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Demographic and developmental factors associated with hepatic fat accumulation and risk of nonalcoholic fatty liver disease (NAFLD) among healthy Hispanic and Non-Hispanic children

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2015

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An abstract of
a thesis submitted to the Faculty of the
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Abstract

Demographic and developmental factors associated with hepatic fat accumulation and risk of nonalcoholic fatty liver disease (NAFLD) among healthy Hispanic and Non-Hispanic children

By Yuichi Murayama

Nonalcoholic fatty liver disease (NAFLD), commonly defined as chronic liver condition, is a common liver disease in the US, especially prominent in the Hispanic population. Prevalence of NAFLD has increased markedly in recent years, among children as well as adults. This study aims to identify factors associated with the accumulation of hepatic fat among healthy Hispanic vs. Non-Hispanic children. We conducted a cross-sectional study utilizing data collected from the ongoing Project Exposome Analysis in Child Health (PEACH) cohort study conducted by the Department of Pediatrics Emory University School of Medicine. We created a series of linear regression models to explore the relationship between liver fat accumulation (%), as assessed using MRI, and age, as well as the interaction between age and ethnicity. We also developed multivariable models to explore the association between possible demographic risk factors and the role of puberty onset and % liver fat using risk-factor models in order to assess the impact of adjustment for potential confounding factors identified from previous research. Among the 30 children in the study, 10 were Hispanic and 20 were Non-Hispanic, the mean age (±Standard Deviation) of those of Hispanic ethnicity was 13.3 ±3.2 years (range, 7.5 to 18.1), and of those Non-Hispanic was13.5±2.8 years (range, 7.8 to 18.1), (p=0.872). The mean % liver fat (±SD) of Hispanic children was 4.2±1.8 (range, 1.6 to 7.0), and among Non-Hispanic it was 3.5±2.1 (range, 1.6 to 10.1), (p=0.365). There was no significant difference for this interaction term age and ethnicity (p=0.108), but the Hispanic group exhibited a steeper slope compared to the Non-Hispanic group. On the other hand, stepwise results showed "Age," "Ethnicity," and "Obesity" as statistically significant predictors. Our results demonstrated that percent hepatic fat increased with age among all participants in our sample of healthy children but the rate of increase was greater among those of Hispanic ethnicity compared to Non-Hispanic ethnicity. The results of this study can be used to inform the development of improved screening and intervention methods.

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Table of Contents

Background

Nonalcoholic fatty liver disease (NAFLD) is a common liver disease in the United States, which is prominent especially in Hispanic and Asian populations; however, there is still no consensus of its prevalence in the pediatric population. This is due in part to difficulty diagnosing NAFLD. Liver biopsy is currently the most reliable method for determining the presence of steatohepatitis and fibrosis in patients with NAFLD, but it is generally recognized that biopsy is limited by cost, sampling error, and procedure-related complications. [1] [2] This inhibits early prevention due to the insufficient accumulation of data and lack of definitive diagnosis. [3-9]

NAFLD is one of the common chronic liver diseases. It is estimated that about 10% of all children in the U.S. have NAFLD including both nonalcoholic fatty liver (NAFL) and non-steatohepatitis (NASH). [3,10] NAFL is defined as a condition with more than 5% of hepatic steatosis without evidence of hepatocellular injury in the form of hepatocyte ballooning. On the other hand, NASH is defined as a condition of hepatic steatosis with more than 5% of hepatic steatosis with hepatocyte injury, with or without any fibrosis. [1] In the long term, NASH can ultimately progress to liver cirrhosis, which is one of the leading causes of liver transplant and hepatocellular carcinoma (HCC). However, histologic evaluation with liver biopsy remains the only definitive diagnosis as surrogate markers still cannot replace it. [11,12] Early prevention and intervention would decrease future irreversible liver cirrhosis and liver failure, reducing the need for more invasive measures such as a liver transplant. Liver transplant is indicated in patients with end-stage liver disease. The most common indication to liver transplant for end-stage liver disease in adults is cirrhosis. Patients need to be referred to transplant centers when major complications of cirrhosis, such as variceal hemorrhage, ascites, hepatorenal syndrome, and encephalopathy occur. [13]

NAFLD is strongly associated with factors of metabolic syndrome, insulin resistance, diabetes mellitus (DM)[14], obesity, and ethnicity. [15] Obese children have seven times the prevalence of NAFLD compared to normal-weight children. [10,16] The Hispanic population has four times elevated risk of hepatic steatosis compared to Non-Hispanic children (11-22 years-old).[7,17] However, the specific onset of fat accumulation during puberty by sex is understudied. [18,19] Thus, elucidating this natural time course would aid in timely prevention at the early stage of disease onset. This study aims to identify factors associated with the accumulation of hepatic fat among healthy Hispanic vs. Non-Hispanic children.

