [doi]

Lazo, M., & Clark, J. M. (2008). The epidemiology of nonalcoholic fatty liver disease: A global perspective. *Seminars in Liver Disease, 28*(4), 339-350. doi:10.1055/s-0028-1091978 [doi]

Lazo, M., Hernaez, R., Eberhardt, M. S., Bonekamp, S., Kamel, I., Guallar, E., . . . Clark, J. M. (2013). Prevalence of nonalcoholic fatty liver disease in the united states: The third national health and nutrition examination survey, 1988-1994. *American Journal of Epidemiology, 178*(1), 38-45. doi:10.1093/aje/kws448 [doi]

Leite, N. C., Salles, G. F., Araujo, A. L., Villela-Nogueira, C. A., & Cardoso, C. R. (2009). Prevalence and associated factors of non-alcoholic fatty liver disease in patients with type-2 diabetes mellitus. *Liver International : Official Journal of the International Association for the Study of the Liver, 29*(1), 113-119. doi:10.1111/j.1478-3231.2008.01718.x [doi]

Li, C., Ford, E. S., Mokdad, A. H., & Cook, S. (2006). Recent trends in waist circumference and waist-height ratio among US children and adolescents. *Pediatrics, 118*(5), e1390-8. doi:118/5/e1390 [pii]

Lim, J. S., Mietus-Snyder, M., Valente, A., Schwarz, J. M., & Lustig, R. H. (2010). The role of fructose in the pathogenesis of NAFLD and the metabolic syndrome. *Nature Reviews.Gastroenterology & Hepatology, 7*(5), 251-264. doi:10.1038/nrgastro.2010.41 [doi]

Loomba, R., Sirlin, C. B., Schwimmer, J. B., & Lavine, J. E. (2009). Advances in pediatric nonalcoholic fatty liver disease. *Hepatology (Baltimore, Md.), 50*(4), 1282-1293. doi:10.1002/hep.23119 [doi]

Ludwig, J., Viggiano, T. R., McGill, D. B., & Oh, B. J. (1980). Nonalcoholic steatohepatitis: Mayo clinic experiences with a hitherto unnamed disease. *Mayo Clinic Proceedings, 55*(7), 434-438.

Machado, M., Marques-Vidal, P., & Cortez-Pinto, H. (2006). Hepatic histology in obese patients undergoing bariatric surgery. *Journal of Hepatology, 45*(4), 600-606. doi:S0168-8278(06)00384-9 [pii]

Marengo, A., Rosso, C., & Bugianesi, E. (2016). Liver cancer: Connections with obesity, fatty liver, and cirrhosis. *Annual Review of Medicine, 67*, 103-117. doi:10.1146/annurev-med-090514-013832 [doi]

Mittal, S., & El-Serag, H. B. (2013). Epidemiology of hepatocellular carcinoma: Consider the population. *Journal of Clinical Gastroenterology, 47 Suppl*, S2-6. doi:10.1097/MCG.0b013e3182872f29 [doi]

Mittal, S., Sada, Y. H., El-Serag, H. B., Kanwal, F., Duan, Z., Temple, S., . . . Davila, J. A. (2015). Temporal trends of nonalcoholic fatty liver disease-related hepatocellular carcinoma in the veteran affairs population. *Clinical Gastroenterology and Hepatology : The Official Clinical Practice Journal of the American Gastroenterological Association, 13*(3), 594-601.e1. doi:10.1016/j.cgh.2014.08.013 [doi]

Nobili, V., Manco, M., Devito, R., Di Ciommo, V., Comparcola, D., Sartorelli, M. R., . . . Angulo, P. (2008). Lifestyle intervention and antioxidant therapy in children with nonalcoholic fatty liver disease: A randomized, controlled trial. *Hepatology (Baltimore, Md.), 48*(1), 119-128. doi:10.1002/hep.22336 [doi]

