RELATIONSHIPS BETWEEN CENTRL OBESITY, FRUCTOSE INTAKE, AND NON-ALCOHOLIC FATTY LIVER DISEASE IN OVERWEIGHT HISPANIC ADOLESCENTS

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ABSTRACT

Introduction: The purpose of this study was to evaluate whether central (visceral) fat measured as waist circumference and daily fructose intake were associated with measures of non-alcoholic fatty liver disease (NAFLD) in Hispanic overweight adolescents.

Methodology: Thirty four obese boys (n=15) and girls (n=19) between the ages of 13 and 18 years were measured for body composition, waist circumference, and dietary fructose intake. Fasting alanine (ALT) and aspartate (AST) aminotransferase levels in blood, ALT/AST ratio, and hepatic fat fraction (HFF) by magnetic resonance imaging were measured and used as surrogates for NAFLD. Pearson Product-Moment correlation analyses were used to assess correlations between variables. Linear regression models were created to examine independent correlations after controlling for the covariates age, sex, and body mass index (BMI).

Results: Overall, 26% of participants had fatty liver (HFF > 5.5%) compared to 20% of boys and 32% of girls, however, differences were not significant (χ^2 (1) = 0.57, p = 0.47). All but one participant had normal ALT and AST levels in blood (< 40 U/L). An ALT/AST ratio > 1.0 was seen in 44% of all participants. Statistically significant correlations included age and log ALT/AST (r = 0.42, $p < 0.05$), fat mass (r = 0.63, $p < 0.01$), fat-free mass (r = 0.80, $p < 0.01$); sex (1=male, 2=female) and BMI (r = 0.36, $p < 0.05$), log HFF (r = 0.37, $p < 0.05$), total caloric intake (r = -0.36, $p < 0.05$), fat mass (r = 0.36, $p < 0.05$), fat-free mass (r = -0.34, $p < 0.05$); fructose intake and total caloric intake (r = 0.48, $p < 0.01$); BMI and log HFF (r = 0.99, $p < 0.01$), log ALT/AST (r = 0.37, $p < 0.05$), fat mass (r = 0.80, $p < 0.01$), fat-free mass (r = -0.43, $p < 0.01$); log HFF and log ALT/AST (r = 0.40, $p < 0.05$), fat mass (r = 0.79, $p < 0.01$), fat-free mass (r = -0.45, $p < 0.01$); waist circumference and BMI (r = 0.62, $p < 0.01$), HFF (r = 0.65, $p < 0.05$), log ALT (r = 0.41, $p < 0.05$), log ALT/AST (r = 0.57, $p < 0.01$), fat mass (r = 0.53, $p <$

0.01), fat-free mass (r = 0.49, $p < 0.01$). In multivariate linear regression analysis, waist circumference was significantly associated with log HFF (β = 0.06, $p < 0.01$) and log ALT/AST (β = 0.49, $p < 0.05$), independent of age, sex, and BMI. Fructose intake was not significantly associated ($p > 0.05$) with log HFF (β = -0.05), log ALT (β = 0.19), log AST (β = 0.24) or log ALT/AST (β = 0.07), independent of total caloric intake.

Conclusion: The current study demonstrated that waist circumference, an indirect measure of central (visceral) fat, was significantly associated with log HFF and log ALT/AST, independent of age, sex, and BMI. Dietary fructose intake was not significantly associated with any of the measures of NAFLD. The prevention of obesity should be a primary intervention in children at risk for NAFLD.

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GLOSSARY

Aminotransferases – Enzymes in blood commonly used as indicators of liver disease, specifically, alanine, ALT and aspartate, AST.

Biopsy – Invasive examination of tissue, such as a liver biopsy to examine liver tissue for fat.

Cytokine – Signaling molecules used for cellular communication of the immune system.

De novo lipogenesis – The synthesis of new fatty acids.

Free fatty acid – An uncombined carboxylic acid that is used for energy.

Hepatic fat fraction - Percentage of liver fat by non-invasive imaging techniques like ultrasound and magnetic imaging resonance.

Obese –In the field of medicine the term "obesity" has been used to characterize a BMI $\geq 95^{th}$ percentile in children and adolescents. The terms overweight and obesity are often used interchangeably in pediatric patients. The term obesity was used in this report to express the seriousness of this medical condition.

Overweight – According to the Centers for Disease Control and Prevention (CDC 2001) growth charts by age and weight, normal weight is defined as a BMI $> 5^{th}$ and $< 85^{th}$ percentile. At risk for overweight is defined as a BMI $\geq 85^{th}$ and $< 95^{th}$ percentile. A BMI $\geq 95^{th}$ percentile is considered overweight.

Oxidation – A series of reactions in which energy is derived from a molecule. For example, the oxidation of sugar or fatty acids would include the chemical reactions necessary so that energy is derived.

Steatohepatitis – Liver fat buildup plus inflammation.

