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Relationships Between Central 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) $\geq 5\%$. 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

AST levels in blood and severity of liver disease. Rashid & Roberts (2000) followed 36 children with NAFLD for 10 years in Ontario, Canada. Overall, 83 percent of participants were obese and 97 percent had elevated liver enzyme levels in blood. The average age at diagnosis was 12, however, ranged from 4 to 16 years. One 10-year old child presented with cirrhosis of the liver at diagnosis. Studies suggest that elevated liver enzyme levels are correlated with NAFLD. When compared to a biopsy, assessing liver enzyme levels in blood is less invasive, inexpensive, and practical for screening large populations.

Ultrasound is routinely used to assess liver fat and has been shown to have a sensitivity of 89-95% and a specificity of 84–95% (Love-Osborne et al., 2008). A study by Fishbein et al. (2005) assessed liver fat in 31 participants by ultrasound and biopsy. Liver fat by ultrasound was significantly correlated ($r = 0.90$, $p < 0.001$) with liver fat by biopsy. Researchers reported that ultrasound was effective in detecting excessive but not minimal liver fat. Ultrasound may therefore fail to detect simple liver fat buildup in newly developed NAFLD, however, should be considered as non-invasive, alternatives to biopsies.

Magnetic resonance imaging (MRI) is also routinely used to assess liver fat buildup. Fishbein et al. (2005) investigated liver fat percentage by MRI and biopsy in 38 participants and reported a strong correlation between the two techniques ($r = 0.92$, $p < 0.00$). When compared to ultrasound, MRI was more effective in detecting excessive and minimal liver fat. Although a liver biopsy is the gold standard for diagnosing NAFLD, MRI has become commonly used because it is one of the most sensitive, reliable, and accurate imaging techniques available (Fishbein et al., 2006).

Pathogenesis. The pathogenesis of NAFLD is not completely understood. A ‘two-hit’ theory has been proposed (Day & James, 1998). Liver fat buildup is believed to be the first-hit in

which the liver becomes vulnerable for further insults. The second-hit is thought to be the subsequent oxidative and inflammatory insults. Oxidative stress is important in liver injury.

The first-hit, liver fat buildup, results from an unbalanced ratio of triglyceride import and synthesis to export and oxidation (Duvnjak et al., 2007). Triglycerides are constructed with fatty acids that derive from de novo lipogenesis (newly synthesized), dietary intake, and pre-existing storage pools (Vedala, Wang, Neese, Christiansen, & Hellerstein, 2006). Chylomicrons (protein coated lipid carriers) transport fatty acids derived from dietary intake to the liver. Fatty acids are also obtained from the lipolysis of fat or from the breakdown of lipoproteins in the blood. The rate at which fatty acids are made available for triglyceride assembly is dependent upon the equilibrium of many biochemical pathways.

After triglycerides are assembled, they are stored, exported, or oxidized. Triglycerides may be directed to liver cytoplasm storage pools via the delayed secretory pathway (Vedala, Wang, Neese, Christiansen, & Hellerstein, 2006) or coupled with apolipoprotein B (Apo B) via the immediate secretory pathways for export as very low density lipoprotein triglycerides (Figure 1). Triglycerides may also be oxidized as fatty acids in liver cell mitochondria. When triglyceride input and output fail to exist in equilibrium, liver fat buildup is likely.

Stable-isotope tracer techniques are useful in identifying the origins of fatty acids used in triglyceride assembly. A study by Donnelly et al. (2005) used a tracer technique to assess the proportion of fatty acids derived from de novo lipogenesis for triglycerides assembly in adults with NAFLD. Compared to healthy controls (5%), adults with NAFLD had a higher proportion (25%) of fatty acids derived from de novo lipogenesis for triglyceride production. Diraison, Moulin, and Beylot (2003) also used a tracer technique to assess the source of fatty acids used for triglyceride assembly in adults with (n=6) and without (n=5) NAFLD. Although both groups

had similar rates of triglyceride assembly, adults with NAFLD had a higher proportion of fatty acids that derived from de novo lipogenesis when compared controls. Increased de novo lipogenesis is believed to accelerate the first-hit, liver fat buildup in the development of NAFLD.

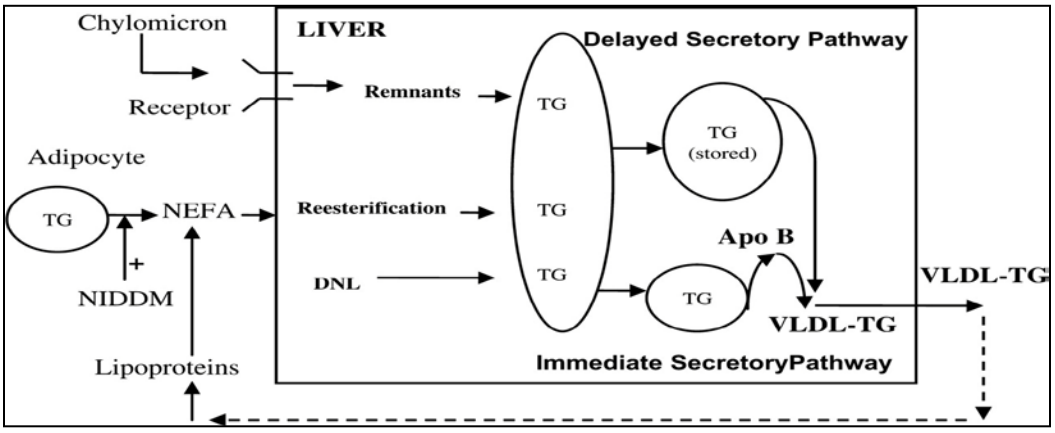

Figure 1. Liver Triglyceride Secretory Pathways. Triglycerides TG, de novo lipogenesis DNL, apolipoprotein B Apo B, very low density lipoprotein triglyceride VLDL-TG, non-esterified fatty acid NEFA, Non-insulin dependent diabetes mellitus NIDDM. Source: Vedala et al. (2006).

The second-hit is believed to result from the accumulation of oxidative and inflammatory metabolic reactions. Specifically, free radicals such as those produced by fatty acid oxidation are thought to be toxic to liver cells. When reactive species accumulate, oxidative stress occurs. Oxidative stress may impair mitochondrial function by altering deoxyribonucleic acid (DNA) structure and function. Sanyal et al. (2001) assessed adults with (n=10) and without (n=6) NAFLD for oxidative stress by performing an immunohistochemical stain on biopsied liver tissue for 3-nitrotryosine, a surrogate for oxidative stress. Adults with fatty liver plus inflammation had the highest level of oxidative stress, followed by adults with simple fatty liver, and then controls. Oxidative stress was further investigated by electron spectroscopy. Structural abnormalities in mitochondria were assessed in liver cells of adults with NAFLD. Approximately 90 percent of adults with fatty liver plus inflammation (steatohepatitis) had abnormal enlargement of mitochondria and low cristae, a component needed for the production of

adenosine triphosphate (ATP). Adults with simple fatty liver were observed to have normal mitochondria without any abnormalities. Oxidative stress is a major component of the second-hit that is believed to contribute to the progression of NAFLD.

Inflammatory processes are also believed to be a major component of the second-hit. Cytokines such as tumor necrosis factor–alpha (TNF-α) are produced by fatty tissue and believed to advance liver injury by stimulating pro-inflammatory mechanisms. Chronically elevated levels of TNF-α have been reported in adults with NAFLD (Zivkovic, German, & Sanyal, 2007). Crespo et al. (2001) assessed the expression of TNF-α messenger ribonucleic acid (mRNA) in 52 obese NAFLD adults by reverse transcriptase polymerase chain reaction (RT-PCR). Messenger RNA is the genetic precursor from which TNF-α is assembled. Adults with fatty liver plus inflammation had significantly higher levels of TNF-α mRNA and fat in liver cells than adults with simple fatty liver without inflammation.

Anti-inflammatory peptides called adipokines are also produced and released by fatty tissue. These molecules protect against inflammatory second-hits. For example, adiponectin is thought to increase fatty acid oxidation while decreasing fatty acid synthesis thus reducing the potential for liver fat buildup. Adults with fatty liver plus inflammation have been observed to have lower levels of adiponectin and complimentary receptors in liver cells when compared to adults with simple fatty liver without inflammation (Duvnjak et al., 2007). Adiponectin, although anti-inflammatory, may contribute to second-hits when in limited amounts.

Insulin resistance is believed to contribute to NAFLD, however, not a direct player in the ‘two-hit’ theory. Insulin resistance is thought to contribute to liver fat buildup by increasing the lipolysis of fatty tissue and stimulating fatty acid synthesis and triglyceride assembly in the liver

(Duvnjak et al., 2007). Furthermore, insulin resistance is believed to decrease fatty acid oxidation and inhibit triglyceride export resulting in liver fat buildup (Zivkovic et al., 2007).

The 'two-hit' theory suggests that the first-hit is liver fat buildup. Excessive liver fat is believed to leave the liver vulnerable to numerous secondary insults. The second-hit is composed of numerous, smaller, oxidative and inflammatory mechanisms that are believed to lead to inflammation, necrosis, fibrosis, and cirrhosis.

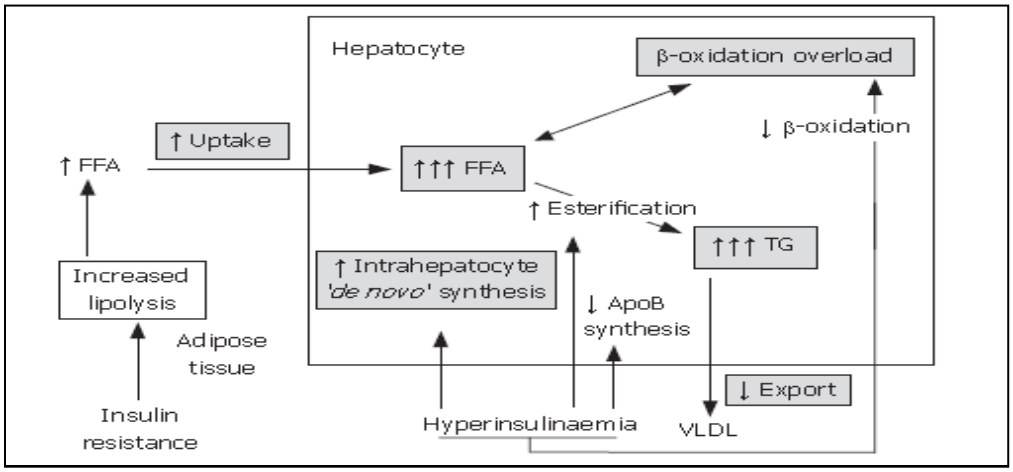

Figure 2. Insulin Resistance in Triglyceride Production. Free fatty acid FFA, triglyceride TG, very low density lipoprotein VLDL, apolipoprotein B Apo B. Source: (Duvnjak et al., 2007).

Risk factors. Obesity, elevated triglycerides, and insulin resistance are important risk factors (Barshop et al., 2008). In addition to overall obesity, central (visceral) fat is believed to play a role in NAFLD. Changes in dietary intake have been suggested to contribute to NAFLD, specifically high-fructose consumption which has steadily increased in the past three decades and common in western diets (Cave et al., 2007).