Methods

Study Design and Sample

We conducted a cross-sectional study utilizing data collected from the ongoing Project Exposome Analysis in Child Health (PEACH) cohort study conducted by the Department of Pediatrics Emory University School of Medicine. The PEACH cohort study aims to describe the healthy exposome in children. Data collected will be used to investigate the mechanism, treatment, diagnosis, and prevention of diseases that occur in children. As part of the PEACH study, samples of plasma, urine, serum, stool, teeth, toenails and fingernails, and saliva were collected from 41 healthy children ages 6 to 18 years living in the greater Atlanta area. In addition, a whole-body MRI was done among a subgroup (N=31) to assess body and liver fat. Children whose parents reported them as healthy were included. Those who had an acute illness

(fever) within the past last 14 days, who reported consuming illicit drug or alcohol, taking antibiotics, or were pregnant were excluded from the enrollment.
The 30 children enrolled in the PEACH study for whom data were available on key variables of interest, including demographic data, puberty status, and percent (%) hepatic fat formed the sample for the current study.

Outcome Variable

"% hepatic fat" was measured by MRI utilizing a proton density fat fraction (PDFF) method to evaluate the hepatic steatosis [16,20]

Other Variables

During a research study visit, demographic data, including age, sex, and race/ethnicity (self-identified category, such as Hispanic or Non-Hispanic) were collected using self-administered surveys. Subjects also self-reported their stage of puberty as Tanner Stage I-V. In addition, blood samples were collected from all participants to assess ALT (ALT: Alanine-aminotransferase, elevated level Male > 25.8 U/L, Female > 22.1U/L) levels[21 22], which indicates inflammation or cellular injury, a surrogate marker for screening NAFLD. Blood samples also captured lipid panels including Total Cholesterol (mg/dL), LDL Cholesterol (mg/dL), HDL Cholesterol (mg/dL), and Triglycerides (mg/dL). Waist circumference (cm) and Body Mass Index (BMI) weight in kilograms divided by height in meters squared, (kg m^{-2}).

Data Analysis

For the statistical analysis, R version 4.0.2 (R Core Team (2020). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.) was used. Visual inspection of the samples' scatter plot revealed that % liver fat increased visually when the subjects' age increased. Hence, we created a linear regression model to explore the relationship between % liver fat and age. We created multivariable models to explore the association between possible risk factors in demographic data and % liver fat elevation using a series of basic/risk-factor models in order to assess the impact of adjustment for potential confounding factors from previous research. [23]

We developed a "basic model" with % liver fat as a dependent variable and ethnicity as independent variable (model A), then we added age into to the model A (model B). We also added the interaction term between age and ethnicity to explore the difference in steepness of two slopes depending on ethnicity (model C):

$$(\%\ \text{liver fat} = b_0 + b_1 * \text{Age} + b_2 * \text{Ethnicity} + \text{Age} * \text{Ethnicity} + \text{Error})$$

To explore the association between % liver fat and puberty stage, we added Tanner Stage into to the model B (model D). Tanner stages were based on secondary sexual characteristics. Male and female children self-reported their stage on a 5-point scale. In male children, Tanner Stages were evaluated with the changes in genitalia (I: Pre-adolescent testes, scrotum, and penis are of about the same size and proportion as in early childhood ~ V: Genitalia adult in size and shape). In female children, Tanner Stages were evaluated with the changes in breast stages (I: Pre-adolescent, Elevation of papilla only ~ V: Mature stage projection of papilla only) [24 25] In the last models, obesity or waist circumference was added, as they are one of the well-established risk factors of NAFLD (model E and F). In order to explain % liver fat and to create a more

parsimonious model, we utilized the stepwise regression method and included the statistically significant variables into the final model at a ten percent significance level (α=0.10). We then included the interaction term between age and ethnicity and evaluated the statistical significance by controlling variables from the stepwise results.

Because there was a small sample size, continuous variables were dichotomized to keep the residuals normally distributed. Total Cholesterol over 170 (mg/dL) was categorized as "Elevated". LDL Cholesterol over 100 (mg/dL) was categorized as "Elevated". HDL Cholesterol under 45 (mg/dL) was categorized as "Low". Triglycerides over 150 (mg/dL) was categorized as "Elevated". BMI over 95 (percentile) was categorized as "Obesity." Waist Circumference above 90 percentile (cm) was categorized as "WC above 90 percentile". [26] [27] [28] [29] (Table.1)

We utilized Cook's distance and identified outliers using the cutoff of 4/N. When sample size is small, outliers would have a stronger influence on the overall result and a series of sensitivity analyses were conducted with and without outliers to assess their impact. [30] In our study, samples who had strong influence due to high hepatic fat were excluded.