Ochiai, H., Shirasawa, T., Nishimura, R., Nanri, H., Ohtsu, T., Hoshino, H., . . . Kokaze, A. (2015). Waist-to-height ratio is more closely associated with alanine aminotransferase levels than body mass index and waist circumference among population-based children: A cross-sectional study in japan. *BMC Pediatrics, 15*, 59-015-0378-8. doi:10.1186/s12887-015-0378-8 [doi]

Ouyang, X., Cirillo, P., Sautin, Y., McCall, S., Bruchette, J. L., Diehl, A. M., . . . Abdelmalek, M. F. (2008). Fructose consumption as a risk factor for non-alcoholic fatty liver disease. *Journal of Hepatology, 48*(6), 993-999. doi:10.1016/j.jhep.2008.02.011 [doi]

Pais, R., Charlotte, F., Fedchuk, L., Bedossa, P., Lebray, P., Poynard, T., . . . LIDO Study Group. (2013). A systematic review of follow-up biopsies reveals disease progression in patients with non-alcoholic fatty liver. *Journal of Hepatology, 59*(3), 550-556. doi:10.1016/j.jhep.2013.04.027 [doi]

Pais, R., Silaghi, H., Silaghi, A. C., Rusu, M. L., & Dumitrascu, D. L. (2009). Metabolic syndrome and risk of subsequent colorectal cancer. *World Journal of Gastroenterology, 15*(41), 5141-5148.

Perumpail, R. B., Wong, R. J., Ahmed, A., & Harrison, S. A. (2015). Hepatocellular carcinoma in the setting of non-cirrhotic nonalcoholic fatty liver disease and the metabolic syndrome: US experience. *Digestive Diseases and Sciences, 60*(10), 3142-3148. doi:10.1007/s10620-015-3821-7 [doi]

Petersen, K. F., Dufour, S., Befroy, D., Lehrke, M., Hendler, R. E., & Shulman, G. I. (2005). Reversal of nonalcoholic hepatic steatosis, hepatic insulin resistance, and hyperglycemia by moderate weight reduction in patients with type 2 diabetes. *Diabetes, 54*(3), 603-608. doi:54/3/603 [pii]

Pocha, C., Kolly, P., & Dufour, J. F. (2015). Nonalcoholic fatty liver disease-related hepatocellular carcinoma: A problem of growing magnitude. *Seminars in Liver Disease, 35*(3), 304-317. doi:10.1055/s-0035-1562949 [doi]

Roberts, W. L., CDC, & AHA. (2004). CDC/AHA workshop on markers of inflammation and cardiovascular disease: Application to clinical and public health practice: Laboratory tests available to assess inflammation--performance and standardization: A background paper. *Circulation, 110*(25), e572-6. doi:110/25/e572 [pii]

Sanyal, A. J., Chalasani, N., Kowdley, K. V., McCullough, A., Diehl, A. M., Bass, N. M., . . . NASH CRN. (2010). Pioglitazone, vitamin E, or placebo for nonalcoholic steatohepatitis. *The New England Journal of Medicine, 362*(18), 1675-1685. doi:10.1056/NEJMoa0907929 [doi]

Schindhelm, R. K., Dekker, J. M., Nijpels, G., Stehouwer, C. D., Bouter, L. M., Heine, R. J., & Diamant, M. (2007). Alanine aminotransferase and the 6-year risk of the metabolic syndrome in caucasian men and women: The hoorn study. *Diabetic Medicine : A Journal of the British Diabetic Association, 24*(4), 430-435. doi:DME2100 [pii]

Schindhelm, R. K., Diamant, M., Bakker, S. J., van Dijk, R. A., Scheffer, P. G., Teerlink, T., . . . Heine, R. J. (2005). Liver alanine aminotransferase, insulin resistance and endothelial dysfunction in normotriglyceridaemic subjects with type 2 diabetes mellitus. *European Journal of Clinical Investigation, 35*(6), 369-374. doi:ECI1502 [pii]

Schwimmer, J. B., Deutsch, R., Kahen, T., Lavine, J. E., Stanley, C., & Behling, C. (2006). Prevalence of fatty liver in children and adolescents. *Pediatrics, 118*(4), 1388-1393. doi:118/4/1388 [pii]