Obesity. Obesity and obesity-related diseases have increased within developed countries in the past 20 to 30 years (Havel, 2005). Obesity is almost always present in adults with NAFLD and is often the most important clinical indicator of NALFD in children (Barshop et al., 2008). The association between obesity and NAFLD has been demonstrated in studies of adults and

children throughout the world (Sabir et al., 2001). Obesity is believed to contribute to the pathogenesis of NAFLD by accelerating the lipolysis of fatty tissue which results in increased levels of fatty acids in blood that are shunt to the liver (Burgert et al., 2006). The liver separates fatty acids for storage, conversion to triglycerides, or oxidation for energy (Carter-Kent et al., 2009).

Researchers in Asia, North America, and Europe estimate the prevalence of NAFLD in obese adolescents to range from 10 to 77 percent (Barshop et al., 2008). When compared to non-obese individuals, the NAFLD prevalence is approximately three to four times higher in obese individuals (Barshop, Sirlin, Schwimmer, & Lavine, 2008; Chavez-Tapia et al., 2007). Previous studies indicate that obesity is associated with NAFLD independent of age, elevated triglycerides, and insulin resistance (Bantle et al., 2000; Burgert et al., 2006; J. B. Schwimmer, Deutsch, Rauch, Behling, Newbury, & Lavine, 2003b). Obese children appear to be at higher risk for NAFLD when compared to non-obese children (Barshop et al., 2008; Cave et al., 2007). Central (visceral) obesity is suggested to play role in NAFLD, independent of peripheral and subcutaneous fat (Barshop et al., 2008).

Burgert et al. (2006) assessed 72 obese adolescents (BMI > 95^{th} percentile) for fatty liver (HFF > 5.5%) by MRI. Thirty-two percent of participants had fatty liver. Participants with and without fatty liver had a similar percentage of overall body fat; however, participants with fatty liver had significantly higher visceral but lower subcutaneous fat. A study by Sabir et al. (2001) assessed 68 obese and 40 non-obese female adults for liver, visceral, and subcutaneous fat by ultrasound. Of obese participants, 66 percent had fatty liver and when compared to non-obese females, had a significantly higher percentage of visceral fat. Fatty liver was significantly associated with visceral ($r = 0.573$, $p < 0.0001$) and subcutaneous ($r = 0.37$, $p < 0.05$) fat. A

stronger correlation was demonstrated between fatty liver and visceral than subcutaneous fat. Fishbein et al. (2006) examined the association between visceral fat and fatty liver in 29 obese children in a retrospective review of health charts. Visceral ($r = 0.37$, $p < 0.05$) but not overall or subcutaneous fat was significantly related to fatty liver (HFF > 6.0%).

A study by Busetto et al. (2002) assessed fatty liver by ultrasound in six morbidly obese females undergoing gastric banding surgery for weight loss. Females were evaluated before (baseline) and two and six months after surgery for reductions in overall, visceral, and liver fat. When compared to baseline, total body fat was significantly reduced at two and six months after surgery. Visceral and liver fat were significantly reduced when compared to baseline at two months but not at six months after surgery. Liver fat was not reduced at six months after surgery in contrast to reductions of overall body fat when compared to baseline. Reductions in liver and visceral fat paralleled each other more closely than reductions in liver and overall fat. Investigators concluded that visceral fat was a better indicator of liver fat when compared to overall fat.

Obesity is almost always present in adults with NAFLD and to a lesser extent the same goes for children (Cave et al., 2007). The prevalence of obesity (Ogden et al., 2006) and NAFLD (Quirós-Tejeira et al., 2007) in children has increased similarly in recent years such that, obesity is now one of the most important and widely accepted risk factors for NAFLD (J. B. Schwimmer, Deutsch, Rauch, Behling, Newbury, & Lavine, 2003b). Central (visceral) obesity is believed to play a major role in NAFLD and hypothesized to be better indicator of fatty liver than overall fat alone (Lonardo, Bellini, Tartoni, & Tondelli, 1997).

Fructose. In addition to obesity, changes in dietary consumption have been suggested to contribute to the development of NAFLD (Cave et al., 2007). Between 1975 and 2000, according

to the U.S. Department of Agriculture, the availability of food has increased by 20 percent (Havel, 2005). Total caloric intake has also increased in the U.S. in the last 20-30 years (Barshop et al., 2008). The U.S. Department of Agriculture (2006) recently reported that Mexican American adolescents aged 12-19 years consumed on average 2,194 kcal per day. A study by (Ogden et al., 2006) conducted with data from CDC NHANES (2003-2004) found increases in total caloric intake by seven percent and carbohydrate intake by 22 percent in adults. Furthermore, the annual per capita dietary carbohydrate intake had increased from 64 to 80 kg.

Sugar consumption has also increased, specifically fructose, primarily by the consumption of high-fructose containing processed foods and beverages (Havel, 2005). Increases in fructose intake are analogous to increases in the prevalence of obesity and NAFLD in children (Cave et al., 2007). Dietary recommendations in the U.S. indicate that at maximum, 50% of total caloric intake should derive from carbohydrates and only 25 percent of carbohydrates should derive from sugars (Havel, 2005). Fructose is a naturally occurring sugar commonly found in fruits and vegetables, however, fructose has become a major component of sweeteners used in processed foods and beverages in developed countries because it is sweeter, less expensive, and smaller than other sugars (Faeh et al., 2005). Fructose comprises 50% of all sweeteners consumed in the U.S. annually (Zhang, Perdomo, Kim, Qu, Ringquist, Trucco, & Dong, 2008a). Sweeteners like high-fructose corn syrup (HFCS) and sucrose are comprised of 50-55% fructose (Schulze et al., 2004). The U.S. consumption of HFCS has increased by 20-30 % in the past two decades (Cave et al., 2007).

Studies in animals (Ackerman et al., 2005) and humans (Bantle et al., 2000) have demonstrated that high-fructose intake is related to poor health such as obesity, elevated triglycerides, and fatty liver. Fructose derived from natural and artificial sources are metabolized

identically (Bantle et al., 2000). Natural fruits and vegetables provide small amounts of fructose and pose little risk in contributing to high-fructose intake, however, artificial sources are believed to be the biggest provider of fructose in high-fructose consumption (Schulze et al., 2004). For example, a cup of raw tomatoes has about 2.5 g of fructose, whereas a 12-ounce non-diet cola has about 23 g (Wharton & Hampl, 2004). One 32-ounce non-diet cola has about 62 g of fructose and is a much larger contributor to total fructose intake than most fruits and vegetables combined (Vos, Kimmons, Gillespie, Welsh, & Blanck, 2008). High-intake fructose intake is believed to have poor effects on health regardless of source i.e. natural versus artificial (Teff et al., 2004). Natural fruits and vegetable are not likely a major contributor to high-fructose intake when compared to artificial sources like sugar-sweetened carbonated beverages (Schulze et al., 2004).

Sugar-sweetened beverages are the largest source of dietary fructose (Havel, 2005) and account for twice the caloric intake of fruit and fruit juice combined in children, approximately eight and four percent, respectively. Studies in adolescents, have demonstrated that 20-30% of total caloric intake derived from sugar-sweetened beverages (Gaby, 2005). Adolescent males are the largest consumers of sugar-sweetened beverages and are estimated to consume over two times the average caloric intake or about 16 percent (Havel, 2005). A survey of 1,400 eighth-grade students demonstrated that 32.4% of participants consumed at least 200 g/d of sugars derived from sugar-sweetened beverages, approximately half of which, 100 g/d, was fructose (Havel, 2005). Based on a 2,000 kcal/d diet, the upper limit of normal for fructose intake is 60 g (Havel, 2005). Vos et al. (2008) used data from NHANES (2004-2005) of 21,483 adolescents aged 12-19 and reported an average daily fructose intake of 72.8 g. An average daily fructose intake of 54.7 g was reported for all ages combined.

Sugar-sweetened beverages provide a large amount of fructose. Overconsumption of such beverages is therefore believed to contribute to high-fructose intake and potentially increases the risk for adverse health effects in adolescents who are major consumers of such beverages. The availability of total calories and the amount of calories consumed have both increased in the U.S. in a relatively short time and thought to be related to increases in the prevalence of NAFLD in children.

*Metabolism***.** Fructose has a five-carbon molecular ring structure and is the sweetest of all naturally occurring carbohydrates (Cave et al., 2007). Fructose is a small, energy dense, sugar often referred to as an 'empty calorie' because it lacks vitamins, minerals, and amino acids (Cave et al., 2007). Fructose is almost entirely absorbed in the small intestine and then sent to the liver where it is converted to other molecules such as glucose, fatty acids, and glycogen, or oxidized (Havel, 2005). Although fructose is a glucose isomer (same chemical structure, different molecular structure), it is thought to contribute to poor health because of three major distinctions in metabolism that differ from glucose according to a review by (Gaby, 2005). First, the uptake of fructose by liver cells is insulin-independent whereas glucose uptake is insulin-dependent. Therefore, fructose uptake occurs without the regulatory interaction of insulin. Second, fructose metabolism is not rate-limited like glucose. In addition to insulin, glucose metabolism is also regulated by the negative feedback inhibition of previously metabolized end products (substrates) whereas fructose is not. Third, fructose metabolism does not directly stimulate insulin secretion like glucose does. Insulin is vital to the metabolism of simple sugars because it is responsible for the signaling of important biochemical processes. Overall, these three major differences are believed to contribute to both the unregulated uptake and metabolism of fructose by liver cells and low levels of metabolism regulation associated with insulin secretion.

A thorough understanding of the mechanisms by which fructose induces liver fat is not yet complete. Fructose metabolism is insulin-independent therefore uptake and metabolism are highly unregulated (Gaby, 2005). After fructose is sent to the liver it may be converted to other molecules or oxidized (Havel, 2005). When liver storage capabilities are exceeded, excessive fructose is directed to fatty acid synthesis and believed to contribute to NAFLD by increasing liver fat buildup and fatty acid oxidation (Fishbein et al., 2003).

*Animal models***.** Bergheim et al. (2008) used a mouse model to evaluate the effects of fructose-supplemented diets on liver fat. Mice were fed diets supplemented with solutions sweetened with glucose, sucrose, or an artificial sweetener for eight weeks. Mice were assessed for weight gain, liver enzyme levels in blood, and caloric intake and then sacrificed to investigate liver fat. Liver enzyme levels in blood were comparable among mice in all diet conditions before and after treatment. Mice fed glucose-sweetened solutions had the highest total caloric intake and weight gain of all mice; however, mice fed fructose-supplemented diets had significantly higher liver fat when compared to mice fed diets supplemented with glucose, sucrose, and an artificial sweetened. Mice fed diets supplemented with fructose had significantly higher liver fat after controlling for total caloric intake when compared to all other diet conditions, including sucrose which is 50% fructose. Furthermore, liver enzyme levels in blood were normal for all mice regardless of sugar supplementation.

Ackerman et al. (2005) investigated the association between high-fructose intake and measures of NAFLD in rats. Rats were fed a high-fructose or control diet for three weeks and then evaluated triglyceride and cholesterol levels in the liver and in blood. Rats fed high-fructose diets had similar increases in weight gain when compared to controls. Liver enzyme levels in the blood did not differ significantly between both groups. Rats fed a high-fructose diet, however,

had a significantly higher increase in triglycerides and cholesterol levels in the liver when compared to controls at 198 and 89%, respectively and a 223% higher increase in triglycerides in blood, independent of total caloric intake.