Results

Among the 30 children in the study, 10 were Hispanic and 20 were Non-Hispanic, the mean age (±Standard Deviation (SD)) of those of Hispanic ethnicity was 13.3 ±3.2 years (range, 7.5 to 18.1), and of those Non-Hispanic was13.5±2.8 years (range, 7.8 to 18.1), (p=0.872). The mean % liver fat (±SD) of Hispanic children was 4.2±1.8 (range, 1.6 to 7.0), and Non-Hispanic was 3.5±2.1 (range, 1.6 to 10.1), (p=0.365), The mean ALT (±SD) of those of Hispanic ethnicity was 25.3 ±18.0 (range, 14.0 to 74.0), and of those Non-Hispanic was 21.0±6.15 (range, 11.0 to 35.0), (p=0.354). (Table 2)

The samples' distribution showed a linear relationship between % liver fat and age, and residuals were was fairly normally-distributed. As the age increased, % liver fat also increased. Age was not significant in explaining % liver fat at a ten percent significance level (age: coefficients=0.197, p=0.123), but it became significant after removing the outlier (age: coefficients=0.219, p=0.030).

We developed a linear regression model with the % liver fat and age, and identified one outlier (MRI:10.1 (%), Age:12.1) based on Cook's distance, and the influence from this outlier was Cook's SD > 0.20.

As preliminary analysis prior to outlier removal, we developed a model only with % liver fat, as the dependent variable and ethnicity as the independent variable; ethnicity was not significant in this model (ethnicity: coefficients = -0.71, p=0.365). After removing the outlier, ethnicity became significant (ethnicity: coefficients = -1.0595, p=0.097) in the univariate model (Table. 3: model A). We then added age into model and it showed both age and ethnicity as significant variables (age: coefficients = 0.226, p=0.0196; ethnicity: coefficients = -1.11, p=0.055) (Table. 4: model B). Then, we added the interaction term between % liver fat and age into model B (Figure.1, Table. 5: model C), and the interaction term was not found to be significant (p=0.108). We adjusted for Tanner Stage into model B (Table. 6: model D), and only ethnicity remained significant (ethnicity: coefficients = -1.25, p=0.047). We added obesity into the model D, and only ethnicity remained significant (ethnicity: coefficients = -1.28 p=0.0622) (Table. 7: model E). We replaced obesity with waist circumference above 90 percentile in model D, then only ethnicity remained significant (ethnicity: coefficients = -1.65 p=0.00386), (Table. 8: model F). for models C, D, E, and F, only ethnicity was significant.

To find a more parsimonious model explaining MRI liver fat accumulation (%), we integrated other variables known as possible risk factors of NAFLD into the model. We also dichotomized continuous variables and conducted the stepwise method using age, ethnicity, sex, obesity, and WC above 90 percentile. (Table. 2) The stepwise method without the outlier indicated age, ethnicity, and obesity were chosen as predictors with an adjusted R^2 of 0.51. (Table. 9)

It is known that DM is related to having NAFLD. There were no subjects diagnosed with DM prior to the participation in the research in our data. In our dataset, the highest blood sugar levels noted were 99 (mg/dL), which was still within a normal range based on diagnostic criteria [31]. For this reason, we omitted the DM variable, and considered other known risk factors such as "Total Cholesterol (mg/dL)," "HDL_Cholesterol (mg/dL)," "Triglycerides(mg/dL)," "BMI (percentile)," and "Waist Circumference (cm)."

Discussions

We discovered differences in the percentage of liver fat between healthy children of Hispanic compared to Non-Hispanic ethnicity in % liver fat. Our findings are consistent with previous studies that were conducted among Hispanic and Non-Hispanic children that had been previously diagnosed with NASH/NAFLD. Race/ethnicity is an established risk factor in the prevalence of NAFLD in the United States. [32,33] This finding suggests that this disparity is also true among healthy children's populations in the United States.

