Distribution Agreement

In presenting this thesis or dissertation as a partial fulfillment of the requirements for an advanced degree from Emory University, I hereby grant to Emory University and its agents the non-exclusive license to archive, make accessible, and display my thesis or dissertation in whole or in part in all forms of media, now or hereafter known, including display on the world wide web. I understand that I may select some access restrictions as part of the online submission of this thesis or dissertation. I retain all ownership rights to the copyright of the thesis or dissertation. I also retain the right to use in future works (such as articles or books) all or part of this thesis or dissertation.

Signature:

_____ _____

Alexandra K. Lee Date

Added Sugar Consumption and HDL in Adolescent Girls: A Longitudinal Analysis

By

Alexandra K. Lee
MSPH

Epidemiology

_____

Julie Gazmararian, PhD, MPH
Faculty Thesis Advisor

_____

Jean Welsh, PhD, MPH, RN
Thesis Field Advisor

Added Sugar Consumption and HDL in Adolescent Girls: A Longitudinal Analysis

By

Alexandra K. Lee

B.A.
Amherst College
2009

Faculty Thesis Advisor: Julie Gazmararian, PhD, MPH, BBA

An abstract of
A thesis submitted to the Faculty of the
Rollins School of Public Health of Emory University
in partial fulfillment of the requirements for the degree of
Master of Science in Public Health
in Epidemiology
2009

Abstract

Added Sugar Consumption and HDL in Adolescent Girls: A Longitudinal Analysis
By Alexandra K. Lee

Background: Added sugar consumption has been linked to obesity, type 2 diabetes, and dyslipidemia. In the United States, added sugar consumption is highest among adolescents, accounting for over 22% of daily calories in the year 2000.

Objective: To determine if added sugar consumption is associated with change in HDL among adolescent girls, and whether race or obesity modify this relationship.

Methods and Participants: The National Heart Lung and Blood Institute's Growth and Health Study (NGHS) was a 10-year cohort study that recruited 2,379 9 and 10 year old girls in 1987 and 1988. Participants completed a three-day food record annually and lipids were assessed biennially. Participants' added sugar consumption was categorized for each year lipids were available and a longitudinal mixed model was fit.

Results: After controlling for obesity, race, physical activity, smoking, maturation stage, age, and other nutritional factors, consumption of 15-20% of calories from added sugar was significantly associated with a 0.30mg/dL annual decline in high-density lipoprotein (HDL) compared to consumption of <10% calories from added sugar ($p=0.03$). Consumption of ≥25% of calories from added sugar was marginally not significantly associated with a 0.27mg/dL annual decline in HDL compared to consumption of <10% calories from added sugar ($p=0.06$).

Conclusion: High consumption of added sugar has deleterious effects on HDL cholesterol among adolescent girls.

Added Sugar Consumption and HDL in Adolescent Girls: A Longitudinal Analysis

By

Alexandra Kathryn Lee

B.A.
Amherst College
2009

Faculty Thesis Advisor: Julie Gazmararian, PhD, MPH, BBA

ACKNOWLEDGEMENTS

I would like to acknowledge Dr. José Binongo for his statistical instruction and guidance, and Dr. Aryeh Stein for his conceptual advice. I would also like to acknowledge Ritam Chowdhury for his contributions of clinical expertise, modeling guidance, and general tips on research and preparing manuscripts.

Table of Contents

INTRODUCTION

Since World War II, the American food landscape has changed dramatically. As the U.S. urbanized, the country went through the nutrition transition, during which consumption of complex carbohydrates and fiber declined and consumption of sugars and fats rose (1). The nutrition transition accompanied the better-known epidemiologic transition, in which infant mortality rates fell, life expectancy rose, and fertility declined, as infectious diseases gave way to chronic diseases (1).

Food processing capability increased as new substances were developed. Corn syrup, when first produced in the early 1950's, was a boon to the food industry—no longer did food manufacturers have to rely on sugar cane from tropical climates, where the availability and pricing was unstable (2). Instead, the new sweetener could be sourced in the U.S. from a stable crop (2). In the early 1970's, the development of high-fructose corn syrup was another revolution for the food industry, because it had better chemical properties than sucrose. High-fructose corn syrup maintains its flavor and is stable in water and acidic solutions. In contrast, at low pH and cold temperatures, sucrose frequently hydrolyzes into its component monosaccharides, fructose and sucrose, which can cause changes in the sweetness, texture, viscosity and flavor of the food item (2, 3).

As the availability of cheap sweeteners increased, so did the consumption of added sugars in the American diet. In the late 1970's, added sugar accounted for 13.1% of all calories consumed (4). By 2000, calories from added sugar accounted for 18.1% of daily caloric intake, a 38% increase (5). Added sugar intake is highest among adolescents age 12-17, who consumed 22.3% of their daily calories from added sugar in

2000 (5). Because the increase of added sugar intake occurred concomitantly with the rise in obesity prevalence, some have hypothesized that increasing added sugar intake contributes to weight gain. This hypothesis has gained traction, particularly as research has shown that consumption of sugar-sweetened beverages is associated with obesity and type 2 diabetes (6). Dietary sugars, particularly fructose-containing sugars, have been shown to effect lipid metabolism (7): experimental studies have documented significant increases in triglycerides and high-density lipoprotein (HDL) cholesterol in subjects fed fructose but not glucose (8). Cross-sectional studies have shown that high levels of added sugar are associated with lower HDL levels in adults as well as adolescents (9, 10). To date, no long-term prospective studies have examined the association between added sugar intake and blood lipid levels.

This aim of this study was to determine if added sugar consumption in adolescent girls affected HDL levels over time. A secondary aim was to determine if the relationship between added sugar and HDL differed by race among adolescent girls. A tertiary aim was to assess whether there was any effect modification of the relationship between added sugar consumption and HDL level by obesity.