A study by Zhang et al. (2008) assessed the effects of high-fructose intake on liver fat in 5-week old hamsters. Hamsters were fed a high-fructose or control diet for eight weeks and then evaluated for liver fat, liver enzymes in blood, and the expression of liver fat synthesizing enzymes. High-fructose fed hamsters had significantly higher liver fat, liver enzymes levels in blood, and expression of liver fat synthesizing enzymes when compared to controls after eight weeks of treatment. Higher liver fat synthesizing enzymes in high-fructose fed hamsters indicates that high-fructose intake induced the production of enzymes needed to synthesize liver fat. Fructose may have been converted to fatty acids or stimulated fatty acid synthesis.

*Human studies***.** Ouyang et al. (2008) conducted a prospective case-control study to evaluate the association between the consumption of sugar-sweetened beverages and NAFLD. Biopsy-proven NAFLD (n=49) adults were matched for gender, age, and body mass index to controls (n=24). An in-person 24-hour dietary recall was conducted to estimate the consumption of sugar-sweetened beverages. Participants with NAFLD had a significantly higher, two-fold increase in the average daily consumption of sugar-sweetened beverages when compared to controls, 365 versus 170 kcal/d, respectively. The difference in sugar-sweetened beverages was independent of total caloric intake. Twelve participants were randomly selected for further investigation. Biopsy-diagnosed NAFLD patients (n=6) and controls (n=6) were evaluated for the genetic expression of two liver enzymes, fatty acid synthase and fructokinase. Fatty acid synthase is an enzyme used in the synthesis of fatty acids and fructokinase is used in fructose metabolism. Controls were patients who had previously undergone a liver biopsy for the

suspicion of NAFLD; however, after histological examination were confirmed to not have any evidence of such a condition. Liver tissue samples from both groups were assessed for the levels of liver enzymes. The expressions of both liver enzymes were significantly higher in NAFLD patients when compared to controls, indicating an acceleration of fructose metabolism and fatty acid synthesis.

Faeh et al. (2005) evaluated the effects of high-fructose intake in a crossover study design of normal-weight, healthy adult males (n=7). Participants were instructed to avoided vigorous physical activity for the duration of the study and were randomly assigned to a series of four differently ordered dietary treatment conditions. Diets included (1) fish oil, (2) high-fructose, (3) fish oil plus high-fructose, and a (4) control. Triglyceride levels in blood and rates of liver fatty acid synthesis were examined after each diet. Tracer techniques were used to evaluate rates of liver fatty acid synthesis. Participants had a washout period after each diet to minimize carryover effects. When compared to the (4) control diet, participants fed a (2) high-fructose diet experienced a significant 6-fold increase in the rate of fatty acid synthesis and a 79 percent increase in triglyceride levels in blood, independent of total caloric intake. Furthermore, participants fed a (2) high-fructose diet had significantly higher triglyceride levels in blood when compared to participants fed (1) fish oil and (3) fish oil plus high-fructose. Results indicate that high-fructose consumption favors liver fatty acid synthesis and elevates triglyceride levels in blood and the liver.

Swanson et al. (1992) used a crossover study design to evaluate the effects of high-fructose intake on fasting cholesterol levels in blood of healthy adults (n=14). Participants consumed at random, a high-fructose diet and a control diet for 28 days each with a washout period between diets. At the end of the high-fructose diet, participants exhibited significantly

higher total and low-density lipoprotein (LDL) cholesterol levels in blood, 9 and 11%, respectively, when compared to the control diet. Results indicated that high-fructose intake increased fats (cholesterol).

The association between non-fasting triglyceride levels and high-fructose intake was evaluated in a study of healthy, normal-weight adults (n=21) by Cohen & Schall (1988). Participants were randomly assigned to one of four fatty meals supplemented with (1) glucose, (2) fructose, (3) sucrose, or (4) fatty meal alone (control). Participants fed fatty meals supplemented with (1) glucose did not experience a significant raise in triglyceride levels in blood when compared to participants fed meals supplemented with (2) fructose, (3) sucrose, or (4) fatty meal alone. Participants who were fed fatty meals supplemented with (2) fructose or (3) sucrose (50% fructose) did not vary amongst each other, however, both groups had significantly higher triglyceride levels in blood when compared to participants fed (4) fatty meal alone. Evidence from this study indicates that fructose and fructose-containing sugars (sucrose) may increase triglyceride levels in blood possibly from increasing liver fatty acid synthesis or by another unknown mechanism in which dietary fat contributes to significant increases in triglyceride levels in blood when consumed with fructose or fructose-containing sugars than when fatty meals are consumed alone.

Summary of Literature Review

A review of the literature suggests that the prevalence of obesity, high-fructose intake, and NAFLD have comparably increased in children in the past three decades. Commonly accepted NAFLD risk factors include obesity, elevated triglycerides, and insulin resistance. Hispanics appear to at a higher risk for NAFLD when compared non-Hispanic whites, likely because of a higher prevalence of risk factors. Studies in humans demonstrate that central (visceral) fat may play an important role in the development of NAFLD. In addition, high-fructose intake is believed to contribute to NAFLD and has been demonstrated in studies of animals and to a lesser extent in humans. Visceral obesity and high-fructose intake are believed to be related to NAFLD; however, further insight is needed to understand the mechanisms by which they contribute to the development and progression of NAFLD.

CHAPTER 2

Purpose and Significance of Research

The childhood prevalence of obesity, high-fructose intake, and NAFLD has similarly increased in the U.S. Hispanics are at higher risk for NAFLD, when compared to non-Hispanic whites and black, in-part attributable to a higher prevalence of risk factors. Clinicians often depend upon the presence of risk factors such as obesity, elevated triglycerides, and insulin resistance to determine risk of NAFLD. Indirect measures of NAFLD include liver enzyme levels in blood and fatty liver on MRI.

The understanding of NAFLD in children remains incomplete, not only because NAFLD is a novel and mostly asymptomatic condition, but also because large, gold-standard biopsy-confirmed, epidemiological studies are scare. A 'two-hit' theory has been proposed in the pathogenesis of NAFLD and often referenced in the literature. The first-hit is liver fat buildup which is thought to leave the liver vulnerable to subsequent insults. The second-hit is the cumulative impact of many oxidative and inflammatory-causing insults which are believed to induce inflammation, necrosis, and cirrhosis.

Obesity is present in most individuals with NAFLD. A review of the literature suggests that obesity, especially visceral fat contributes to the development and progression of NAFLD by two primary mechanisms. First, excessive visceral fat is believe to accelerate the lipolysis of fatty tissue which results in an increase of fatty acids that are shunt to the liver. Increased fatty acids may lead to liver fat buildup and increases in fatty acid oxidation. Second, visceral fat is believed to produce and release increased levels of pro-inflammatory molecules that accelerate oxidative stress.

In addition to obesity, high-sugar intake has been recently suggested in the literature to be a risk factor for NAFLD. High-fructose intake is believed to contribute to NAFLD by two primary mechanisms. First, high-fructose intake favors fatty acid synthesis and second, it increases fatty acid oxidation which may result in liver fat buildup and oxidative stress. Children are major consumers of fructose-containing foods and beverages and therefore may be at high risk for NAFLD.

A complete understanding has not been established for the association of visceral fat and high-fructose intake on measures NAFLD in Hispanic children. A thorough understanding will assist in the prevention and management of NAFLD. The purpose of this study was to investigate the associations between central fat measured as waist circumference, and dietary fructose intake on measures of NAFLD in obese Hispanic adolescents.

Specific Aims

- Aim 1: To investigate the associations between central fat, an indirect measurement for visceral fat, and measurements of NAFLD in obese Hispanic adolescents
  - Null hypothesis: Central fat will not be significantly related to measures of NAFLD.
  - Alternative hypothesis: Central fat will be significantly related to measures of NAFLD, and this will be independent of age, sex, and BMI.
- Aim 2: To investigate the associations between dietary fructose intake and measurements of NAFLD in obese Hispanic adolescents
  - Null hypothesis: Dietary fructose will not be significantly related to measures of NAFLD.
  - Alternative hypothesis: Dietary fructose will be significantly related to measures of NAFLD and the association will be independent of total caloric intake.

Variables

Figure 3 displays variables used in the statistical analysis. Fructose intake was calculated as the total average daily intake over three dietary recalls. Total daily fructose was comprised in grams from both artificial and natural sources. Fructose derived from high-fructose corn syrup for example, was not measured separately from fructose consumed from natural sources such as fruits and fruit juices.

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Independent	Covariate	Dependent
Waist circumference (centimeters) Fructose intake (grams)	Age (years) Sex Body mass index (kg/m^2) Total caloric intake (kilocalories)	Hepatic fat fraction (%) ALT (U/L) AST (U/L) ALT/AST ratio

Figure 3. Variables for Statistical Analysis The contribution of waist circumference and fructose intake on measures of NAFLD were assessed independent of covariates listed in Figure 3.

CHAPTER 3

Methods

Participants. A total of 15 male and 19 female obese Hispanic adolescents between the ages of 13-18 years participated in current study located in El Paso, Texas. In addition to word of mouth, multiple media approaches were used for recruitment, including radio, television, the internet, and newspapers. Presentations were conducted and written media such as fliers and brochures were distributed throughout the community. A proposal was submitted and approved by the El Paso Independent School District Institutional Review Board for permission to recruit students within the El Paso School District. Health care professionals were invited to refer potentially eligible and interested individuals. Adolescents were required to meet inclusion criteria outlined in Table 1 to participate in this study. Adolescents that met exclusion criteria outlined in Table 2 were ineligible for the current study.

Table 1. Inclusion Criteria

- Hispanic Ethnic Origin – Defined as all grandparents of Hispanic ethnicity as determined by self-report by participant and/or parents (legal guardian) during the screening interview.
- Age – Adolescents between 13-18 years.
- Sex – Male and female.
- Obesity Status – Body mass index (BMI) in the 95th percentile or greater by age and sex according to the Centers for Disease Control and Prevention Control Growth Charts (CDC 2001).

Table 2. Exclusion Criteria

- Past Medical History - Participants and/or parents who self-reported during the screening process a medical history of conditions or diseases known to cause liver damage such as toxin exposure, viral or autoimmune hepatitis, alpha-1-antitrypsin deficiency, and Wilson's disease. In addition, adolescents who self-reported a medical history of conditions or diseases known to influence insulin action or body composition during the screening interview.
- Social History – Participants who self-reported during the screening process the use of illicit drugs, alcohol, and tobacco within the past 30 days.
- Medication Use – Participants and/or parents who self-reported during the screening process the use of medications known to alter liver function, insulin activity, or body composition such as growth hormones, certain antibiotics, and anti-thyroid medications during the screening interview.
- Pregnancy – Female participants who self-reported during the screening process Female adolescents who self-reported as being pregnant.

Overview of Testing Procedures

Participants completed two outpatient visits at least one but no more than four weeks apart as outlined in Table 3. Adolescents and at least one biological parent or legal guardian were invited to the Border Clinical Research Center (BCRC) for a first visit. Each adolescent was

provided with an assent document and their parent an informed consent document in the language of choice, English or Spanish. After reading, verbalizing a thorough understanding, and asking questions if needed, each adolescent and parent signed their appropriate document. The informed consent document was signed by the adolescent, his/her parent, and a research team member. The adolescent assent document was signed by the adolescent and a research team member. Adolescents and parents were given copies of each signed consent document.