According to the MRI/Age plots figure, children tend to accumulate liver fat with increased age in a monotonic relationship. Ethnicity remained statistically significant all throughout before and after controlling age, Tanner Stages, Obesity, Waist Circumference, and the coefficient for ethnicity was highest (-1.65) when controlling for age, Tanner Stage, and Waist Circumference without the outlier. The interaction between age and ethnicity was not statistically significant in the model. However, the graph presents a difference in steepness. This implies that Hispanics accumulate liver fat faster than Non-Hispanic groups before they develop fatty liver disease. In addition to increase with age, Hispanic ethnicity and obesity explain MRI liver fat accumulation according to the stepwise analysis. In this analysis, to keep the residuals normally distributed, we dichotomized most of the numerical variables such as lipid profile, body weight, and height. This could have introduced excessive rounding, leading to an unavoidable loss of information and power and increase the potential for misclassification. Despite these issues, most of the models have shown that age and ethnicity are important factors to explain % liver fat. In the models in which the age variable is replaced with Tanner Stage, the stepwise method shows ethnicity is not significant. However, as shown in Table 2, there was not enough data for analysis in each cell among stages (I - V); some cells are absent in the analysis. Therefore, due to the small sample size in these models, identifying specific stages within puberty or other risk factors contributing to liver fat accumulation was not clear. We note that at least one research group has suggested that compensatory changes in insulin secretion fails after Tanner III in both sexes. [34] Thus, a larger sample size and would allow for the exploration of the possible relationship between Tanner Stages and liver fat accumulation.

Multiple studies have confirmed genetic disparities as well as environmental disparities among NAFLD patients. Studies point to an allele in the Patatin-like phospholipase domain-containing protein 3 (PNPLA3) for increased risk of developing NAFLD. [32]Hispanics have been shown to present the highest frequency of the PNPLA3 compared to their other ethnicity counterparts suggesting a potential genetic mechanism. [35] Furthermore, Mexican Americans tend to consume

more carbohydrates, saturated fats, cholesterol, and less linoleic acids than Anglo Americans implicating behavioral risk factors as well. There was a small impact of socioeconomic status among different ethnicities on the consumption of atherogenic diet. [36] Davis et al., implies GG genotype of PNPLA3 makes Hispanic children more susceptible to increased hepatic fat when dietary carbohydrate intake, specifically sugar, is high. [37] Among Hispanics, there will be genetic variation depending on their origin of countries and their ancestries. To explore the racial disparities of NAFLD, not only the cultural context and socioeconomic status could be helpful, but exploring the genetic dispositions would allow a better understating of NAFLD prevalence.

We only used self-identified ethnicity in the questionnaire, many of the participants who answered as Hispanics did not answer the race question, and our samples size was small. Despite this shortcoming, our results using % liver fat of healthy children follow the findings of previous reports that indicate that Hispanics are more likely to accumulate liver fat. While we did not find any statistical differences, our data visually suggest that healthy Hispanic children may accumulate liver fat faster than Non-Hispanics (Figure.1). Because this study only used clinical data, future studies would benefit from the use of genomic data and further demographic data such as socioeconomic status to determine the difference between Hispanics and Non-Hispanics.

Conclusion

Our results demonstrated that the % of hepatic fat increased with age among all in our sample of healthy children but the rate of increase was greater among those of Hispanic compared to Non-Hispanic ethnicity. There was no positive linear association between puberty stage and % liver fat.

1. Chalasani N, Younossi Z, Lavine JE, et al. The diagnosis and management of nonalcoholic fatty liver disease: Practice guidance from the American Association for the Study of Liver Diseases. *Hepatology.* 2018;67(1):328-357.
2. Musso G, Gambino R, Cassader M, Pagano G. Meta-analysis: natural history of non-alcoholic fatty liver disease (NAFLD) and diagnostic accuracy of non-invasive tests for liver disease severity. *Ann Med.* 2011;43(8):617-649.
3. Dunn W, Xu R, Wingard DL, et al. Suspected nonalcoholic fatty liver disease and mortality risk in a population-based cohort study. *Am J Gastroenterol.* 2008;103(9):2263-2271.
4. Newton KP, Hou J, Crimmins NA, et al. Prevalence of Prediabetes and Type 2 Diabetes in Children With Nonalcoholic Fatty Liver Disease. *JAMA Pediatr.* 2016;170(10):e161971.
5. Pinero F, Pages J, Marciano S, et al. Fatty liver disease, an emerging etiology of hepatocellular carcinoma in Argentina. *World J Hepatol.* 2018;10(1):41-50.
6. Rausch JC, Lavine JE, Chalasani N, et al. Genetic Variants Associated With Obesity and Insulin Resistance in Hispanic Boys With Nonalcoholic Fatty Liver Disease. *J Pediatr Gastroenterol Nutr.* 2018;66(5):789-796.
7. Rehm JL, Connor EL, Wolfgram PM, Eickhoff JC, Reeder SB, Allen DB. Predicting hepatic steatosis in a racially and ethnically diverse cohort of adolescent girls. *J Pediatr.* 2014;165(2):319-325 e311.