LITERATURE REVIEW

Sugars

Carbohydrates, which are characterized by the empirical formula $C_x(H_2O)_y$, typically provide the largest source of energy for humans (11). Monosaccharides like glucose are the basic unit of carbohydrates; larger molecules are composed of monosaccharides bonded together in chains. Polysaccharides, such as cellulose and starch, consist of chains of more than 11 carbohydrate rings and are complex carbohydrates (11). Cellulose must be cooked to break down the carbohydrate chains before they can be digested, while starch can be processed by salivary and pancreatic amylases alone (12). Disaccharides and monosaccharides are both categorized as sugars. Monosaccharides are composed of a single carbohydrate ring ($C_6H_{12}O_6$), and the three types of monosaccharides, glucose, fructose, and galactose, are isomers of each other. Disaccharides consist of two monosaccharides bonded to each other. There are three types of disaccharides: sucrose (glucose bonded to fructose), lactose (glucose bonded to galactose), and maltose (two glucose bonded together). Disaccharides are broken apart into monosaccharides during digestion, and so in studies of nutritional effects of sugars, sucrose is considered as 50% glucose and 50% fructose (13, 14).

Recently, nutritionists have begun to distinguish natural sugars from added sugars (4, 5, 9, 10, 12, 15, 16). Although they are chemically identical, natural sugars are intrinsic to a given food product, such as fructose in apples and pears, lactose in milk, and sucrose in beets (12). In contrast, added sugars are any form of sugar added to a food product during processing or preparation, such as sucrose to ice cream and cakes, and high fructose corn syrup to beverages (12, 15). The primary concern about added sugars

in the diet is that most foods high in added sugar contribute a lot of calories but do not contain many important nutrients, vitamins, or minerals (16). Thus, people consuming items with high levels of added sugar may suffer a nutritional deficient in key dietary components. Another concern is that high levels of sugar in the diet can adversely affect lipid metabolism and heart disease risk (9, 10, 17). Finally, it has been established in a recent systematic review that high consumption of sugar-sweetened beverages, such as soda, is associated with weight gain, obesity, and diabetes in both children and adults (6).

History of and Trends in Sugar Consumption

For the majority of the 20th century, sucrose (table sugar) was the most common form of added sugar (2). Table sugar is typically produced from sugar cane or beets, which grow in tropical regions. However, both the weather and politics in these equatorial regions are unstable, which can cause crop failures and spikes in prices for beets and cane sugar (2). This fluctuating supply of sucrose, as well as several negative chemical properties, made it difficult to use in the food industry. The development of corn syrup in the 1950's was welcomed by the food industry because of its low cost and price stability due to government subsidies and the favorable growing climate for corn in the Midwestern United States. The development of high-fructose corn syrup (HFCS) in the early 1970's was a huge milestone in the food processing industry. Unlike sucrose, it is stable in acidic foods and beverages. Ironically, high-fructose corn syrup is a bit of a misnomer: the vast majority of high-fructose corn syrup in the food supply is composed of nearly equal amounts of fructose and glucose (similar to sucrose which contains 50% of each). HFCS-55, which is 55% fructose, is used primarily in beverages, while HFCS-

42 contains 42% fructose and is used primarily in baked goods. High-fructose corn syrup was so named because all previous corn syrups were 100% glucose, and so in comparison this new product was high in fructose.

As new sweeteners were developed in the 20th century, sucrose consumption decreased while HFCS consumption increased. In 1960, approximately 90% of added sugar consumption by dry weight was sucrose (13), while regular corn syrups accounted for the remaining 10% of added sugar. With the invention of HFCS in the early 1970s, corn syrups began to increase in the food supply (13). By 1985, HFCS alone accounted for 35% of added sugar consumption (13). In 2004, 42% of all added sugar in the food supply was HFCS, while sucrose had shrunk to just 44% of all added sugars, which was a 50% decline over 44 years (14).

Despite the changing types of added sugar, the average ratio of glucose to fructose consumed by Americans has remained relatively stable over time. In the early 1960's, this ratio was 1.2:1, meaning that an average person consumed 20% more glucose than fructose (13). With the increase of glucose-only corn syrups in the 1960's, the ratio of glucose to fructose increased to 1.4:1 by 1970 (13). Since 1970, this ratio of glucose to fructose has remained stable because the change of added sugars from sucrose to HFCS has not affected the glucose to fructose ratio, as both sucrose and HFCS are approximately 50% glucose and 50% fructose (13, 14).

While the ratio of glucose to fructose has not changed since 1970, the absolute amount of added sugar consumed per capita has increased dramatically. This increase in sugar consumption was documented in the U.S. and 126 other countries from 1962 to 2000 by Popkin et al. (4). Using the USDA's Nationwide Food Consumption Survey in

1977-1978 and the Continuing Survey of Food Intake by Individuals in 1989-1991 and 1994-1996, Popkin showed a 22% increase in added sugar as a percentage of total caloric intake in the U.S., from 13.1% in 1977-1978 to 16.0% in 1994-1996 ($p<0.01$). Interestingly, there was no significant increase in added sugar consumption in the period spanning 1977-1978 to 1989-1991 (13.1% to 13.5%) (4). These data suggest that there was a rapid increase in added sugar beginning in the early 1990's. This increase in added sugar consumption occurred at the same time that the U.S. government was strongly promoting low-fat and high-carbohydrate diets (18).

Building upon this work, Welsh et al. used five 2-year National Health and Nutrition Examination Survey (NHANES) cycles from 1999 to 2008 to document the consumption of added sugars during this period (5). In 1999-2000, Americans aged 2 and older consumed an average of 18.1% of their total calories from added sugar, a 13% increase from Popkin's 1994-1996 estimate (5). However, the 1999-2000 estimate appears to have been the peak of added sugar consumption: the percent of total calories from added sugar declined over subsequent years to 14.6% in 2007-2008 (p-value for trend <0.001) (5).

Further analysis of this data indicated that changes in added sugar consumption differed by age group. Young adults ages 18-34 had the largest decline, from 21.4% in 1999-2000 to 16.3% in 2007-2008 ($p<0.001$) (5). Adolescents 12-17 years of age had the second largest decline, with percent of total calories from added sugar falling from 22.3% in 1999-2000 to 17.3% in 2007-2008 ($p<0.001$) (5).

With regards to gender, both the USDA's 1977-1978 data and NHANES 1999-2004 data showed that female adolescents consume a higher percentage of their total calories as fructose than males of the same age (13, 14).