After consent, each participant had their height and weight measured to the nearest 0.1 cm and 0.1 kg, respectively. A body mass index (BMI) percentile was established by age and sex according to the Centers for Disease Control and Prevention Growth Charts (CDC 2001). Participants that had a BMI $\geq 95^{th}$ percentile were included in the study. A registered nurse then performed a past medical history and physical exam on each participant. Participants self-reported a 24-hour dietary recall before being measured for waist circumference and body composition to complete the first outpatient visit.

Participants returned to the BCRC at 0800 for a second visit at least one but no more than four weeks after the first. A venous puncture was performed by a registered nurse and approximately 2cc of blood was collected into a serum-separator tube which was immediately centrifuged. Serum was separated and sent for laboratory testing. Samples were analyzed for liver enzymes (ALT, AST) by spectrophotometry. Participants were also asked to self-report a 24-hour recall of dietary intake before reporting to Arango Imaging for magnetic resonance imaging (MRI) of the liver. In addition, a 24-hour dietary recall was conducted by phone in between the first and second visit. In total, three (3) self-reported 24-hour dietary recalls were conducted for the current study (one weekend, two weekdays).

Participants were compensated with a $50 gift card for completing the study. Study results were sent directly to the BCRC and reviewed by a registered nurse who contacted parents (legal guardian) and participants by phone to explain results and answer questions, if any. In addition, a hardcopy of results was sent by mail. Participant and parental (legal guardian) permission were required to share study results with outside parties.

Table 3. Testing Procedures

1st Outpatient Visit	2nd Outpatient Visit
• Consent and assent	• 24-hour dietary recall – 2 of 3
• Height and weight to establish eligibility	• Fasting blood draw for liver enzymes (ALT, AST)
• Medical history and physical exam	• MRI for liver fat
• 24-hour dietary recall – 1 of 3	
• Waist circumference	
• Body composition	

Note: An over-the-phone 24-hour dietary recall (3 of 3) was conducted in between the 1st and 2nd visit.

Testing Facilities

Testing took place at three different locations within El Paso, Texas. Specifically, participants completed their first outpatient visit at the Border Clinical Research Center (BCRC), located at 1100 N. Stanton and the Exercise Physiology Laboratory (EPL), located inside the Memorial Gymnasium at the University of Texas at El Paso at 500 W. University Avenue. Participants completed their second outpatient visit at the BCRC and then reported to Arango Imaging, located at 643 S. Mesa Hills Dr for an MRI of the liver.

The BCRC is an outpatient clinical laboratory which has an administrative office area, patient exam rooms, and a small specimen laboratory and thus was used for a majority of participant testing in the current study. The office area was used for the consenting process and a computer in the area was used to calculate participants' BMI percentiles. Patient exam rooms were used to conduct for medical and physical exam and for venous blood collection. Blood samples were processed in the specimen laboratory before being sent for further testing.

The EPL is an outpatient clinical laboratory equipped with exercise physiology testing instruments. Specifically, the BOD POD, a body composition instrument, was used to estimate fat mass and fat-free mass for each participant. The BOD POD is located in a private room within the EPL.

Arango Imaging is an outpatient clinic equipped with magnetic resonance imaging. Participants reported to Arango imaging for an assessment of liver fat. Liver fat was reported as hepatic fat fraction (HFF). Arango Imaging sent study results directly to the BCRC. A registered nurse contacted parents and participants by phone to provide results and answer questions, if any. In addition, hardcopies of study results were sent by mail.

Data Collection

Medical history and physical examination. A registered nurse performed a general medical examination to include a medical history and physical exam in a private exam room. Parents assisted participants in reporting a complete medical history since birth to include conditions or diseases known to cause liver damage such as toxin exposure, viral or autoimmune hepatitis, alpha-1-antitrypsin deficiency, and Wilson's disease. Furthermore, conditions or medications known to influence insulin action or body composition were also investigated.

Parents were asked to step out of the exam room when participants were interviewed for social health history that included the use of alcohol, cigarettes, and illicit drugs within the past 30 days. Female participants were asked to self-report pregnancy status. A systemic physical exam followed the medical history. The exam began with vital signs which included a resting arterial blood pressure, pulse, and body temperature. A thorough investigation beginning at the head and finishing with the extremities followed. The main organs systems were investigated by inspection, palpation, percussion, and auscultation. The liver was palpitated for enlargement. Participants with abnormal or significant findings on exam were further investigated by the nurse examiner for suspected exclusion criteria.

24-Hour dietary recall. Participants self-reported one weekend and two weekday 24-hour dietary recall interviews for a total of three. Two of the interviews were conducted in-person and one was done by phone. The Nutrition Data System for Research (NDSR) dietary software (NDSR 2007, University of Minnesota, Nutrition Coordinating Center, and Minneapolis, MN) was used to conduct each interview. The software is updated annually and has 18,000 foods and beverages in addition to 8,000 brand-name products. National fast food chains are also included in the database such as Subway, Mc Donald's, and Sonic.

The interview began by asking participants general questions about dietary intake for the previous day that included time, meal type, and place. For example, a participant might have reported 0700, breakfast, home to indicate they had eaten breakfast at 7am from home. Participants were then asked to report all foods and beverages consumed at each time reported previously. After all dietary intake was reported, participants were asked to recall the proportion or size of intake. Food props were on display to assist with recalling the amount of intake in addition to measuring cups, bowls, and glasses of various sizes. The NDSR software repeatedly

prompts the interviewer to ask for additional dietary intake that may have been overlooked. The recall ends with asking the participant if the recently reported intake was representative of average daily consumption i.e. higher or lower than usual.

An average of total caloric intake and fructose intake (natural fructose and high-fructose corn syrup) per day were provided by NDSR over three self-reported 24-hour dietary recalls. Average fructose intake was used as an independent variable and total caloric intake was used as a confounding variable in the statistical analyses. The U.S. Department of Agriculture (2006) recently reported that Mexican American adolescents aged 12-19 now consumed on average 2,194 kcal per day. Based on a 2,000 kcal/d diet, the upper limit of normal daily fructose intake is 60g (Vos, Kimmons, Gillespie, Welsh, & Blanck, 2008).

Blood pressure. A registered nurse measured arterial blood pressure in participants with an electronic blood pressure machine that provided results in 30 seconds (Spot Vital Signs® Device, Welch Allyn, Skaneateles Falls, NY). Participants rested for five minutes before having a sitting blood pressure taken on the right arm. Two additional blood pressure readings were taken, each five minutes apart. The measurements were averaged and reviewed in accordance to the Update on the 1987 Task Force Report on High Blood Pressure in Children and Adolescents (Anonymous, 1996).

Height and weight. Participants had their height and weight measured to the nearest 0.1 cm and 0.1 kg, respectively with a Seca balance beam scale that had an attached height measuring rod (Seca® Model 700D, Ontario, CA). A BMI percentile was calculated in accordance with the 2000 Centers for Disease Control and Prevention growth charts (CDC 2001) with public software for research, Epi Info™ (Version 3.5.1, Centers for Disease Control and Prevention, Atlanta, GA),

Body composition. Participants were evaluated for body composition with a two-chambered machine called the BOD POD (Life Measurement, Inc., Concord, CA). The first chamber is called the testing chamber in which the participant sat and the second chamber is called the reference chamber (Fields, Goran, & McCrory, 2002). The reference chamber is connected to the testing chamber by a diaphragm that oscillated to changes in pressure (Dempster & Aitkens, 1995). Like underwater (hydrostatic) weighing, the BOD POD calculates body composition from body volume displacement; however, air instead of water was displaced (Fields et al., 2002). Studies have shown that the BOD POD estimates body composition within one percent of hydrostatic weighing (King et al., 2006). For accuracy purposes, participants were asked to wear minimal clothing and a spandex-like swim cap (King et al., 2006).

The entire testing process took approximately five to ten minutes. Female participants were tested by a research team member of the same sex. Participants were first weighed on an electronic scale provided by the BOD POD for body mass. Second, participants were asked to sit inside the testing chamber and breathe normally. The testing chamber has a window to maximize comfort and lessen anxiety. In addition, an emergency button is located inside the testing chamber had a participant want to stop the test. Third, while inside the testing chamber participants were asked to remain motionless and breathe normally.

After baseline data was acquired, participants were asked to breathe into a plastic tube located inside the testing chamber. Breathing into the tube estimates air lung volume and assists BOD POD in calculating an accurate body volume (Dempster & Aitkens, 1995). After the BOD POD had acquired the participants' body volume and mass, a body density was finally derived. Density was added into a standardized equation used for two-chambered air-displacement instruments such as BOD POD (Fields et al., 2002). Fat and fat-free mass were then calculated

and provided by BOD POD computer software. The BOD POD provided an accurate, safe, and fast approach for evaluating body composition in study participants.

Liver fat. Participants underwent magnetic resonance imaging (MRI) for approximately 30 minutes by a certified technician at Arango Imaging. Images were reviewed by a licensed physician, Dr. Arango. A Toshiba Vantage 1.5 Telsa (Siemens Medical, Erlangen, Germany) was used to perform a dual double-gradient-echo MRI in accordance with the commonly used, Modified Dixon Technique (M. H. Fishbein et al., 2006). The participants had multiple images taken at different segments and phases of respiration (M. H. Fishbein & Stevens, 2001). Three segments of the liver were imaged in total, segment V and VIII of the right lobe and segment II of the left lobe (M. H. Fishbein & Stevens, 2001). Each segment was imaged twice, once after exhaling (in-phase) and again after inhaling (out-of-phase) (M. H. Fishbein et al., 2006). The difference between in-phase and out-of-phase image signal intensity was calculated then entered into a standard equation to derive a hepatic fat fraction by segment. The final HFF was calculated as averaging HFF over three segments of the liver. Browning (2004) recently conducted a large study to investigate the distribution of HFF values in a study sample of 2,500 multi-ethnic adults. Researchers concluded that the upper limit of normal for HFF was 5.5% and has been referenced elsewhere including studies of children (Rashid & Roberts, 2000). The current study adopted the range for normal HFF at 0-5.5%. Participants with a higher percentage liver fat were referred to as having fatty liver.

$$HFF = (\text{Signal Intensity (SI)}_{\text{In-phase}} - SI_{\text{Out-of-phase}} \times 100) / (2)\, SI_{\text{In-phase}}$$

Figure 4. Calculating Hepatic Fat Fraction (HFF) Hepatic fat fraction is reported in percent as the difference between signal intensity when inhaling and exhaling multiplied by a 100 and divided by two (2) times the signal intensity when inhaling.

Liver enzymes. Participants underwent venous puncture after a 12-hour fast for the collection of one blood sample that was evaluated for liver enzymes. Specifically, the quantity of alanine and aspartate aminotransferase levels in blood investigated. The blood draw was performed by a registered nurse who used new latex gloves for each participant. Participants sat during the blood draw and had a tourniquet placed around one arm. Using clean technique, the nurse cleaned the puncture site will an alcohol pad. A 22-gauge sterile needle was used to puncture a vein and 2cc of blood was acquired into a serum separator collection tube.