8. Saab S, Manne V, Nieto J, Schwimmer JB, Chalasani NP. Nonalcoholic Fatty Liver Disease in Latinos. *Clin Gastroenterol Hepatol.* 2016;14(1):5-12; quiz e19-10.
9. Schwimmer JB, Zepeda A, Newton KP, et al. Longitudinal assessment of high blood pressure in children with nonalcoholic fatty liver disease. *PLoS One.* 2014;9(11):e112569.
10. Schwimmer JB, Deutsch R, Kahen T, Lavine JE, Stanley C, Behling C. Prevalence of fatty liver in children and adolescents. *Pediatrics.* 2006;118(4):1388-1393.
11. Fazel Y, Koenig AB, Sayiner M, Goodman ZD, Younossi ZM. Epidemiology and natural history of non-alcoholic fatty liver disease. *Metabolism.* 2016;65(8):1017-1025.
12. Wong RJ, Aguilar M, Cheung R, et al. Nonalcoholic steatohepatitis is the second leading etiology of liver disease among adults awaiting liver transplantation in the United States. *Gastroenterology.* 2015;148(3):547-555.
13. EASL Clinical Practice Guidelines: Liver transplantation. *J Hepatol.* 2016;64(2):433-485.
14. Beauchamp G, Barr MM, Vergara A, Ashraf A, Bril F. Treatment of hyperglycemia is not associated with NAFLD improvement in children with type 2 diabetes mellitus. *International Journal of Pediatrics and Adolescent Medicine.* 2021.
15. Paik JM, Henry L, De Avila L, Younossi E, Racila A, Younossi ZM. Mortality Related to Nonalcoholic Fatty Liver Disease Is Increasing in the United States. *Hepatol Commun.* 2019;3(11):1459-1471.
16. Deboer MD, Wiener RC, Barnes BH, Gurka MJ. Ethnic differences in the link between insulin resistance and elevated ALT. *Pediatrics.* 2013;132(3):e718-726.
17. Vos MB, Abrams SH, Barlow SE, et al. NASPGHAN Clinical Practice Guideline for the Diagnosis and Treatment of Nonalcoholic Fatty Liver Disease in Children: Recommendations from the Expert Committee on NAFLD (ECON) and the North American Society of Pediatric Gastroenterology, Hepatology and Nutrition (NASPGHAN). *J Pediatr Gastroenterol Nutr.* 2017;64(2):319-334.
18. Suzuki A, Abdelmalek MF, Schwimmer JB, et al. Association between puberty and features of nonalcoholic fatty liver disease. *Clin Gastroenterol Hepatol.* 2012;10(7):786-794.
19. Temple JL, Cordero P, Li J, Nguyen V, Oben JA. A Guide to Non-Alcoholic Fatty Liver Disease in Childhood and Adolescence. *Int J Mol Sci.* 2016;17(6).
20. Tazawa Y, Noguchi H, Nishinomiya F, Takada G. Serum alanine aminotransferase activity in obese children. *Acta Paediatr.* 1997;86(3):238-241.
21. Research MFfMEa. Pediatric Test Reference Values. https://www.mayocliniclabs.com/test-info/pediatric/refvalues/reference.php. Published 1995–2021. Accessed.
22. Welsh JA, Karpen S, Vos MB. Increasing prevalence of nonalcoholic fatty liver disease among United States adolescents, 1988-1994 to 2007-2010. *The Journal of pediatrics.* 2013;162(3):496-500.e491.
23. Patel S, Lawlor DA, Ferreira DL, et al. The association of nonalcoholic