Shih, M. H., Lazo, M., Liu, S. H., Bonekamp, S., Hernaez, R., & Clark, J. M. (2015). Association between serum uric acid and nonalcoholic fatty liver disease in the US population. *Journal of the Formosan Medical Association = Taiwan Yi Zhi, 114*(4), 314-320. doi:10.1016/j.jfma.2012.11.014 [doi]

Sirota, J. C., McFann, K., Targher, G., Johnson, R. J., Chonchol, M., & Jalal, D. I. (2013). Elevated serum uric acid levels are associated with non-alcoholic fatty liver disease independently of metabolic syndrome features in the united states: Liver ultrasound data from the national health and nutrition examination survey. *Metabolism: Clinical and Experimental, 62*(3), 392-399. doi:10.1016/j.metabol.2012.08.013 [doi]

Strauss, R. S., Barlow, S. E., & Dietz, W. H. (2000). Prevalence of abnormal serum aminotransferase values in overweight and obese adolescents. *The Journal of Pediatrics, 136*(6), 727-733. doi:S0022347600246453 [pii]

Taitano, A. A., Markow, M., Finan, J. E., Wheeler, D. E., Gonzalvo, J. P., & Murr, M. M. (2015). Bariatric surgery improves histological features of nonalcoholic fatty liver disease and liver fibrosis. *Journal of Gastrointestinal Surgery : Official Journal of the Society for Surgery of the Alimentary Tract, 19*(3), 429-36; discussion 436-7. doi:10.1007/s11605-014-2678-y [doi]

Tran, T. T., Changsri, C., Shackleton, C. R., Poordad, F. F., Nissen, N. N., Colquhoun, S., . . . Martin, P. (2006). Living donor liver transplantation: Histological abnormalities found on liver biopsies of apparently healthy potential donors. *Journal of Gastroenterology and Hepatology, 21*(2), 381-383. doi:JGH3968 [pii]

Wanless, I. R., & Lentz, J. S. (1990). Fatty liver hepatitis (steatohepatitis) and obesity: An autopsy study with analysis of risk factors. *Hepatology (Baltimore, Md.), 12*(5), 1106-1110. doi:S0270913990002610 [pii]

Welsh, J. A., Karpen, S., & Vos, M. B. (2013). Increasing prevalence of nonalcoholic fatty liver disease among united states adolescents, 1988-1994 to 2007-2010. *The Journal of Pediatrics, 162*(3), 496-500.e1. doi:10.1016/j.jpeds.2012.08.043 [doi]

Welzel, T. M., Graubard, B. I., Zeuzem, S., El-Serag, H. B., Davila, J. A., & McGlynn, K. A. (2011). Metabolic syndrome increases the risk of primary liver cancer in the united states: A study in the SEER-medicare database. *Hepatology (Baltimore, Md.), 54*(2), 463-471. doi:10.1002/hep.24397 [doi]

Xu, C., Yu, C., Xu, L., Miao, M., & Li, Y. (2010). High serum uric acid increases the risk for nonalcoholic fatty liver disease: A prospective observational study. *PloS One, 5*(7), e11578. doi:10.1371/journal.pone.0011578 [doi]

Younossi, Z. M., Stepanova, M., Afendy, M., Fang, Y., Younossi, Y., Mir, H., & Srishord, M. (2011). Changes in the prevalence of the most common causes of chronic liver diseases in the united states from 1988 to 2008. *Clinical Gastroenterology and Hepatology : The Official Clinical Practice Journal of the American Gastroenterological Association, 9*(6), 524-530.e1; quiz e60. doi:10.1016/j.cgh.2011.03.020 [doi]

Zhang, Y. X., Wang, Z. X., Chu, Z. H., & Zhao, J. S. (2017). Blood pressure profiles of children and adolescents categorized by waist-to-height ratio cutoffs: Study in a large sample in shandong, china. *Blood Pressure Monitoring,* doi:10.1097/MBP.0000000000000249 [doi]