After the blood draw, a sterile gauze pad was taped in place over the puncture site and the participant was asked to apply direct pressure and remain sitting for approximately 15 minutes. The collection container was inverted eight time, allowed to clot for 30 minutes at room temperature, and then centrifuged for 15 minutes at 2,500 rpm at 4º C to separate serum. Approximately 1.0cc of serum was extracted by pipette into two equivalent 0.5cc micro-vials and immediately placed on ice and shipped to Quest Diagnostics (CLIA-88 certified, CAP-licensed) for testing. Liver enzymes, alanine (ALT) and aspartate (ALT) aminotransferases were evaluated by spectrophotometry (Olympus 5400 random access clinical chemistry analyzer, Coulter Ltd, High Wycombe, UK). Results were sent to BCRC for review by a registered nurse. The upper limit of normal for ALT and AST was 40 U/L in the current study and adopted from an often cited study by (Clark et al., 2003) of data from 15,676 adults from the Third National Health and Nutrition Examination Survey (1988-1994). In addition, an ALT/AST ratio was also calculated from the separate ALT and AST values. A ratio greater than one has been suggested to increase the risk for NAFLD (Matteoni et al., 1999; Younossi et al., 1999)

Risks

Participants had minimal risks associated with participating in the current study. Risks were related to undergoing a fasting blood draw and body composition testing. There were no known risks associated with magnetic resonance imaging. Precautions were taken to minimize risk whenever possible.

Risks associated with venous puncture for blood collection were minimal, however, participants may have experienced slight pain, bleeding, and bruising during fasting blood draws. Although uncommon, participants may have also experienced a local infection at the site of the blood draw. A trained nurse wore gloves while performing all blood draws and minimized the risk of infection by using clean techniques. The puncture site was cleaned with an alcohol and a sterile needle was used to collect venous blood into a serum separator collection container. A gauze pad was then used to cover the puncture site and direct pressure was applied until bleeding stopped. A sterile gauze pad was taped over the puncture site and participants were instructed on the care of the site after the blood draw was completed.

Blood draws were conducted in the fasting state, therefore participants may have felt brief symptoms related to an overnight fast. Such symptoms may have included dizziness, nauseas, weakness, and visual changes which usually resolved after resting. Participants were seated during the blood draw; however, beds were also available if participants needed to lie down. A registered nurse monitored participants for the duration of the blood draw and encouraged participants to consume food and water immediately after the procedure.

Risk associated with body composition testing were minimal, however, participants may have felt anxious or claustrophobic when testing inside the BOD POD. The BOD POD has a large chamber in which participants sat to be tested for. The only known risk associated with the

BOD POD is anxiety from being in an enclosed chamber. Participants with claustrophobia may have been especially vulnerable. There was, however, a window on the front door of the chamber so that participants could see out at all times. An emergency button was also located inside the chamber for participants. When activated, the button immediately opened the front door of the chamber and testing was stopped. Participants were instructed on the use of the emergency button before any testing was performed.

Participants and parents were at risk for psychology stress associated with receiving abnormal study results. To minimize psychological stress a registered nurse reviewed and provided study results by phone and mail. The nurse explained whether study results were within normal limits according to standards provided by Quest Diagnostic Laboratories (testing facility). Abnormal study results and their implications were explained to parents and participants to include recommendations to follow-up with a physician, if necessary. The contact information for the study nurse, physician, and principle investigator were provided at the beginning of the study and participants and their parents were encouraged to call if they had questions or concerns.

Participants were also at risk for consequences associated with the bias and stigma of obesity. The CDC (2001) recommends that a BMI percentile be used instead of weight to minimize obesity-related social consequences such as depression, stress, and social isolation. The current study obtained a BMI percentile as recommended during the first part of the study and weight was not referenced again throughout the study. Adolescents are at a vulnerable stage in social development for obesity-related bias and stigma. To minimize stress associated with medical facilities that are not equipped for obese individuals, the current study used medical equipment such as scales and blood pressure cuffs that were the appropriate size. Potential

participants were not singled-out during recruitment presentations. While recruiting in public educational institutions, the school nurse was utilized as the point of contact for referrals. The school nurse was asked to keep strict confidentiality when speaking with potential participants. The current study avoided using terms with negative connotation in recruitment materials. To deter the avoidance of participation in medical services, recruitment presentations emphasized the importance of medical screening for a healthy lifestyle. Study participants were tested in private medical rooms. Members of the research team were trained in the testing of human participants and awareness to the needs and concerns of participants were emphasized. Specifically, team members were asked to consider the negative experiences obese children may have endured in previous medical facilities, recognize the complex condition of obesity, and avoid stereotyping.

Benefits

Adolescents benefited from participating in this study by receiving a complete medical history and physical examination. Participants had liver tests performed that are routinely used in detecting liver disease such as NAFLD. Participants had a fasting blood draw to evaluate the levels of liver enzymes in blood and an MRI of the liver performed to detect excessive liver fat. In addition, participants were compensated with a $50 gift card for completing the study.

This study served as a benefit to society by investigating the associations between central fat, fructose intake, and NAFLD in Hispanic adolescents. Hispanic youth are at higher risk for NAFLD when compared to non-Hispanic whites, possibly as a result of a higher prevalence of risk factors such as obesity (Barshop et al., 2008). In addition to recent increases in fructose intake, the prevalence of obesity and NAFLD has also increased in children (Sharp, Santos, & Cruz, 2009). The development and progression of NFLD in children is only partially understood.

Therefore this study contributed to increasing the understanding of NAFLD in children and has assisted in directing future research efforts and interventions.

Confidentiality and Safety

Maximum efforts and strict guidelines were taken to maintain the confidentiality of participant information as allowed by law. Participant and parental (legal guardian) permission were required for the sharing of study information with outside parties. Personal identifiers were not used in any publications. Study documents were labeled with a unique identification code that was cross-matched to a database with personal information and only accessible to research personnel. Additionally, documents were stored under lock and key.

Research personnel were trained in the protection of human participants for research. Compared to the benefits of participating, the disadvantages associated with participating in the current study were outweighed. However, each participant and their parent were given an opportunity to ask questions and asked to consider both the risks and benefits before deciding to participate.

Data Analysis

Boys (n=15) and girls (n=19) were evaluated for differences in physical and blood chemistry characteristics with a general linear model. Specifically, univariate linear regression was used in which sex was entered as a fixed factor (independent variable, IV) and participant characteristics were entered separately as dependent variables (DV). Homoscedasticity was investigated with Levene's test for homogeneity of variance and models that violated this assumption were changed to a one-way analysis of variance (ANOVA) model. The Brown-Forsythe test for equality of means is robust for violations of homoscedasticity and therefore

used in the ANOVA model to investigate differences by sex. A chi-square test was used to test for significant differences in the proportion of participants with fatty liver by sex.

Boys and girls were combined (n=34) for additional data analysis. Measures of NALFD (HFF, ALT, AST, the ALT/AST ratio) that were not normally distributed were log transformed. A constant of 10 was added to HFF so that all values were positive before log transformation. Pearson parametric correlations were used to investigate associations. Multivariate linear regression was used to examine the independent contribution of predictors (IV) on outcome (DV) variables when controlling for age, sex, and BMI. All analyses were performed with SPSS version 17.0 (SPSS, Chicago, IL) with an error rate set at $p < 0.05$.

This study was part of a larger study investigating insulin sensitivity and measures of NAFLD in obese adolescents. A power calculation was done using preliminary data from the principal investigator's laboratory. In the preliminary study, 37 obese Hispanic adolescents with a mean ± standard deviation (SD): age16.1 ± 0.2 years and BMI 36.1 ± 0.9 kg/m^2 were recruited. In univariate linear regression, where ALT was the dependent variable, fasting insulin (a surrogate measure of insulin sensitivity) was negatively and significantly related to ALT levels ($R^2 = 0.11$, $p < 0.05$). Thus, we used the SD for the independent and dependent variables and the slope of the regression to calculate sample size using a PS:Power & Sample Size Calculations program. Assuming a power of 0.8 and an α of 0.05, a sample size of 66 subjects were required to detect a significant association between fasting insulin and ALT levels. Given that the correlation between fasting insulin and insulin sensitivity is approximately 0.8, and that insulin sensitivity measured via the oral glucose tolerance test is a more accurate reflection of whole body insulin sensitivity, we were confident that we could detect significant associations between directly measured insulin sensitivity and ALT and liver fat content with a sample size of 34.

Chapter 4

Results

Participant characteristics. Descriptive statistics are provided as mean ± standard deviation (SD) by sex in Table 4. Boys and girls did not significantly differ in age, weight, BMI percentile, or waist circumference. In addition, fat-free mass (FFM), HFF, liver enzyme levels in blood (ALT, AST, and ALT/AST ratio) and daily fructose intake did not differ by sex. Total body fat (mass and percentage), height, BMI, and daily total caloric intake did, however, significantly differ between boys and girls. Boys were taller, had a higher total caloric intake, and lower BMI when compared to girls. Girls differed in measures of body composition such that, they had a significantly higher percentage and mass of total body fat when compared to boys.

Table 4-Descriptive Statistics by Sex

Variable	Boys (*n*=15)	Girls (*n*=19)	Total (*n*=34)
Age (years)	14.9 ± 1.8	14.4 ± 1.4	14.6 ± 1.6
Height (cm)	167.6 ± 11.0	158.4 ± 5.8†	162.5 ± 9.5
Weight (kg)	87.7 ± 18.0	86.0 ± 14.4	86.7 ± 15.9
BMI (kg/m^2)	30.9 ± 3.7	34.2 ± 4.8†	32.7 ± 4.6
BMI (percentile)	97.8 ± 1.1	98.3 ± 1.3	98.0 ± 1.2
Waist circumference (cm)	101.2 ± 9.6	101.0 ± 8.7	101.1 ± 9.0
Body fat percent (%)	32.0 ± 6.2	39.4 ± 5.6†	36.1 ± 6.9
Body fat mass (kg)	27.6 ± 6.1	34.3 ± 9.5†	31.3 ± 8.8
Body fat-free mass (kg)	60.1 ± 15.2	51.7 ± 7.3	55.4 ± 12.0
Liver fat, HFF (%)	4.8 ± 10.7	4.5 ± 6.7	4.6 ± 8.5
Liver enzyme, ALT (U/L)	25.1 ± 20.4	18.0 ± 8.1	21.1 ± 15.0
Liver enzyme, AST (U/L)	20.8 ± 6.1	17.8 ± 5.3	19.1 ± 5.8
Liver enzyme ratio (ALT/AST)	1.1 ± 0.5	1.0 ± 0.3	1.1 ± 0.4
Total caloric intake (kcal/d)	2,256.9 ± 675.6	1,763.9 ± 618.2†	1,981.4 ± 681.1
Total fructose intake (g/d)	29.1 ± 13.0	30.7 ± 18.4	30.0 ± 16.1

Note: Data are means ± SD between boys and girls; BMI, body mass index; ALT, alanine aminotransferase; AST, aspartate aminotransferase; $^{\dagger}p < 0.05$.

Approximately 68 and 18 percent of participants had a BMI $\geq$ 30 and 35 kg/m^2, respectively. In addition, about 24 percent of participants were in the 99th BMI percentile or greater by age and sex according to CDC growth charts. Twenty six percent of all participants had a HFF > 5.5% in comparison to 20 percent of boys and 32 percent of girls. A chi-square test, χ^2 (1) = 0.58 p = 0.48, indicated that the proportion of participants with fatty liver did not differ by sex. All participants had normal ALT and AST levels in blood (< 40 U/L) except for one, who in addition to higher liver enzymes (ALT, 95 U/L; AST 40 U/L; ALT/AST Ratio 2.11), also had a higher proportion of liver fat (HFF 32.32%). An ALT/AST ratio > 1.0 was seen in 44 percent of all participants. Similarly, 46 percent of boys and 42 percent of girls also had an ALT/AST ratio > 1.0.