High consumption of soft drinks and fruit drinks appears to be the leading cause of high added sugar intakes. In Popkin's study, the average number of calories from soft drinks and fruit drinks doubled from 1977-1978 to 1994-1996, from 70 to 136 calories per day (4). This increase corresponded to a 3.0% increase of total calories from added sugar, which almost exactly mirrored the total change in percent calories from added sugar during that time period (13.1% to 16.0%). In contrast, the percent of calories from desserts remained constant, "sugars and jellies" decreased, and "candy" increased during this time period (4). Welsh's study from 1999 to 2008 showed similar patterns: as the percent calories from added sugars decreased (18.1% to 14.6%), so too did the number of calories from soft drinks and fruit drinks (192 to 121 kcals) (5).

Biological Mechanisms

The biological mechanisms for how added sugar intake influences cholesterol levels are still unclear (12). Because added sugar consists of both fructose and glucose, and they are processed in fundamentally different ways by the body (8, 17, 19), it is difficult to know whether one compound or the other is responsible for the effects of added sugar.

After food is digested in the stomach, it passes though the small intestines where nutrients are absorbed into the bloodstream. The nutrient-rich blood travels via the hepatic portal vein to the liver. At the liver, fructose is primarily metabolized via

fructokinase (20), which has no rate-limiting steps, allowing the liver to quickly absorb almost all of the ingested fructose. High levels of fructose in the liver is thought to speed up the rate of fatty acid synthesis in the liver, known as de novo lipogenesis (DNL), by directly contributing to the increase in triacylglycerol (17, 19, 21). This triacylglycerol is then released into the blood as very-low-density lipoprotein (VLDL), and sustained high triacylglycerol levels in the bloodstream can decrease HDL levels (17).

In contrast, glucose is metabolized in the liver via phosphofructokinase, which is limited by the availability of adenosine triphosphate (ATP) and citrate (20). Due to this rate-limited process, most glucose passes by the liver and continues on in the bloodstream, triggering the release of insulin (22). Glucose can be absorbed by peripheral tissue to produce energy via glycolysis or it can be converted to glycogen for storage in the liver and muscle tissue (22). When there is excess glucose in the bloodstream, it can be converted by the liver into fatty acids, which are then deposited in adipose tissue as triglyceride (22).

In an experimental study, high consumption of fructose was linked to an increase in visceral adipose tissue, while high consumption of glucose was linked to an increase in subcutaneous adipose tissue (19). Additionally, high fructose consumption may promote insulin resistance, but the mechanisms for this process are still being debated (19).

The effects of added sugar could be due to high fructose consumption, high glucose consumption, or an interaction of fructose and glucose. The effects of added sugar are not directly attributable to either fructose or glucose because added sugar is essentially a one-to-one ratio of fructose and glucose. However, since added sugar in the

diet is modifiable, whereas the ratio of glucose to fructose is intractable, the effects of added sugar are more relevant from a public health perspective.

Sugar and Cholesterol

Some experimental studies have shown significant effects of high sucrose diets on cholesterol (23-25). In the 1980's, one experimental study fed four groups of six male participants differing levels of sucrose in a high-carbohydrate, low-fat diet for ten days (23). All test diets contained 15% fat, 15% protein, and 70% carbohydrates; subjects were randomized to diets that contained 0%, 18%, 36%, or 52% of total calories from sucrose. Cholesterol measurements were taken on days 1, 4, 8, and 11. HDL declined significantly over the 10-day diet among the 36% sucrose and 52% sucrose participants, but HDL changes from baseline to day 11 were not significantly different across levels of sucrose consumption. This result suggests that a high-carbohydrate diet may have adverse effects on HDL cholesterol, and a high-sucrose diet may exacerbate these negative effects.

In contrast, a more recent cross-over experimental study in 2006 found no effect of sucrose on HDL (24). Fourteen adult males were given a high-sucrose or low-sucrose diet for 6 weeks, returned to their normal diets for 4 weeks for a washout period, and then received the other sucrose diet for 6 weeks. Diets contained the same number of calories and identical macronutrient compositions: 55% carbohydrates, 30% fat and 15% protein. The high-sucrose diet contained 25% of total energy from sucrose, while the low-sucrose diet contained 10% of total energy from sucrose. Total cholesterol and LDL cholesterol were significantly higher after the 25% sucrose diet ($p<0.001$), but HDL cholesterol

remained the same. The authors noted that the high-sucrose diet contained more saturated fat and less polyunsaturated fat than the low-sucrose diet, which could explain the increase in LDL cholesterol.

Another experimental study looked at the effects of fructose-sweetened compared to sucrose-sweetened diets and found no difference in any cholesterol measure over 2 weeks (25). Although these experimental studies have conflicting results, potentially due in part to their small sample sizes, they provide some context for larger observational studies.

Several cross-sectional studies using NHANES data show a significant association between high added sugar intake and low HDL cholesterol (9, 10). In a study of adults participating in the 1999-2006 NHANES, participants were categorized into one of six categories of percentage of total calories from added sugar: <5%, 5 - <10%, 10 - <17.5%, 17.5 - <25%, and ≥25% (9). Higher added sugar consumption was significantly associated ($p<0.01$) with higher mean daily caloric intake: the lowest consumers of added sugar had a mean caloric intake of 2,038 (95% CI: 1,975-2,100) while the highest consumers of added sugar had a mean caloric intake of 2,312 (95% CI: 2,242 – 2,382) (9). The outcomes of interest were dichotomous assessments of low HDL (<50 mg/dL for women and <40 mg/dL for men), high triglycerides (>150 mg/dL), high LDL (>130 mg/dL), and a high triglyceride to HDL-C ratio (>3.8). Of all dyslipidemia measures, the strongest association was found for HDL-C and added sugar: the odds ratio comparing the highest and lowest categories of added sugar consumption was 2.6 (95% CI 2.0-3.4), adjusting for only age, sex, and race/ethnicity. In a fully adjusted model that adjusted for the demographic characteristics listed above, poverty, BMI, waist circumference, weight

change, physical activity, total energy intake, nutrient residuals for intake of mono-unsaturated fatty acids, polyunsaturated fatty acids, saturated fatty acids, cholesterol, fiber, and other carbohydrates (excluding fiber and added sugars), hypertension, cigarette smoking, and alcohol use, the odds ratio jumped to 3.2 (95% CI 2.3 – 4.3). The linear trend for all categories of sugar consumption and HDL was significant at p=0.01. The linear trend for sugar consumption and high triglycerides was also statistically significant at p=0.05, although for each individual category of sugar consumption, the 95% confidence interval around the odds ratio included the null value in both the minimally adjusted and fully adjusted models. High LDL was not significantly associated with sugar consumption in both models. The trend for high triglyceride to HDL ratio was also statistically significant at p=0.01.