Steatosis – Simple liver fat buildup.

Chapter 1

Non-alcoholic fatty liver disease (NAFLD) is characterized as the development of fatty liver in persons without a significant history of alcohol use (Browning et al., 2004). The condition is not thoroughly understood and it can range from simple liver fat buildup (steatosis) to liver fat with inflammation (steatohepatitis) to necrosis and cirrhosis (Day & James, 1998). Risk factors include obesity, elevated triglycerides, and insulin resistance (Barshop, Sirlin, Schwimmer, & Lavine, 2008). Non-alcoholic fatty liver disease is higher in Hispanics when compared to non-Hispanic whites and blacks, likely because of a higher prevalence of risk factors (Browning et al., 2004).

The U.S. has seen an increase in the prevalence of NAFLD comparable to childhood obesity in the past 30 years (Chavez-Tapia et al., 2007). Obesity is almost always present in children with NAFLD (M. H. Fishbein, Mogren, Gleason, & Stevens, 2006). Obesity accelerates the lipolysis of fatty tissue (Fishbein, Miner, Mogren, & Chalekson, 2003) and inflammatory processes (Busetto et al., 2002) which are believed to increase in the liver, fat buildup (Brunt, 2005) and oxidative stress (Da Silva, Rabello Coelho, Rabello Coelho, & Fazzio Escanhoela, 2009). In addition to overall obesity, central (visceral) fat is believed to play a role in fatty liver buildup (Sabir, Sermez, Kazil, & Zencir, 2001). Further insight is needed to establish the mechanisms by which visceral fat contributes to NAFLD in children.

Components of the Western diet are also thought to contribute to NAFLD within developed countries (Ackerman et al., 2005). Sugar consumption has risen in parallel to the prevalence of NAFLD and childhood obesity (Chavez-Tapia et al., 2007). Specifically, high-fructose intake is thought to result from the excessive consumption of sugar-sweetened, processed foods and beverages (Ouyang et al., 2008). High-fructose intake accelerates fatty acid

synthesis (Donnelly et al., 2005) and oxidation (Bantle, Raatz, Thomas, & Georgopoulos, 2000) which is believed to lead to liver fat buildup (Cohen & Schall, 1988) and oxidative stress (Da Silva et al., 2009). The mechanisms by which high-fructose intake contributes to NAFLD remains largely unknown, therefore continued investigations are recommend.

Obesity, high-fructose intake, and NAFLD have increased similarly in children in recent years (Browning et al., 2004). Central (visceral) obesity and high-fructose intake have been shown to accelerate liver fat buildup in experimental animal studies (Kallwitz et al., 2008; Ouyang et al., 2008). In humans, large epidemiological studies are few, especially in children. Further efforts are needed to identify and understand the role different risk factors play. A review of the literature examines the background information that focused on the impact of visceral fat and high-fructose intake on the development and progression of NAFLD. In addition, the diagnosis and pathogenesis of NAFLD were reviewed. Reviewing these topics may provide insight in the prevention and management of NAFLD in children.

Literature Review

Non-alcoholic fatty liver disease ranges from simple liver fat buildup (steatosis) to liver fat with inflammation (steatohepatitis) and may possibly progress to cirrhosis and liver failure in individuals without a history of significant alcohol use, defined as no more than 2 drinks or 20g of alcohol per day (Duvnjak et al., 2007). The term NAFLD was coined in the 1980s after liver disease was found in a group of obese females without a history of alcohol use (Ludwig, Viggiano, McGill, & Oh, 1980). Since then NAFLD has been used to illustrate a range of liver diseases in which alcohol does not play a factor.

Epidemiology. The NALFD prevalence in the U.S. is unknown because liver biopsies are unethical in asymptomatic individuals and impractical in large population-based studies

(Barshop et al., 2008). Estimates from epidemiological data suggest that 10-30 percent of adults (Gaby, 2005) and 20 percent of all children (McCullough, 2006) have NAFLD. Schwimmer et al. (2006) evaluated health records of 742 children between 2 and 19 years of age who had an autopsy performed by a county medical examiner during a 10-year retrospective study. Children had died of accidental, non-liver related fatal injuries. Fatty liver was defined as liver fat (hepatic fat fraction, HFF) ≥ 5%. Fatty liver was seen in 13 percent of children and obesity was present in almost all children with NAFLD. Although mostly asymptomatic, estimates indicate that NAFLD is the most common liver disorder of children (Cave et al., 2007; Manco et al., 2008; Schwimmer, Deutsch, Rauch, Behling, Newbury, & Lavine, 2003b; Schwimmer et al., 2005).