Self-reported total caloric intake demonstrated that 44% of participants consumed an average of 2,000 kcal/d or more, as compared to 8 percent of which consumed 3,000 kcal/d or more. The U.S. Department of Agriculture (2006) reported that Mexican American adolescents aged 12-19 consume on average 2,194 kcal/d. The average intake in the current study was approximately 2,257, 1,764, and 1,981 kcal/d in boys, girls, and overall, respectively. In the current study on average, girls consumed about 400 kcal/d less, boys consumed about 60 kcal/d more, and overall participants consumed about 200 kcal/d less when compared to the study of Mexican American adolescents conducted by the U.S. Department of Agriculture (2006).

Average daily fructose intake was also less in the current study sample across boys, girls, and overall, when compared to previously reported national estimates of adolescents of similar age. Vos et al. (2008) used data from the National Health and Nutrition Survey (n=21,483) and reported that the average daily fructose intake in adolescents aged 12-19 was 72.8 g and 54.7 g in adults and children combined. The current study sample reported approximately 30 g per day of

fructose intake in boys, girls, and overall. Studies of obese Hispanic adolescents, especially males, have demonstrated an average fructose intake as high as 100g per day (Barshop et al., 2008). Based on a 2,000 kcal/d diet, the upper limit of normal fructose intake is 60 g per day, according to U.S. recommendations (Vos et al., 2008). The current study sample overall reported lower total caloric and fructose intakes on average per day when compared to previous reports dietary intake in adolescents.

Correlation analysis. Variables were investigated for a normal distribution with the Shapiro-Wilk test of normality. Variables that were not normally distributed (HFF, ALT, AST, ALT/AST ratio) were log transformed. A constant of 10 was added to HFF to eliminate negative and zero values prior to logarithmic transformation. Normality tests were performed again after transformations and all variables met normality assumptions. Correlations among variables were investigated with Pearson Product-moment correlation analysis (Table 5).

Table 5. Pearson Product-Moment Correlation												
Variable	Age	Sex	Waist	Fructose	BMI	Log HFF	Log ALT	Log AST	Log ALT/AST	Total Intake	Fat Mass	Fat Free Mass
Age	1.00	-0.16	0.30	0.00	0.28	0.05	0.31	0.03	0.42†	-0.01	0.63‡	0.80‡
Sex		1.00	-0.01	0.00	0.36†	0.37†	-0.25	-0.27	-0.14	-0.36†	0.36†	-0.34†
Waist			1.00	0.08	0.62‡	0.65†	0.41†	-0.01	0.57‡	0.05	0.53‡	0.49‡
Fructose				1.00	-0.14	-0.13	0.09	0.12	0.03	0.48‡	0.06	-0.20
BMI					1.00	0.99‡	0.17	-0.17	0.37†	-0.19	0.80‡	-0.43‡
Log HFF						1.00	0.20	-0.15	0.40†	-0.20	0.79‡	-0.45‡
Log ALT							1.00	0.71‡	0.83‡	-0.09	-0.10	0.30
Log AST								1.00	0.20	-0.11	-0.40	0.02
Log ALT/AST									1.00	-0.04	0.16	0.40†
Total Intake										1.00	-0.15	0.19
Fat Mass											1.00	0.15
Fat Free Mass												1.00

*n =34, Sex coded 1=male 2=female, †$p<0.05$, ‡ $p<0.01$

Note: Waist, waist circumference; BMI, body mass index; HFF, hepatic fat fraction; ALT, alanine aminotransferase; AST, aspartate aminotransferase; total intake, total daily caloric intake; fructose, total daily fructose intake; †$p<0.05$, ‡ $p<0.01$.

Multivariate linear regression analysis. The enter method was used in multivariate linear regression analysis. Separate models for waist circumference and fructose were constructed. When waist circumference was entered as an IV, the following covariates were entered; age, sex, and BMI (Table 6). A separate model was constructed in which total caloric intake was entered as a covariate and fructose was entered as an IV (Table 7). Dependent variables were Log-HFF, Log-ALT, Log-AST, and Log-ALT/AST.

Table 6. Contribution of Waist Circumference on Measures of NALFD

Dependent Variable	Independent Variable	B ± SEE	β	*P*
Model 1 Log ALT/AST	Age	0.02 ± 0.01	0.26	0.10
$R^2 = 0.42$ $p = 0.00$	Sex	-0.03 ± 0.05	-0.13	0.53
$F_{observed} = 5.23$	BMI	0.00 ± 0.01	0.04	0.86
	Waist Circumference	**0.01 ± 0.00**	**0.49**	**0.02**
Model 2 Log ALT	Age	0.02 ± 0.02	0.18	0.30
$R^2 = 0.26$ $p = 0.06$	Sex	-0.08 ± 0.08	-0.20	0.29
$F_{observed} = 2.50$	BMI	-0.00 ± 0.01	-0.05	0.84
	Waist Circumference	0.01 ± 0.01	0.39	0.08
Model 3 Log AST	Age	0.00 ± 0.01	-0.01	0.99
$R^2 = 0.08$ $p = 0.64$	Sex	-0.05 ± 0.05	-0.22	0.30
$F_{observed} = 0.65$	BMI	-0.00 ± 0.01	-0.12	0.62
	Waist Circumference	0.00 ± 0.00	0.07	0.78
Model 4 Log HFF	Age	0.00 ± 0.00	0.03	0.10
$R^2 = 1.00$ $p = 0.00$	Sex	0.01 ± 0.00	0.05	0.01
$F_{observed} = 1.35 \times 10^3$	BMI	0.01 ± 0.00	0.93	0.00
	Waist Circumference	**0.00 ± 0.00**	**0.06**	**0.00**

Note: $n = 34$, $F_{critical}(4, 39) = 2.70$ at $\alpha = 0.05$, Sex coded 1=male 2=female; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; β, parameter estimate; SEE, standard error of the estimate.

Table 7. Contribution of Fructose Intake on Measures of NALFD				
Dependent Variable	Independent Variable	B ± SEE	β	*p*
Model 1 Log ALT/AST	Total Intake	-0.00 ± 0.00	-0.07	0.73
R^2 = 0.05 *p* = 0.92 $F_{observed}$ = 0.08	Fructose Intake	0.00 ± 0.00	0.07	0.73
Model 2 Log ALT	Total Intake	-0.00 ± 0.00	-0.18	0.37
R^2 = 0.04 *p* = 0.58 $F_{observed}$ = 0.56	Fructose Intake	0.00 ± 0.00	0.19	0.36
Model 3 Log AST	Total Intake	-0.00 ± 0.00	-0.23	0.26
R^2 = 0.06 *p* = 0.40 $F_{observed}$ = 0.94	Fructose Intake	0.00 ± 0.00	0.24	0.24
Model 4 Log HFF	Total Intake	-0.00 ± 0.00	-0.18	0.37
R^2 = 0.09 *p* = 0.51 $F_{observed}$ = 0.70	Fructose Intake	0.00 ± 0.01	-0.05	0.82

Note: *n* =34, $F_{critical}$ (2, 31) = 3.30 at α = 0.05; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; β, parameter estimate; SEE, standard error of the estimate, total intake, total daily caloric intake; fructose intake, total daily fructose intake

Waist circumference significantly associated with Log-ALT/AST and Log-HFF, independent of age, sex, and BMI. Fructose was not significantly associated with any of the measures of NAFLD, independent of total caloric intake.

Chapter 5

Conclusions and Recommendations

The prevalence of NAFLD has risen in parallel to obesity and fructose intake in children within the U.S. (Barshop et al., 2008). The pathogenesis of NAFLD is not entirely understood, however, obesity and insulin resistance are believed to play major roles (Kabir et al., 2005). Hispanic children are especially vulnerable. Studies suggest that excessive central (visceral) fat, in addition to overall adiposity, is associated with the development of childhood NAFLD (De Bruyne et al., 2010). Furthermore, excessive fructose intake is believed to increase the risk of NALFD in adolescents, who are major consumers of sugar-sweetened foods and beverages.

The first aim of this study was to investigate the associations between waist circumference, central (visceral) fat, and markers of NAFLD in obese, Hispanic adolescents. The alternate hypothesis stated that waist circumference would be significantly related to measures of NAFLD, independent of age, sex, and BMI. Waist circumference was significantly associated with Log-ALT/AST and Log-HFF, independent of age, sex, and BMI. The null hypothesis was rejected. Waist circumference was associated with Log-ALT and Log-ALT/AST ratio, however, not independent of age, sex, and BMI. Large, multiethnic, studies in children are needed to validate waist circumference as a reliable screening tool. If established, waist circumference could provide clinicians with a rapid, inexpensive, and non-invasive instrument for identifying children at risk for NAFLD.

The second aim of the study was to investigate the associations between dietary fructose intake and markers of NAFLD in obese Hispanic adolescents. The alternative hypothesis stated that dietary fructose intake would be significantly associated to markers of NAFLD, independent of total caloric intake. Significant correlations were not observed between fructose intake and

measures of NAFLD. Furthermore, fructose intake did not predict any of the measures of NAFLD. The current study failed to reject the null hypothesis. Adolescents are believed to be large consumers of artificially sweetened, high-fructose containing foods and beverages. The current study did not observe excessive high-fructose intake in adolescents. Recall bias may explain the lower than expected fructose intake. Future studies should focus on establishing reliable techniques for obtaining fructose intake in children. Furthermore, studies should investigate whether fructose intake differences by age, sex, and ethnicity. Dietary interventions may be tailored for children according to fructose consumption (De Bruyne et al., 2010).

A review of the literature suggests that the prevalence of childhood NAFLD differs by sex; greater in boys than girls. In the current study, however, boys did not significantly differ from girls in the proportion of participants with fatty liver or liver enzyme levels in blood. The overall proportion of fatty liver, defined as HFF > 5.5% (Schwimmer et al., 2006) was 26% and all but one participant had normal liver enzyme (ALT, AST) levels in blood. Although elevated liver enzyme levels in blood are common in children with NAFLD, normal levels have also been reported. Therefore, other indicators of NAFLD should be evaluated in addition to liver enzyme levels in blood, which have not been standardized in children. An ALT/AST ratio greater than 1.0 has been suggested as an additional indicator of fatty liver in children. The proportion of participants in the current study with an ALT/AST ratio greater than 1.0 was 44%. Liver enzyme levels in blood are routinely used as clinical indicators of NAFLD; however, a normal range in children has not been established. The liver enzyme ratio may serve as an additional indicator of NAFLD. Future studies should focus on standardizing the range of liver enzyme levels in blood by age, sex, and ethnicity in children. Furthermore, studies should investigate the relationship of the ALT/AST ratio and NAFLD in large, multiethnic, studies of children.