Welsh et al. also performed an analysis of cardiovascular disease risk factors and added sugar intake among adolescents participating in NHANES 1999-2004 (10). Percent of caloric intake from added sugar was categorized as follows: <10%, 10 - <15%, 15 - <20%, 20 - <25%, 25 - < 30%, and ≥30%. In this analysis, models used cholesterol levels as continuous variables and were adjusted for sex, race, age, education, BMI, physical activity, total energy intake of fats (monounsaturated fatty acids, polyunsaturated fatty acids, and saturated fatty acids), sodium, cholesterol, and fiber. The lowest category of sugar intake was used as the referent group. Average HDL decreased with increasing added sugar: mean HDL was 1.40 mmol/L in the referent group and 1.28 in the highest consumers of added sugar. The linear trend for average HDL was highly statistically significant (p=0.001). Average LDL cholesterol increased from 2.24 mmol/L in the referent group to 2.44 in highest sugar consumers, although it

peaked at 2.51 in the 20- <25% group. The linear trend was significant (p=0.01). In contrast, the linear trend was not significant for total cholesterol and was marginally significant (p=0.05) for triglycerides.

A small correlational study of 32 participants ranging in age from 11 to 25 years also found significant correlations between diet and HDL cholesterol (26). In an unadjusted correlation calculation, total sugar consumption was inversely associated with HDL cholesterol (p=0.034). However, this association was not as strong as fructose alone or all carbohydrates alone. Given that these are unadjusted estimates and the sample size is small with a wide range of values, these results should be interpreted with caution.

An analysis of children ages 6 to 18 in NHANES 2003-2006 found no association between added sugar consumption and measures of adiposity (27). The mean added sugar intake for both sexes aged 12-18 was 25 teaspoons or 17% of energy intake. In a model adjusted for sex, race/ethnicity, poverty income ratio, physical activity, and total energy intake, and teaspoons of added sugar was not associated with BMI, waist circumference, triceps skinfold, subscapular skinfold, or the sum of skinfolds. However, this study also found that children with a higher BMIs reported lower caloric intake, suggesting significant under-reporting of daily intake. Even when total caloric intake was excluded from the model, there were no significant associations between added sugar and measures of obesity in adolescents aged 12-18. Finally, only 7% of the variance in obesity measures was explained by the regression model, suggesting that cross-sectional studies are not sufficient to explain variation in obesity.

The primary study objective is to evaluate whether sugar consumption affects change in HDL over time using a longitudinal cohort study of adolescent girls. The secondary objective is to determine whether race modifies the relationship between sugar consumption and change in HDL. The tertiary objective is to assess whether obesity modifies the relationship between sugar consumption and change in HDL.

METHODS

Study Design and Participants

The National Lung, Heart and Blood Institute's Growth and Health Study (NGHS) was a 10-year prospective cohort study that recruited Caucasian and African-American girls age 9 or 10. Study participants were recruited between January 1987 and May 1988 at three study sites: Richmond, California; Cincinnati, Ohio; and Washington, DC. Participants in Richmond were recruited at schools in the Richmond Unified School District, while participants in Cincinnati were recruited from public and private schools in Hamilton County. Participants in Washington DC were part of a local health maintenance organization or a Girl Scout troop from the same geographic area. The primary inclusion criterion was that both parents identified as the same race as the daughter. Full inclusion and exclusion criteria have been documented elsewhere (28).

HDL

Approximately every two years fasting and non-fasting blood samples were obtained and analyzed for lipid levels. Non-fasting HDL levels were used because non-fasting HDL had fewer missing values than fasting HDL and have the same predictive value in adults (29). The Women's Health Study, a prospective cohort study with over 25,000 healthy women aged 44 and over at baseline and 11 years follow-up, found that non-fasting HDL was equally predictive of incident coronary heart disease as fasting HDL (29).

Nutrition

Nutrition information was collected annually except in years 5 and 7. Girls completed a consecutive 3-day food record (two weekdays and one weekend day) with the help of nutritionists who were trained and certified by the University of Minnesota Nutrition Coordinating Center. The results of the validation study are published elsewhere (30).

Because added sugar was not included as a covariate in the NGHS dataset, added sugar content had to be abstracted from the available food records. The goal was to allocate all grams of sugar contained in a food item to either natural or added sugars. First, foods were categorized as containing exclusively natural sugars (fruits, vegetables, and plain milk), exclusively added sugars (soda, desserts, grain products), or a mix of natural and added sugar (fruit pies, puddings). Next, the sugars in each food were categorized as either natural or added depending on the type of sugar and the food category it was assigned. All sugars contained in natural-sugar containing foods were categorized as natural sugars. In added-sugar containing foods, sucrose, fructose, and glucose contained were classified as added, while milk sugars (lactose and galactose) were classified as natural sugar. For products containing a mix of natural and added sugars, milk sugars were classified as natural, sucrose was classified as added, half of glucose and half of fructose was classified as added sugar, and half of glucose and half of fructose was classified as natural sugar.

Although this classification of half added and half natural sugar is unlikely to be the exact breakdown of natural and added sugars in any given food item, on the whole it is expected to average out. Additionally, the majority of added sugar among adolescents

is consumed in beverages, candies, and syrups, which have minimal, if any, sugar misclassification (70%) (5). Nineteen percent of added sugar among adolescents is from sweetened grain-based foods, and another 7% from sweetened milk products (5). These three categories, all of which have minimal or no misclassification of sugars, account for over 96% of all added sugar consumption among adolescents. Thus, misclassification of sugar type is expected to be very minimal.