Obesity is major risk factor for NALFD (Leung, Williams, Fraley, & Klish, 2009). The U.S. has seen a three-fold increase in the percentage of overweight children in the last three decades (Freedman, Khan, Serdula, Ogden, & Dietz, 2006). Obesity in children aged 2 to 19 years has increased from less than five percent to approximately 17.1 % between 1980 and 2002 according to the National Health and Nutrition Examination Survey (NHANES) 2003-2004 (Ogden et al., 2006). Approximately 10 percent of all overweight children are believed to have NAFLD (Strauss, Barlow, & Dietz, 2000). Schwimmer et al. (2003) evaluated children from 1999-2002 at the Children's Hospital San Diego and reported that 88 percent of children with biopsy-confirmed NAFLD were obese. With a rise in the prevalence of childhood obesity, NAFLD is expected to become a major public health concern in the near future.

Non-alcoholic fatty liver disease is the most common cause of abnormal liver enzyme levels in the blood (Strauss et al., 2000). An estimated 10 to 25% of overweight obese children have elevated liver enzymes in blood (Cave et al., 2007). With data from NHANES III, Strauss et al. (2000) evaluated the prevalence of elevated ALT levels in 2,450 adolescents aged 12-18

years. Participants were classified by age and weight as normal, overweight if BMI > 85th percentile, and obese if BMI > 95th percentile. Sixty percent of participants with elevated ALT levels were either overweight or obese. Six percent of all overweight adolescents had elevated ALT levels as did 10% of all obese participants. One percent of obese participants had ALT levels that were more than twice the upper limit of normal. These results suggest that overweight and obesity are related to elevated ALT levels in blood.

Studies have reported ethnic differences in the prevalence of NAFLD (Mager & Roberts, 2006). Hispanics are especially vulnerable to NAFLD when compared to non-Hispanics (Cave et al., 2007); in-part attributable to a higher prevalence of risk factors (Duvnjak et al., 2007). Hispanic children are more likely than whites and blacks to be overweight (Freedman et al., 2006) and insulin resistant (Goran, Bergman, Cruz, & Watanabe, 2002). Clark, Brancati, & Diehl (2003) reported ethnic differences in the prevalence of elevated liver enzymes levels in adults with data from NHANES III (1994-1998). Overall 7.9% of participants had elevated liver enzymes compared to 14.9% of Mexican Americans, 8.1% of blacks, and 7.1% of whites. Mexican Americans had an almost two-fold higher prevalence of elevated liver enzymes in blood than whites and blacks. A study by Kallwitz et al. (2008) reported ethnic differences of elevated liver enzyme levels in a multi-ethnic sample of 567 obese adults from Chicago, Illinois. Elevated liver enzyme levels were highest in Hispanics, then whites and blacks at 39, 28, and 12 percent, respectively. Browning et al. (2004) assessed 2,349 ethnically diverse adults from the Dallas Heart Study for fatty liver by proton magnetic resonance spectroscopy. Overall, approximately 33 percent of participants had fatty liver compared to 45, 33, and 24 percent of Hispanics, whites, and blacks, respectively. Evidence supports the hypothesis that the prevalence of NAFLD differs by ethnicity.

Hispanic children are also at-risk for NAFLD. Schwimmer et al. (2005) evaluated 127 obese but otherwise healthy 12th grade Hispanic, black, and white students from the Child and Adolescent Trial for Cardiovascular Health in California, Louisiana, Minnesota, and Texas. Hispanics had the highest prevalence of elevated liver enzymes when compared to whites and blacks at 36, 22, and 14%, respectively. After controlling for BMI and sex, Hispanics had a significantly higher prevalence of elevated liver enzymes when compared to blacks but not whites. A high prevalence obesity and insulin resistance is thought to increase the risk of NAFLD in Hispanic children.

Diagnosis. Symptoms may include upper abdominal or right upper quadrant discomfort and fullness; however, symptoms are unlikely. A liver biopsy is the gold standard in diagnosis (Schwimmer et al., 2005); however, it is invasive, expensive, and unethical for individuals with an unremarkable symptomology (J. B. Schwimmer, Deutsch, Rauch, Behling, Newbury, & Lavine, 2003b). Elevated liver enzyme levels in blood, enlargement of the liver, and increased liver fat (Cave et al., 2007) are signs of NAFLD when alcohol use, Wilson's disease, viral hepatitis, and environmental toxin exposure are absent (Schwimmer et al., 2003).

Positive correlations between elevated liver enzyme (ALT, alanine; AST, aspartate; aminotransferases) levels in blood and NAFLD have been shown in previous research (J. B. Schwimmer et al., 2005). Little consensus exists on normal levels among children, however, many clinicians and researchers (Schwimmer et al., 2005; Siest et al., 1975) acknowledge 40 U/L as the upper limit of normal. Love-Osborne, Nadeau, Sheeder, Fenton, & Zeitler (2008) assessed the prevalence of fatty liver and elevated liver enzymes in children. Authors reported that 76 percent of Hispanic children with an ALT > 40 U/L had fatty liver by ultrasound. A study by Patton et al. (2008) of 176 children aged 6 to 17 years reported a significant association between