The current study was a prospective, observational investigation and thus limited in establishing causality; correlations between variables was the main focus. This study utilized a small, convenience sample of participants (n=34) and was limited in statically power. Future studies should include large, multiethnic populations of children and focus on investigating the long-term outcomes of NAFFD. Recall bias may have played a role in extracting dietary intake from participants. Care must be taken so that children do not feel stigmatized; such barriers may lead to biased diet recalls. Future efforts should focus on establishing a reliable instrument in assessing dietary intake in children. Liver fat was assessed by MRI and although not as specific and sensitive as biopsies, a practical approach to screening large populations.

Guidelines in the treatment of childhood NAFLD have not been determined. Eliminating and minimizing risk factors are the primary interventions. Obesity is almost always present in children with NAFLD. Studies have demonstrated improvements in surrogates of NAFLD associated with weight loss in overweight and obese children (Havel, 2005; Cave et al., 2007; Sanyal, 2002), thus lifestyle modifications that facilitate a healthy diet and exercise are recommended. In a prospective study of 84 children (BMI 15.2–38.4) with biopsy-proven NAFLD and elevated liver enzyme levels in blood, participants significantly improved BMI, liver enzymes levels in blood, and liver fat on ultrasound after receiving 12 months of counseling on diet and exercise (Nobili et al., 2006). Researchers concluded that the improvements in liver fat and enzyme levels in blood of normal-weight participants was associated with the insulin-sensitizing effects of exercise in muscles, independent of weight loss.

Pharmaceutical therapy aimed at reducing insulin-resistance and oxidative stress is proposed in addition to lifestyle modifications for the treatment of NAFLD in children. An open-label pilot study by (Schwimmer et al., 2005b) of ten non-diabetic children with NAFLD

demonstrated an improvement in liver enzyme levels in blood and liver abnormalities on MRI after 6 months of treatment with an insulin sensitizer, metformin (1.0 g/d). In another study by Nadeau et al., (2008) metformin (1.7 g/d) was used in a randomized, double-blinded, controlled trial of 50 multiethnic, obese, and insulin-resistant adolescents. Participants received lifestyle modification counseling and either metformin or a placebo for 6 months. The prevalence and severity of fatty liver on ultrasound significantly improved with metformin compared to placebo. In a study by Nobili et al. (2008), 57 overweight and obese children received lifestyle modification counseling and either metformin (1.7 g/d) or a placebo for 24 months. Participants treated with metformin did not significantly differ in measures of NAFLD when compared to controls. Future studies should focus on evaluating the efficacy of metformin in the treatment of varying degrees of NAFLD in children.

In addition to insulin-sensitizers, anti-oxidants have been proposed as a treatment option for childhood NAFLD; however, research is limited and results inconclusive. A 40-week study of vitamin E supplementation (400-1200 IU/d) in 11 children with NAFLD demonstrated improvements in liver enzyme levels in blood, independent of weight loss, however, liver fat by ultrasound remained unchanged (De Bruyne et al., 2010). Sufficient evidence is lacking on the relationship between vitamin E supplementation and improvements in measures of childhood NAFLD, independent of weight loss and diet modification.

As of yet, there has not been substantial support for a treatment that reverses the effects of NAFLD in children. Minimizing risk factors, especially obesity and insulin resistance, is a primary interest in prevention and treatment. Studies suggest that lifestyle modifications that facilitate a healthy diet and exercise are associated with improvements in measures of NAFLD (De Bruyne et al., 2010). A few small studies have suggested pharmaceutical treatment that

includes insulin-sensitizers and anti-oxidant such as metformin and Vitamin E, respectively (Nobili et al., 2006, Nadeau et al., 2008). Large, multiethnic, randomized, double-blind, placebo-controlled trials will help establish effective treatments for NAFLD in future research. Research should also investigate dietary guidelines for children in which weight loss is recommended. Eliminating childhood obesity should be the primary intervention in preventing and managing childhood NAFLD.

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APPENDIX

1. UTEP IRB Protocol # 80289 – Assent for research study
2. UTEP IRB Protocol # 80289 – Informed consent form for parental permission
3. Screening questionnaire
4. History and physical

UTEP IRB Protocol # 80289

ASSENT FOR RESEARCH STUDY

Copy to be provided to subject or legal guardian

Title: Insulin Resistance and Non Alcoholic Fatty Liver Disease in Hispanic Adolescents

Investigators:

Principal Investigator: Chantal A Vella, PhD (Tel. 915 747 8228)

Consultants: Luis Santos, MD (Tel. (Tel. 915 577-1380)

Carmen P. Arango, MD (Tel 915 856-7533)

CONTACT TELEPHONE NUMBERS:

You may contact the investigators/consultants at the numbers listed above at any time if you have any questions or concerns about the study, or if you have any unexpected problem related to the study.

INSTITUTION: University of Texas at El Paso (UTEP), College of Health Sciences.

WHY IS THIS STUDY BEING DONE?

You re being asked to participate in a research study about a condition known as fatty liver. As the name implies, fatty liver occurs when there is an excess accumulation of fat in the liver. We are not sure why adolescents develop fatty liver disease but it may be linked to being overweight. Adolescents of Hispanic or Mexican descent may be at higher risk for fatty liver disease. To participate, your and one parent (legal guardian) must agree.

WHAT WILL HAPPEN DURING THIS STUDY?

If you agree to participate in the study you will come to the University of Texas in El Paso's Border Clinical Research Center on two different occasions.

1st Visit (purpose: to make sure your are healthy and qualify for the study)

During this visit we will:

- Take your height, weight and blood pressure and measure your waist
- Examine you to make sure you are ok
- We will do a test called the BodPod to see how much muscle and fat is in your body. You will wear a bathing suit and swimming cap for this test. We will take a picture over your belly button to see how much fat is in your belly and in your liver
- Ask you some questions about the amount and types of foods that you ate the day before
- Take a picture of a big artery in your neck with an ultrasound machine. The procedure does not cause any pain.

If you qualified for the study then we will ask you to come back for a second visit. Before that we may call you at home to ask you about the types and amounts of food that you ate over a weekend day.

2nd Visit

- Your will fast overnight at home before this visit. You may do schoolwork at the center, watch T.V., DVD's or videos, listen to music or play electronic games.

During this visit we will:

- Take your weight, blood pressure, pulse, and temperature (by mouth)
- You will get a diabetes-screening test. During the test, you will be given sugar water to drink. You will have 6 blood samples taken from your arm. If you want, we will put a cream on your arms that will make your skin numb before we take the samples. This will help to make the needle stick only hurt a little bit.
- Ask you about the types and amount of food that you ate the day before
- Take a picture over your belly button to see how much fat is in your belly and in your liver

WHAT ARE THE POSSIBLE RISKS AND DISCOMFORTS?

Any of the tests will end right away if you are uncomfortable or unhappy and want us to stop. If you don't want to do a test, you don't have to.

- Blood sampling: Your arm may be sore from where we take a blood samples or where the catheter was. A small bruise may appear on your arm.

- Hungry: You may feel hungry after the overnight fast.

WHAT ARE THE POSSIBLE BENEFITS OF TAKING PART IN THIS STUDY?

By participating in this study you will get a physical and we will do a blood test that can rule out hidden diabetes or fatty liver.

WHAT ARE YOUR RIGHTS AS A PARTICIPANT, AND WHAT WILL HAPPEN IF YOU DECIDE NOT TO PARTICIPATE?

If at any time you don't want to be a part of this study, then you can leave the study. You can leave the study even if your parent wants you in the study. There will not be any problems with you not being in the study.

UNDER WHAT CIRCUMSTANCES WILL YOUR PARTICIPATION BE ENDED?

You may be asked to withdraw from the study if:

• You do not follow the study staff's instructions

• Your behavior is unacceptable

WILL I RECEIVE ANYTHING FOR TAKING PART IN THIS RESEARCH STUDY?

You will receive a $50 gift certificate for completing the 2nd Visit. .

WHOM DO YOU CALL IF YOU HAVE QUESTIONS OR PROBLEMS?

You can ask any questions you want about the study. We will answer all of your questions. If you have a question later that you did not think of now, you can ask it. You can call the head researcher, Chantal A Vella, PhD (915 747 8228). You can also ask us next time you see us.

AGREEMENT

If it is okay with you, please print and sign your name in the lines below.

_____ _____

Printed Name of Subject Age of Subject in Years

_____ _____

Signature of Subject Date Time

_____ _____

Signature of authorized research personnel Date Time
who conducted the informed consent discussion

UTEP IRB PROTOCOL # 80289

INFORMED CONSENT FORM FOR PARENTAL PERMISSION

Signed copy to be provided to parent or legal authorized representative

This is a research study for adolescents who voluntarily choose to take part. Please take your time to make a decision, and discuss the study with your child, personal doctor, family and friends if you wish.

Title: Insulin Resistance and Non Alcoholic Fatty Liver Disease in Hispanic Adolescents

Principal Investigator: Chantal A Vella, PhD (Tel. 915 747 8228)

Consultants: Luis Santos, MD (Tel. (Tel. 915 577-1380)

Carmen P. Arango, MD (Tel 915 856-7533)

CONTACT TELEPHONE NUMBERS:

You may contact the investigators/consultants at the numbers listed above at any time if you have any questions or concerns about the study, or if you have any unexpected problem related to the study.

INSTITUTION: University of Texas at El Paso (UTEP), College of Health Sciences.

1. Why is this study being done?

Your child has been invited to participate in a clinical research study on a condition known as fatty liver disease. As the name implies, fatty liver disease occurs when there is an excess accumulation of fat in the liver. We are not sure why adolescents develop fatty liver disease but it may be linked to being overweight. Adolescents of Hispanic or Mexican descent may be at higher risk for fatty liver disease. The following information is provided in order to help you make an informed decision whether or not you would like your child to participate in this research study. To participate, your child and one parent (legal guardian) must agree.

2. How many people will take part in this study?

Approximately ~50 adolescents who qualify will be enrolled in this study.

3. Why is my child being asked to take part in this research study?

Your adolescent is being invited as a possible participant in this study because he/she is considered overweight and is of Hispanic of Mexican descent.

4. What will happen during this study?

The study visits will take place at the University of Texas at El Paso in the Border Clinical Research Center located in the College of Health Sciences.

1st Outpatient Visit (purpose: establish if child qualifies for study and is in good health)

🕘 ~ 2 hour visit at the Border Clinical Research Center, Stanton Building, CHS

During this visit we may:

- Measure your child's height, weight, waist circumference, blood pressure and pulse. Your child will be eligible for the study only if he/she is classified as being overweight
- Perform a medical history to further confirm that your child is in good health.
- We may perform a test to measure body composition. To do this we will use a machine called a BodPod. The BodPod is a sealed chamber that measures body fat and lean body mass based on air displacement of the body. During this procedure your child will sit comfortably inside a sealed chamber for a period no longer than 6 minutes. He/she will be asked to wear a swim cap and bathing suit. The procedure is extremely safe. If your child feels that he/she needs to stop the test, he/she can do so by pushing an eme

If your child qualified for the study then he/she will be asked to come for an additional visit within a 4 week period. We w

During this visit we may:

- Measure your child's weight, blood pressure, pulse, and temperature (by mouth). Perform a Diabetes Screening Test. This test will help us rule out hidden diabetes. We will place a small plastic tube in your child's arm called a catheter. Occasionally we may have difficulty in locating a vein. If this happens, we will ask for your child's consent to try again. If your child agrees we will try to get the needle in a vein will be made. We will take 6 blood samples from your child. Samples will be taken before and after he/she drinks a sugary drink. The total amount of blood taken during this test is about 5 tablespoons (40 ml). The results of these tests will tell us if your child has pre-diabetes, diabetes or has normal blood sugar (3 hours). In addition, we will be able to confirm that your child does not have any other health condition. Your child will be given lunch. If the results indicate that your child has diabetes we will give you the results in which you should discuss with your child's pediatrician or primary care provider. Other Blood Tests: An additional blood sample (1.5 tablespoon or 20 ml) will be drawn during the above test to measure the amount of fats (triglyceride) in your child's blood as well as liver enzymes. At the end of the day

- Escort your child to the Imaging Center (Arango Imaging on Mesa Hills). We may perform a test to measure body fat distribution and the amount of fat in the liver. This test will measure how much fat is in a specific part of your child's belly. A machine called an MRI will be used for this measurement. Your child will be asked to lie on a flat table that moves (head first) into the machine. The machine has a large magnet inside. When the magnet is turned on, it will take a picture of the fat near your child's belly button. Some youngsters may get scared in small places, in the dark, or by the noise that the machine makes.