Five categories of added sugar consumption were created for the modeling process: <10%, 10% to <15%, 15 to <20%, 20% to <25%, and ≥25% of calories from added sugar. The variable for categories of added sugar consumption was a time-varying covariate; an individual could be in one group at one age and a different group at an older age.

Dietary aspects, including average caloric intake, and the amounts of saturated fat, monounsaturated fat, polyunsaturated fat, fiber and other carbohydrates needed to be controlled for to avoid potential confounding. Nutrient residuals for all control nutrients were created by performing a simple linear regression of each nutrient on the total caloric intake. An individual's nutrient residual is the residual from this linear regression model. Nutrient residuals are advantageous because they eliminate the natural correlation of nutrients with total caloric intake (31).

Other Covariates

Based on published literature, demographic characteristics considered were: age at time of visit, race/ethnicity, maturation stage, physical activity score, obesity, smoking, and alcohol. Age at time of visit was rounded down to the nearest full year. At annual

physical examinations, weight and height were taken in accordance with standard protocols (28). Each adolescent's BMI percentile was determined using the CDC's Growth Charts based on her age in months (32). Individuals below the 5th percentile were considered underweight, individuals between the 5th and 85th percentile were considered normal weight, individuals between the 85th and 95th percentile were overweight, and individuals above the 95th percentile were considered obese (33). Due to small numbers, the underweight category was combined with the normal weight category for modeling.

In years 0 through 5, maturation stage on a scale from 1 to 6 was assessed using both areolar stage and Tanner methodology for pubic hair (28). Because maturation stage not collected beyond year 5 and thus missing for all participants, for the purposes of statistical analysis all participants were assigned to maturation stage 6 after year 5.

Two evaluations of physical activity were performed (34). The first tool measured physical activity using a three-day diary with pictures of common activities, adapted from Baranowski et al (35). A second physical activity questionnaire asked about frequency of physical activities during the school year and in summertime, adapted from Ku (36). Duration, frequency, and intensity of the activity were all factored into an overall physical activity score that ranged from 0 to 131 (baseline mean: 30.04). This analysis used the second physical activity measure adapted from Ku because it showed good validity in an informal internal validity test (34).

Questions regarding smoking changed over time. At the baseline visit and through the first 4 years of follow-up, participants were asked "Have you smoked any cigarettes in the past year?" If the answer was yes, participants were asked "How many

cigarettes did you smoke last week?" For these years, participants were classified as non-smokers if they answered no to smoking in the past year. Participants who smoked 5 or fewer cigarettes in the past week were classified as infrequent smokers, and participants who reported smoking more than 5 cigarettes in the past week were classified as regular smokers. From years 6 to 10, there were a number of questions on smoking. Using the question "How much do you smoke cigarettes?" smoking was classified into three categories: non-smoker, infrequent/former smoker, and current smokers. Non-smokers had one of the following responses: "I've never smoked" "I've smoked once or twice," "I've smoked a few times," or "I smoke occasionally but less than once a month." Infrequent/former smokers answered "weekly but not every day" or "I've smoked in the past but not now." Current smokers answered "every day or nearly every day."

Prevalence of smoking increased substantially in the final years of the study. At visit 6, 5% of participants reported smoking every day or nearly every day. At the final visit, 19% of participants reported smoking every day or nearly every day, and 9% reported smoking weekly or being a former smoker.

Alcohol consumption was dichotomized because very few participants reported alcohol use during the three-day food record. At the final visit, when the participants were age 19 or 20, the 90^{th} percentile of alcohol consumption was only 0.4 grams per day. The standard size of any alcoholic beverage contains 14 grams of alcohol. Alcohol consumption was non-significant all models, and thus is not reported here.

Exclusion from Analysis

Participants' data from a single visit were excluded from analysis if they were missing non-fasting HDL (n=3,472), their average daily caloric intake for a visit was <650 calories or >4000 calories (n=156), if they were missing nutritional data (n=865), or if they were ever pregnant (n=474), leaving a final analytic cohort of n=6,928. (See Figure 1 for flow chart.)

Participants who reported ever being pregnant were excluded from analysis because HDL is higher in the second and third trimesters of pregnancy but drops below pre-pregnancy values after a woman gives birth (37). Analysis of a multi-site US cohort of young adults has shown that HDL levels differ significantly by parity level and these differences remain significant over time and after controlling for other risk factors (38).

Statistical Analysis

Descriptive statistics were generated for all covariates. For annual visits, means and standard deviations were calculated for continuous variables and number and percentage were calculated for categorical variables. To test for trends by age, PROC MIXED was used with dependent continuous variables with age as the sole predictor and a random intercept and slope for age. In addition, covariates were compared by race at three time points using t-tests for continuous variables and chi squared tests for categorical variables. Baseline characteristics were examined by the five categories of added sugar consumption as detailed above and by quartiles of baseline HDL. For these baseline characteristics, continuous variables were tested for trends using linear

regression with the median sugar or HDL intake of each category as a single continuous predictor. Categorical variables were tested for trends using Spearman's coefficient.

To determine if study attrition might affect the results, baseline characteristics of participants who contributed at least three visits to the present analysis were compared to participants who contributed two or fewer visits to the analysis. To assess statistical significance, t-tests were performed for continuous variables and chi-square tests were performed for categorical variables.

Correlations of continuous variables were examined at all time points to look for possible collinearity problems. Bonferroni corrections for multiple tests were used to evaluate significance of correlations.

Prior to analysis, trends in individuals' HDL were examined to assess whether a linear, curvilinear, or spline model would best fit the data, as recommended by Singer and Willet (39). Profile plots for a random selection of 20 girls were created, plotting HDL against time for each individual. Smoothed curves and regression lines were fit to the profile plots to assist in visualizing the data. Next, a larger sample of 100 individuals was plotted on one graph, using regression lines with an overlay of the average regression line. Finally, two random samples of 100 African-American girls and 100 Caucasian girls were plotted with individual regression lines and an average regression overlay on side-by-side graphs.