fatty liver disease with central and peripheral blood pressure in adolescence: findings from a cross-sectional study. *J Hypertens.* 2015;33(3):546-552; discussion 553.
24. Marshall WA, Tanner JM. Variations in the pattern of pubertal changes in boys. *Arch Dis Child.* 1970;45(239):13-23.
25. Marshall WA, Tanner JM. Variations in pattern of pubertal changes in girls. *Arch Dis Child.* 1969;44(235):291-303.
26. MedlinePlus. High Cholesterol in Children and Teens. https://medlineplus.gov/highcholesterolinchildrenandteens.html. Published 2019. Accessed.
27. Fernández JR, Bohan Brown M, López-Alarcón M, et al. Changes in pediatric waist circumference percentiles despite reported pediatric weight stabilization in the United States. *Pediatr Obes.* 2017;12(5):347-355.
28. Fryar CD, Gu Q, Ogden CL. Anthropometric reference data for children and adults: United States, 2007-2010. *Vital Health Stat 11.* 2012(252):1-48.
29. Kim EJ, Kim HJ. Nonalcoholic fatty liver disease in obese and nonobese pediatric patients. *Korean J Pediatr.* 2019;62(1):30-35.
30. Cook RD. Influential Observations in Linear Regression. *Journal of the American Statistical Association.* 1979;74(365):169-174.
31. Xu H, Verre MC. Type 2 Diabetes Mellitus in Children. *Am Fam Physician.* 2018;98(9):590-594.
32. Samji NS, Snell PD, Singal AK, Satapathy SK. Racial Disparities in Diagnosis and Prognosis of Nonalcoholic Fatty Liver Disease. *Clin Liver Dis (Hoboken).* 2020;16(2):66-72.
33. Iqbal U, Perumpail BJ, Akhtar D, Kim D, Ahmed A. The Epidemiology, Risk Profiling and Diagnostic Challenges of Nonalcoholic Fatty Liver Disease. *Medicines (Basel).* 2019;6(1):41.
34. Kelly LA, Lane CJ, Weigensberg MJ, Toledo-Corral CM, Goran MI. Pubertal changes of insulin sensitivity, acute insulin response, and β-cell function in overweight Latino youth. *The Journal of pediatrics.* 2011;158(3):442-446.
35. Kallwitz ER, Tayo BO, Kuniholm MH, et al. American Ancestry Is a Risk Factor for Suspected Nonalcoholic Fatty Liver Disease in Hispanic/Latino Adults. *Clin Gastroenterol Hepatol.* 2019;17(11):2301-2309.
36. Haffner SM, Knapp JA, Hazuda HP, Stern MP, Young EA. Dietary intakes of macronutrients among Mexican Americans and Anglo Americans: the San Antonio heart study. *The American Journal of Clinical Nutrition.* 1985;42(6):1266-1275.
37. Davis JN, Lê K-A, Walker RW, et al. Increased hepatic fat in overweight Hispanic youth influenced by interaction between genetic variation in PNPLA3 and high dietary carbohydrate and sugar consumption. *The American journal of clinical nutrition.* 2010;92(6):1522-1527.

Table. 1: Descriptive Statistics of Lipid panel, Obesity, Waist Circumference, Sex

	Hispanic or Latino or Latina (N=10)	Not Hispanic, not Latino, not Latina (N=20)	Total (N=30)	p value
TCHO				1.000
Elevated	1 (10.0%)	2 (10.0%)	3 (10.0%)	
Not Elevated	9 (90.0%)	18 (90.0%)	27 (90.0%)	
TG				0.038
Elevated	2 (20.0%)	0 (0.0%)	2 (6.7%)	
Not Elevated	8 (80.0%)	20 (100.0%)	28 (93.3%)	
LDL_CHO				0.729
Elevated	2 (20.0%)	3 (15.0%)	5 (16.7%)	
Not Elevated	8 (80.0%)	17 (85.0%)	25 (83.3%)	
HDL_CHO				0.472
Low	0 (0.0%)	1 (5.0%)	1 (3.3%)	
Not Low	10 (100.0%)	19 (95.0%)	29 (96.7%)	
Obesity				0.825
N-Miss	0	2	2	
no	8 (80.0%)	15 (83.3%)	23 (82.1%)	
yes	2 (20.0%)	3 (16.7%)	5 (17.9%)	
Waist_90percentile				0.918
N-Miss	1	4	5	
Less	8 (88.9%)	14 (87.5%)	22 (88.0%)	
Over	1 (11.1%)	2 (12.5%)	3 (12.0%)	
SEX				0.091
F	5 (50.0%)	16 (80.0%)	21 (70.0%)	
M	5 (50.0%)	4 (20.0%)	9 (30.0%)	

TCHO: Total Cholesterol, TG: Triglycerides, LDL_CHO: LDL Cholesterol, HDL_CHO: HDL Cholesterol, Obesity: BMI over 95 (percentile), and WC above 90 percentile: Waist Circumference above 90 percentile (cm)