5. **What are the risks?**

Blood Collections The risks and discomfort of blood drain

Fasting Your child may feel hungry from fasting. Because of the overnight fast, your child may experience symptoms of hunger such as dizziness, nausea, visual changes, weakness or faintness. These symptoms are usually brief and are not serious.

6. **Benefits**

   If your child participates in this study they will get a history and a physical and will be given results for diabetes screening test which may rule out hidden diabetes.

7. **What about confidentiality and the privacy of my child's records?** We will keep your child's involvement in this research study confidential to the extent permitted by law. In addition to the staff carrying out this study, others may learn that your child is in the study. This might include federal regulatory agencies, the University of Texas at El Paso (UTEP) and the UTEP Institutional Review board (a committee that reviews and approves research). These people may review and copy your child's records from this research.
8. **8. Who is funding this study?** The Center for Border Health Research (CBHR) is funding this research study. This means that CBHR has provided money to support the activities that are required to carry out the study. The College of Health Sciences at UTEP is providing the space for this study. No one on the research staff will receive
9. **Will there be any costs to me?**

   NO

10. Will my child receive anything for taking part in this research study? $50 compensation in the form of a gift card.

11. Does anyone on the research staff have a personal financial interest in this study? NO

12. **What if my child is hurt by participating in this study?**

• If you have a research related illness or injury, care will be available to you as usual, but you and/or your medical or hospital insurance company will be responsible for the cost of treatment. Before entering this study, you should check whether your insurance company might limit your insurance coverage if you take part in a research study.

• University of Texas at El Paso and its affiliates do not offer to pay for or cover the cost of medical treatment for research related illness or injury. No funds have been set aside to pay or reimburse you in the event of such injury or illness unless specifically stated.

13. • What are your child's rights as a voluntary participant? Your child's participation in this research study is voluntary. You and your child can decide not to participate. Your child has the right to decide not to be in the study even if you would want him/her to participate. Your child may withdraw from the study at any point. If you and your child sign this form, it means that you choose to be in the study. If new information becomes available during the study that may affect • your willingness to have your child take part in the study, you will be told.

14. Can your child stop being in the study? Your child may leave the study at any time. If your child decides to leave the study please form the investigators.

15. Can someone else end the participation of my child in the study? Under certain circumstances, the investigators, UTEP, or Federal Regulatory Agencies may decide to end your participation in this research study earlier than planned.

16. What if I have questions? If you have any questions about this study please contact the principal investigator, Chantal A Vella, PhD Tel. 915 747 8228. If you would like to speak to someone who is not involved in the study about your rights as a participant, research-related injuries, or any other matter related to the study, you can call Lola Norton, IRB Administrator, at (915) 747-8841 or b.orsp@utep.edu.

Your signature indicates that this research study has been explained to you, that you've been given the opportunity to ask questions, and that you agree to take part in this study. You will be given a signed copy of this form.

_____

Printed Name of Subject

_____ _____

Signature of Subject Date Time

_____ _____

Signature of Parent/Guardian Date Time
or Authorized Representative

I have discussed this research study with the subject and his or her authorized representative, using language that is understandable and appropriate. I believe I have fully informed the subject of the possible risks and benefits, and I believe the subject understands this explanation. I have given a copy of this form to the subject.

_____ _____

Signature of authorized research personnel who conducted the informed consent discussion Date Time

Fly on the Border Border Clinical Research Center College of Health Sciences University of Texas at EL Paso	Date of enquiry _____/_____/_____ Person Enquiring_____	ID LABEL HERE

FLY ON THE BORDER
SCREENING QUESTIONNAIRE

Subject Last name:_____ **First name:**_____
Gender: Male ☐ Female ☐ Age:_____ **Date of Birth:** _____/_____/_____

Telephone interview ☐ **Personal Interview** ☐ **Other** ☐ (specify) _____
Name of person interviewed:_____
Relationship to subject: Mother ☐ Father ☐ Legal guardian ☐ Other ☐ (specify)_____

1. Ethnic background (CHECK the appropriate BOX)

Ethnicity	Mother	Father	Paternal G. father	Paternal G. mother	Maternal G. father	Maternal G. mother	Subject
Mexican	☐	☐	☐	☐	☐	☐	☐
Central American	☐	☐	☐	☐	☐	☐	☐
South American	☐	☐	☐	☐	☐	☐	☐
Other (specify)	_____	_____	_____	_____	_____	_____	_____

All relatives of Hispanic ethnicity? YES ☐ NO ☐

2. Does your child appear to be: ☐ overweight ☐ average ☐ underweight ☐ unknown

a) Estimated Wt_____**kg/lb or Not known ☐ c) Estimated BMI**_____**kg/m^2 or Not known ☐**

b) Estimated Ht _____**m/ft or Not known ☐ d) Estimated BMI percentile** _____**or Not known ☐**

3. Has your child been diagnosed with the following: ☐ Diabetes ☐ Liver Disease
If NO, check here ☐, If YES, describe:_____
4. If female:
Is she currently pregnant? ☐ YES ☐ NO

5. Current Medication (possible exclusion):_____

7. Major illness/surgery/medical condition since birth?_____

8. Any known allergies:_____
_____

9. Presence of acute illness: YES ☐ NO ☐ ***IF YES, describe***_____

10. Demographics
Child's birthplace:_____**Age of child when moved to USA?**_____
_____
Does child speak English? YES ☐ NO ☐ Does child read English? YES ☐ NO ☐
Who has custody?_____

Mother's first and last name:_____ **Maiden name**:_____
Mother's Birthplace_____
Mother's Address:_____ **City:**_____ **Zip:**_____
Mother's Home number:_____ **Work number:** _____
Mother's Mobile number:_____
Does Mother speak English? YES ☐ **NO** ☐ **Does Mother read English? YES** ☐ **NO** ☐

Reviewed	Initials	Date (m/d/y)	PI signature	Date (m/d/y)
Nurse/CRC	_____	____/____/____	_____	____/____/_____

Father's first and last name:_____
Father's birthplace:_____
Father's Address:_____ **City:**_____ **Zip:**_____
Father's Home number:_____ **Work number:** _____
Father's Mobile number:_____
Does Father speak English? YES ☐ **NO** ☐ **Does Father read English? YES** ☐ **NO** ☐

Other contact:_____ **Relationship:**_____
Address:_____ **City:**_____ **Zip:**_____
Home number:_____ **Mobile number:**_____

ID #s of siblings in study:_____
How did you hear about this study?_____
Information for study should be sent in: Spanish ☐ **English** ☐ **Both** ☐
Study Information: mailed ☐ **hand delivered** ☐ **other (specify)** ☐_____
Date ____/____/____

General inclusion/exclusion criteria	**YES**	**NO**	**Confirm on Screening**
Age (13-18)	☐	☐	
Hispanic ethnicity	☐	☐	
Estimated BMI $\geq$ 95th Percentile for age/gender?	☐	☐	☐
Is subject taking medication known to influence insulin or body composition	☐	☐	
Does subject have disease/syndrome/illness on exclusion list (liver disease, diabetes, syndrome that influence insulin action/body comp)	☐	☐	☐
Is subject pregnant?	☐	☐	
Subject eligible for screening visit?	☐	☐	☐

Additional comments:_____
_____

Border Clinical Research Center College of Health Sciences University of Texas at El Paso	Date of Exam ___/___/___ Subject Initials _____	ID Label Here

History and Physical Update

Date of last H+P ___/___/___ Performed by _____

ID # from previous study _____

	BP (right arm) sitting	Pulse
Measure 1		
Measure 2		
Measure 3		
Average completed by study team		

Medical History

Does your child have any current health problems?

□ No □ Yes, problem(s)_____

Is she (he) taking any medicine now? □ No □ Yes If yes, name of medicine_____

How long?_____What for?_____Who prescribed the medication?_____

Name of medicine_____

How long?_____What for?_____Who prescribed the medication?_____

Has your child had:	Yes	No	Comments
Any history of toxin exposures?	□	□	_____
Any major illnesses since birth?	□	□	_____
Any history of hepatitis	□	□	_____

Has your child had any hospitalizations since your last visit? □ No □ Yes, explain_____

(**Confidential**) Are you, or do you think you are currently pregnant? □ Yes □ No

Physical Exam

N	A	NE	Normal Findings	Explain
			General (well developed, alert and oriented to person, place, time) **Affect** (good eye contact, normal affect)	
			Skin (no acne or rashes)	
			Eyes (PERRLA, EOMI)	
			Ears (TM's clear, mobile) **Nose** (no septal deviation or obstruction)	
			Oropharynx (moist, no exudaate)	

			Teeth (no cavities, good alignment)	
			Neck (supple, no lymphadenopathy, thyroid not palpable	
			Lungs (clear and equal breath sounds)	

N	A	NE	Normal Findings	Explain
			CV (RRR, no murmur, S1 and S2 noted, pulses normal	
			Abdomen (no masses, non-tender, soft, no hepatosplenomegaly, good BS in all quadrants)	
			Extremities (normal ROM all joinst, no deformities noted)	
			Nuero (normal strength, coordination and gait)	

Problem List/Assessment:_____

Plan

Nurse Signature _____Date___/___/___(m/d/y)

CURRICULUM VITA

Hector Reyes, Jr. was born on November 1, 1979 in El Paso, Texas. He is the son of Hector Sr. and Felipa Leticia Reyes. He has one brother and sister, Danny and Angela. Hector is a veteran of the U.S. armed forces and a recipient of the Marine Corps Commendation medal with Combat Valor for service as an infantry medic with 1st Marine Division of San Diego, California. Peacetime and combat duties included service to Hawaii, Japan, Korea, Thailand, Singapore, China, Iraq, and Kuwait.

In 2007 Hector graduated from the University of Texas at El Paso (UTEP) with a Bachelor's in Science, majoring in biology with a minor in chemistry after receiving the Southwest Association of American Hispanic Physicians Scholarship. Pursing a Master's in Science degree at UTEP, he recently authored a manuscript for the El Paso City Department of Public Health titled, *"How Healthy Are We? Selected Health Measures for El Paso, Texas 2008"*, available online at: http://www.elpasotexas.gov/health/. He graduated with a Master's of Public Health degree from UTEP in fall 2009 and plans to pursue a career in medicine. His interests are chronic disease and Hispanic health disparities along the U.S./Mexico Border.

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