To address the primary objective of assessing the impact of sugar consumption on HDL over time, longitudinal models using SAS's PROC MIXED (version 9.3, Cary, NC) were fit using full maximum likelihood estimation (39). A priori, random effects for the intercept and centered age were included, and an unstructured error covariance structure

was used. Age, physical activity score, nutrient residuals and total caloric intake were entered into the model as continuous variables. All other demographic variables were entered into the model as categorical variables. Forward model selection was performed, starting with the basic model of sugar categories, age, and the interaction of sugar and age. Demographic characteristics and their interactions with age were added one at a time. If the interaction term with age was not significant, it was dropped from the model before adding the next demographic covariate. Next, nutrition variables and the interaction with age were added one nutrient at a time, eliminating the interaction term if it was not significant or if it did not affect the beta estimates of the interaction terms for sugar categories and age. All nutrition variables were left in the model regardless of significance level to account for all components of diet. Birth control pills were not significant in the final model and were thus dropped. In addition, the middle category of smoking (individuals who smoked infrequently) was not significantly different than the category for non-smokers, and so these two categories were combined. A linear trend test was conducted by entering the median percent of calories from added sugar from each sugar category as a continuous variable in the model.

Multicollinearity was checked using several methods. First, the model was simplified to a linear regression model by limiting observations to age 10 only. All variance inflation factors in this simple linear regression were less than five, far below the cutoff of ten that indicates collinearity. In addition, a SAS macro was used to check for collinearity in the mixed model. Although the macro indicated mild collinearity (one non-intercept parameter with a VDP of .83 and three non-intercept parameters with VDPs of 0.5 to 0.53) it was decided that the model did not need to be changed.

To evaluate whether the effect of sugar consumption over time was modified by race, interaction terms were added between race and sugar, race and age, and race, sugar and age. To determine whether the effect of sugar consumption over time was modified by obesity, interaction terms were added for BMI category and sugar, and BMI category, sugar and age. The significance of the interaction terms was evaluated using a likelihood ratio test.

Graphical results were created using the mean physical activity score and mean caloric intake at each age for African-American and Caucasian girls. These values were used to predict HDL for non-smoking adolescents of normal weight at each category of sugar consumption. Predicted values and the 95% confidence intervals were generated using the ESTIMATE statement in SAS.

Institutional Review Board

This author was added to an existing study with Dr. Jean Welsh as the primary investigator by the Emory University Institutional Review Board under expedited review. (See appendix for IRB documentation.)

RESULTS

Participant Characteristics

Participant characteristics by year of exam are displayed in Table 1. At the first visit, participants were an average of 10 years old and the majority was normal weight (68.8%). A small percentage (3.5%) were underweight (<5th percentile), 15.1% were overweight (85th – 95th percentile) and 12.6% were obese (>95th percentile). As participants aged, the percent of participant who were underweight declined to 2.3% at the final visit, while the percent of participants who were obese rose to 17.0% at the final visit. Racial differences in obesity were pronounced (Table 2). At the final visit, 27.4% of African-American participants were obese, compared to only 8.1% of Caucasian participants ($p<.0001$). In addition, 14.9% of African-American participants were overweight, while 11.8% of Caucasian participants were overweight at the final visit.

Physical activity declined drastically over time, from an average score of 32.3 at the initial visit to 15.7 at the final visit (p for trend <.0001). Regular smoking of >1 cigarette/day increased from 6% of participants at year 6 to 16% of participants at year 9 (p for trend<.0001). Regular smoking was much more common among white participants (23.6%) than among African-American participants (7.7%) at the final visit ($p<.0001$).

Added sugar consumption as a percentage of total caloric intake increased steadily from baseline to the final visit (p for trend=0.005). The average sugar consumption was 17.8% at baseline and increased to 21.5% at the final visit. African-American girls tended to consume more of their daily calories from sugar than Caucasian girls (22.5% compared to 20.7% at the final visit) ($p<0.05$ at all visits). Consumption of

both saturated and monounsaturated fats declined ($p<.0001$); non-sugar and non-fiber carbohydrate consumption rose slightly, from 31.5% to 33.6% of caloric intake ($p=0.0001$). Despite statistically significant changes in fiber and protein over time, these changes were not clinically meaningful.

Baseline descriptive statistics differed slightly across the five categories of added sugar (Table 3). There were no obvious trends in obesity, as the middle categories representing 10% to 20% of calories from sugar had the highest percentages of participants who were overweight or obese. Individuals who consumed ≥25% of their calories from sugar consumed less fat and protein than all other individuals ($p<.0001$, Table 4).

Baseline descriptive statistics by quartile of HDL showed a strong trend in obesity (Table 5). Over 22% of individuals were obese in the lowest quartile of HDL, compared to almost 6% obese in the highest quartile of HDL. There were no differences in nutritional intake across quartiles of HDL. Caucasians were more likely to be in the lower quartiles of HDL ($p=0.007$).

Table 6 details the differences between participants who contributed at least three visits to the analysis compared to participants who contributed two or fewer visits to the analysis. Individuals who contributed two or fewer visits to the analysis were significantly more likely to be African-American ($p=0.002$) and be of lower socioeconomic status ($p<0.0001$), as measured by both parents' income and parents' educational attainment. Interestingly, these participants also had significantly lower added sugar consumption. Individuals who contributed two or fewer visits to the analysis

had slightly higher average HDL cholesterol, but the difference between groups was not statistically significant (p=0.08).

Pearson correlation coefficients for all numeric variables being modeled are displayed in Table 7. There were several moderate to strong (>0.4) correlations, all of which were statistically significant at a Bonferroni-corrected alpha of 0.0022. Despite these strong correlations, collinearity was not detected in the final mixed model.

Table 8 displays the number of individuals at each level of sugar consumption by age. At ages 9 and 10, over 75% of participants consumed between 10% and 25% of their daily caloric intake from sugar. By age 18, the distribution had shifted dramatically: over 75% of participants consumed 15% or more of their daily calories from added sugar, and at age 19 almost one-third of participants consumed more than 25% of their daily calories from added sugar.

Graphical Evaluation

Graphical inspection of individuals' HDL over time did not reveal any patterns of change (Figure 2). Because there was no evidence in any of the graphs to support a quadratic or spline model, a linear model was used in modeling. In practice, however, the use of puberty stage as a categorical variable mimicked the effect of a spline model.