Table. 2: Descriptive Statistics of MRI(%), Age, Tanner Stage

	Hispanic or Latino or Latina (N=9)	Not Hispanic, not Latino, not Latina (N=19)	Total (N=28)	p value
MRI_percent				0.195
Mean (SD)	3.922 (1.708)	3.111 (1.407)	3.371 (1.528)	
Range	1.600 - 7.000	1.600 - 7.900	1.600 - 7.900	
AGE				0.517
Mean (SD)	12.733 (2.840)	13.500 (2.908)	13.254 (2.856)	
Range	7.500 - 16.800	7.800 - 18.100	7.500 - 18.100	
ALT				0.765
Mean (SD)	19.889 (5.988)	20.632 (6.130)	20.393 (5.984)	
Range	14.000 - 32.000	11.000 - 35.000	11.000 - 35.000	
Tanner Stage (1)				0.635
I	1 (11.1%)	3 (15.8%)	4 (14.3%)	
II	1 (11.1%)	2 (10.5%)	3 (10.7%)	
III	3 (33.3%)	2 (10.5%)	5 (17.9%)	
IV	2 (22.2%)	8 (42.1%)	10 (35.7%)	
V	2 (22.2%)	4 (21.1%)	6 (21.4%)	

Figure.1 :Regression Model C (Interaction Term between Ethnicity and Age)

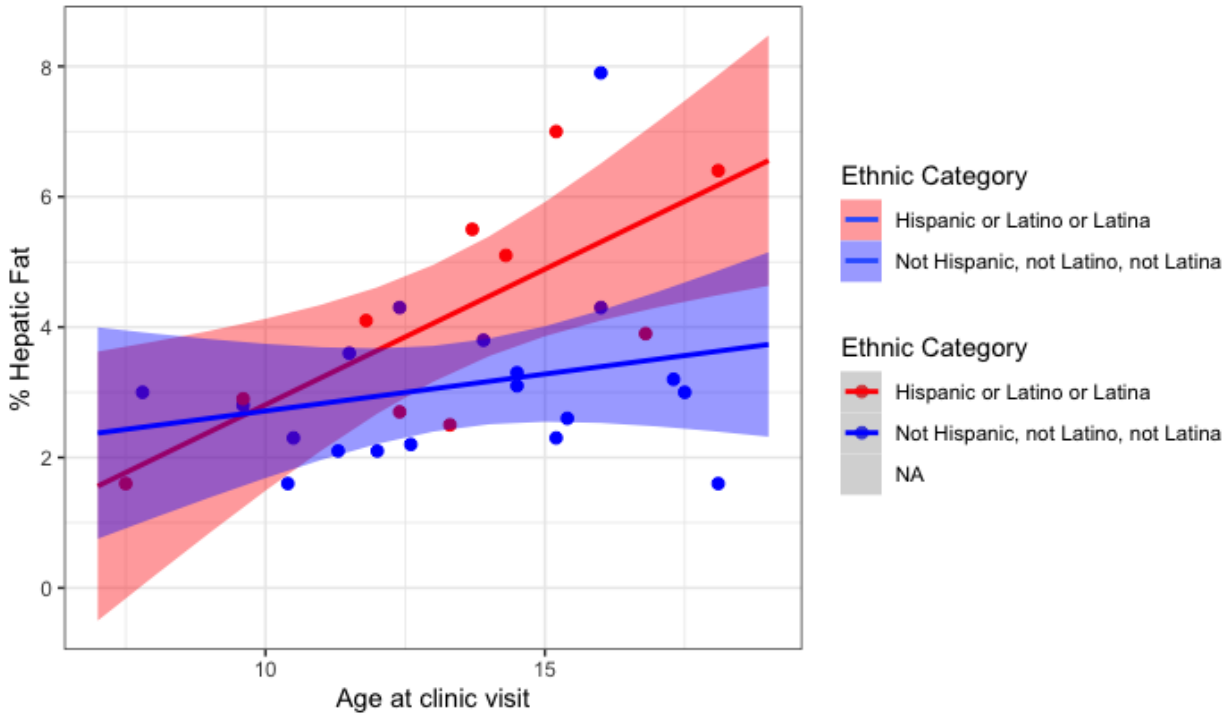

Table.3 :Regression Model A : % liver fat and Ethnicity

	% Hepatic Fat		
Predictors	*Estimates*	*CI*	*p*
(Intercept)	4.17	2.88 – 5.46	**<0.001**
Ethnic Category [Not Hispanic, not Latino, not Latina]	-0.71	-2.29 – 0.87	0.365
Observations	30		
R^2 / R^2 adjusted	0.029 / -0.005		

Table.4 :Regression Model B : % liver fat and Ethnicity, and Age

	% Hepatic Fat		
Predictors	*Estimates*	*CI*	*p*
(Intercept)	1.17	-1.48 – 3.81	0.372
Ethnic Category [Not Hispanic, not Latino, not Latina]	-1.11	-2.25 – 0.03	0.055
AGE	0.23	0.04 – 0.41	**0.020**
Observations	29		
R^2 / R^2 adjusted	0.275 / 0.219		