Mixed Models

The effect of high sugar consumption on HDL became more pronounced as more covariates were added to the mixed model (Table 9). In the unadjusted model (Model 1), there were no significant differences between individuals consuming <10% of calories

from added sugar and the other levels of consumption. In Model 2, adjustment for race, smoking, physical activity, BMI category, and the interaction of BMI category and age slightly reduced the magnitude of the beta coefficients but two of the interaction terms of sugar category and age neared statistical significance (p=0.07). After further adjustment for all nutrient residuals, total caloric intake, and the interaction term of total caloric intake and age, the beta coefficients for all sugar terms increased in magnitude and several became statistically significant. In the fully adjusted model (Model 3), an individual who consumed 15-20% of daily calories from added sugar lost 0.31 mg/dL HDL per year at constant caloric intake compared to an individual consuming the same number of calories and less than 10% of daily calories from added sugar (p=0.03). The effect for individuals who consumed ≥25% of calories from added sugar was similar (β=-0.27), but was marginally not significant (p=0.06). The linear trend test was not significant, indicating that there was no dose-response relationship. Given the relative uniformity of the beta coefficients, there appears to be a threshold effect: any added sugar consumption above 15% of daily calories results in declining HDL over time.

There was no effect modification due to either race or obesity (Table 10). In the model that added interaction terms for race and age and race and sugar, no individual parameter estimate was significant, nor was the overall likelihood test (p=0.57). In the model with added interaction terms for obese/overweight and sugar, and age, obese/overweight and sugar, again no single interaction term was significant, nor was the overall likelihood ratio test (p=0.10). These results showed that neither race nor obesity modified the effect of sugar consumption on HDL over time.

The fully adjusted model was used to predict HDL among normal weight non-smokers for each sugar category in Figure 3. For each race, the average caloric intake, average physical activity, and most common maturation stage at each age were used to generate the predicted HDL. All groups experience a decline in HDL as they age except for the lowest consumers of added sugar, who experience an increase in HDL over time. The 95% confidence intervals for the predicted HDL for the highest and lowest consumers of added sugar are displayed in Figure 4. Because numerous parameters are required for predicted HDL values, the standard errors reflect the variance of all these parameters, and thus the confidence bands are relatively large and overlap for all ages.

DISCUSSION

In the NGHS cohort, adolescent girls who consumed 15-20% of their calories from added sugar experienced a statistically significant decline of 0.3mg/dL HDL per year compared to individuals who consumed <10% of their daily calories from added sugar. Individuals who consumed ≥25% of their calories from added sugar experienced a similar rate of decline that was marginally not significant. These results suggest a threshold effect: consumption of greater than 10% of added calories from sugar causes a yearly decline in HDL among adolescent girls. This effect was not modified by either race or BMI category, indicating that all individuals, regardless of weight or race, are susceptible to the effects of added sugar.

Added sugar consumption among the NGHS cohort is comparable to the NHANES estimates on adolescent females from 1999-2004. Several factors make direct comparisons difficult, however. First, different methodologies were used to calculate added sugar consumption. Second, added sugar consumption increased across American in the 1990's, the decade in which the NGHS occurred (4, 5). Thus, it is difficult to tell whether the observed increase in added sugar consumption over time was due to the secular trend, due to change in diets as participants aged, or due to differential loss to follow up with low sugar consumers failing to return for later study visits.

With these limitations in mind, added sugar consumption in the NGHS cohort was compared to NHANES estimates on adolescent girls from 1999-2004 (10). Compared to NHANES estimates, the NGHS cohort had a narrower distribution of sugar consumption. In the NHANES data, approximately 13% of female adolescents aged 12-18 consumed less than 10% of their daily calories from added sugar (10), while in the

NGHS cohort only 8% of females aged 12 to 18 reported consuming less than 10% of their daily calories from added sugar. On the upper end, 31% of adolescent females reported consuming more than 25% of their daily calories from added sugar in NHANES compared to 24% in NGHS.

Looking exclusively at the older ages of the NGHS cohort, however, the estimates become closer to NHANES. For instance, 30.7% of 19 year olds in the NGHS cohort reported consuming greater than 25% of their daily caloric intake from added sugar, which is very close to the NHANES estimate of 31.1% of adolescent females (10). This data on 19 year olds from NGHS was collected in 1997 and 1998, making it closest in time to the NHANES estimate. Overall, the estimates of added sugar consumption from NGHS appear to be reasonable given the secular trends that occurred during the 1990's.

Previous research found a strong linear trend in cross-sectional data between high sugar consumption and low HDL cholesterol in both adults and adolescents (9, 10). In this longitudinal analysis, there was no evidence of a linear trend between added sugar intake and HDL; instead, the results supported a threshold effect.

Strengths

There are four main strengths of this study. The primary strength of this analysis is its use of longitudinal data to explore the relationship between added sugar and HDL. Unlike many dietary studies, where the time frame is a matter of days or weeks, this study had five HDL measurements spread out over 10 years. The use of multiple measurements on individuals across such a long time span allowed investigation of the trajectory of HDL across all of adolescence. Although many participants did not have

HDL measurements at all 5 visits when HDL was measured, the use of PROC MIXED allowed the incorporation of all available HDL measurements that did not have missing covariates.

Second, the cohort size of 2,379 enrolled participants from three study sites was fairly large. The original study research team made sure to enroll approximately equal numbers of African-American and Caucasian participants at each site, and strived to have different socioeconomic classes represented in both races (28). As mentioned previously, the distribution of sugar consumption in the older ages of this cohort is close to the distribution of sugar consumption of adolescent females in NHANES, indicating that this cohort reflects national trends.

Third, the three-day food record with review by a nutritionist has been shown to have the best correlation between observed and reported intakes (29). Although in any observational nutrition study it is unlikely that all nutrient intakes are measured correctly, the method used in this study the gold standard for observational cohort studies. In addition, in this analysis the primary exposure was categorized, thus reducing potential measurement error. However, it is still possible that non-differential measurement error could result in differential misclassification error (40).

Finally, the cohort was comprised entirely of females. Since the effects of puberty on HDL are likely to be different in males, it is better to model changes in HDL separately by gender during adolescents, despite the lack of generalizability.