Table.5 :Regression Model C : % liver fat, Ethnicity, Age, and Interaction

	% Hepatic Fat		
Predictors	*Estimates*	*CI*	*p*
(Intercept)	-1.35	-5.39 – 2.69	0.497
Ethnic Category [Not Hispanic, not Latino, not Latina]	2.93	-2.19 – 8.06	0.249
AGE	0.42	0.12 – 0.71	**0.008**
Ethnic Category [Not Hispanic, not Latino, not Latina] * AGE	-0.30	-0.68 – 0.07	0.108
Observations	29		
R^2 / R^2 adjusted	0.347 / 0.269		

Table.6 :Regression Model D : % liver fat, Ethnicity, Age, and Tanner Stage

	% Hepatic Fat		
Predictors	*Estimates*	*CI*	*p*
(Intercept)	1.39	-1.98 – 4.76	0.402
Ethnic Category [Not Hispanic, not Latino, not Latina]	-1.25	-2.49 – -0.02	**0.047**
AGE	0.20	-0.12 – 0.53	0.208
Tanner Stage (1) [Tanner Stage (1)II]	0.65	-1.85 – 3.16	0.594
Tanner Stage (1) [Tanner Stage (1)III]	-0.57	-3.02 – 1.87	0.633
Tanner Stage (1) [Tanner Stage (1)IV]	0.22	-2.64 – 3.07	0.877
Tanner Stage (1) [Tanner Stage (1)V]	0.57	-2.01 – 3.16	0.650
Observations	29		
R^2 / R^2 adjusted	0.335 / 0.153		

Table.7 :Regression Model E : % liver fat, Ethnicity, Age, Tanner Stage, and Obesity

	% Hepatic Fat		
Predictors	*Estimates*	*CI*	*p*
(Intercept)	1.90	-1.67 – 5.48	0.279
Ethnic Category [Not Hispanic, not Latino, not Latina]	-1.28	-2.64 – 0.07	0.062
AGE	0.15	-0.20 – 0.50	0.387
Tanner Stage (1) [Tanner Stage (1)II]	0.28	-2.60 – 3.17	0.839
Tanner Stage (1) [Tanner Stage (1)III]	-0.63	-3.34 – 2.09	0.635
Tanner Stage (1) [Tanner Stage (1)IV]	0.42	-2.57 – 3.41	0.771
Tanner Stage (1) [Tanner Stage (1)V]	0.69	-1.99 – 3.38	0.595
Obesity [yes]	0.97	-0.85 – 2.79	0.281
Observations	27		
R^2 / R^2 adjusted	0.373 / 0.141		

Obesity: BMI over 95 (percentile)

Table.8 :Regression Model F : % liver fat, Ethnicity, Age, Tanner Stage, and Waist Circumference 90 percentile

	% Hepatic Fat		
Predictors	*Estimates*	*CI*	*p*
(Intercept)	2.70	-0.32 – 5.72	0.076
Ethnic Category [Not Hispanic, not Latino, not Latina]	-1.65	-2.69 – -0.61	**0.004**
AGE	0.15	-0.11 – 0.42	0.243
Tanner Stage (1) [Tanner Stage (1)II]	0.47	-1.74 – 2.69	0.655
Tanner Stage (1) [Tanner Stage (1)III]	-1.45	-3.52 – 0.61	0.155
Tanner Stage (1) [Tanner Stage (1)IV]	-0.25	-2.47 – 1.97	0.814
Tanner Stage (1) [Tanner Stage (1)V]	0.04	-1.95 – 2.02	0.970
Waist_90percentile [Over]	1.22	-0.65 – 3.08	0.185
Observations	24		
R^2 / R^2 adjusted	0.583 / 0.400		

WC above 90 percentile: Waist Circumference above 90 percentile (cm)

Table.9 :Regression Model from Stepwise method results

	% Hepatic Fat		
Predictors	*Estimates*	*CI*	*p*
(Intercept)	2.28	-0.19 – 4.76	0.068
AGE	0.14	-0.03 – 0.32	0.110
Ethnic Category [Not Hispanic, not Latino, not Latina]	-1.38	-2.31 – -0.46	**0.005**
Obesity [yes]	0.99	-0.26 – 2.23	0.115
Observations	23		
R^2 / R^2 adjusted	0.517 / 0.441		

Obesity: BMI over 95 (percentile)