Limitations

Despite the strengths of this study, there are at least four limitations. The primary limitation of this study is attrition. Only 15% of participants had complete information for all five HDL and covariate assessments. Approximately 42% of participants had at least four measurements of HDL and covariates; 36% of participants contributed two or fewer HDL measurements to the analysis. Participants contributing at least three observations to this analysis differed significantly from participants who contributed two or fewer observations to the analysis. Participants with at least three visits were more likely to be white, consume more sugar, and have a higher socioeconomic status.

Second, the calculation of added sugar did not use a standard methodology. Previous studies of added sugar used the MyPyramid Equivalents Database or the USDA Database for the Added Sugars Content of Selected Foods (5, 9, 10, 27). Because the NGHS data did use the same food codes as either of these databases, added sugar content had to be assigned by the investigator based on the food item and sugar types contained in each food.

Third, measurement error of physical activity and smoking is possible. Surprisingly, smoking was non-significant in the final model. This could be due to underreporting of smoking, or it could be due to the fact that most adolescent females have not smoked for very long. Physical activity is known to provide many health benefits (41), but there is little evidence supporting a relationship between HDL and physical activity among youth (42-45). In this study no association between physical activity and HDL was found in either unadjusted or adjusted models, regardless of whether physical activity was used as a continuous variable or categorized by METS or

by quartiles. It could also be possible that the signal of these usually strong effects was lost in the noise of adolescence: HDL levels were fluctuating due to puberty stage and changing obesity, and so physical activity and smoking had comparatively small contributions. If true, this would amplify the importance of the sugar finding: in spite of the noise from adolescence, the consumption of added sugar still influenced HDL's rate of change.

Fourth, HDL was measured using whole numbers in mg/dL. HDL changes are slight during this period of adolescence (46), and so it was not uncommon for individuals' HDL measurements to change by only two to three mg/dL over two years. Given the small yearly changes in HDL, any amount of measurement error could have dramatic effects on the change in HDL. For instance, if at age 10 HDL was measured at 50 but was actually 51, and at age 12 HDL was measured as 55 but was actually 53, the measured difference between ages would be 5 mg/dL, when in fact the real change was only 2mg/dL. It is difficult to predict whether this non-differential measurement error would have biased the results towards or away from the null.

Finally, this cohort was composed exclusively of African-American and Caucasian participants. Diets and thus added sugar consumption may differ by racial or ethnic group. However, given that there was no effect modification of added sugar by race in the NGHS cohort, it is unlikely that other races would have a different relationship of added sugar and HDL levels.

Implications and Future Directions

The findings of this study support the World Health Organization (WHO) Expert Consultation's recommendation that no more than 10% of caloric intake be from added

sugar (47). The WHO's recommendations are based on concerns that added sugars will contribute to excess weight gain in both adults and children. This study supports a 15% threshold but for a different reason: added sugar can negatively impact HDL levels.

Other organizations have recommended different upper intakes of added sugar consumption. The American Heart Association (AHA) recommended consuming no more than half of the discretionary calorie allowance recommended by the U.S. Department of Agriculture (12). Depending on total caloric intake, the AHA recommendations translate to 4-6% of energy from added sugar. The present study is unable to evaluate this claim because so few subjects in the NGHS cohort consumed less than 6% of energy intake from added sugar.

In contrast, the Institute of Medicine (IOM) issued a guideline stating that individuals should not consume more than 25% of calories from added sugar, because above that level it is difficult to consume all necessary macro and micronutrients (48). The IOM did not use health outcomes to set the 25% guideline because they believed there was not enough evidence on health outcomes to establish a recommended upper limit. The present study adds to the body of evidence that could help the IOM make a recommendation about added sugar consumption based on health outcomes.

This field of study clearly merits more research. Although cross-sectional data has shown that high levels of sugar consumption are correlated with lower HDL (9, 10), it remains to be seen if this association is supported in longitudinal studies among adults. The effects of added sugar on HDL in the NGHS cohort might be slightly weaker than expected because of the fluctuation of HDL in adolescent girls due to puberty; other cardiovascular risk factors such as obesity may be easier to study because it is more

likely that change will occur in a single direction. It has been shown that sugar-sweetened beverages are associated with an increased risk of weight gain, diabetes, and adverse cardiovascular profile in both children and adults (6, 49-54), and it is biologically plausible that all sources of added sugar would have the same effect as sugar-sweetened beverages. Looking at the effects of added sugar consumption on insulin resistance and incident type 2 diabetes is a next possible area of exploration.

It is also biologically plausible that natural sugars could have the same effect as added sugars, since added sugars are chemically indistinguishable from naturally-occurring sucrose, glucose, and fructose. Future studies could examine the relationship between total sugar intake and cholesterol.

Added sugar is a unique nutrient category because there is no lower limit to added sugar consumption. In contrast, almost all other nutrients are required at some minimum level for biological function. Since added sugars are digested the same way as natural sugars, there is no reason to consume added sugars aside from taste. Researchers should work towards establishing a healthy upper limit of the consumption of added sugars.

Research on added sugar consumption has a profound public health impact because almost all Americans consume some added sugar on a regular basis. Processed foods can contain large amounts of added sugar, and added sugar is frequently found even in foods that are not typically considered sweet. Thus, individuals may not be aware of the amount of added sugar they are consuming. If added sugar does indeed cause deleterious health effects, as the literature and this analysis suggest, then greater awareness about added sugars should become a public health objective. The lay public

will need to be educated about the specific negative health consequences of added sugar consumption and the easiest ways to decrease one's consumption of added sugars.

In conclusion, this study adds to the body of evidence suggesting high added sugar consumption causes an increase in cardiovascular risk. These results support the WHO's recommendation that individuals consume no more than 10% of calories from added sugar. Further research should establish if chemically distinct sugars have differential effects and whether natural and added sugars are equally deleterious.

REFERENCES

1. Drewnowski A, Popkin BM. The nutrition transition: new trends in the global diet. *Nutrition Reviews* 1997;55(2):31-43.

2. White JS. Straight talk about high-fructose corn syrup: what it is and what it ain't. *American Journal of Clinical Nutrition* 2008;88(6):1716S-21S.

3. Hein GL, Storey ML, White JS, et al. Highs and lows of high fructose corn syrup: a report from the center for food and nutrition policy and its ceres (R) workshop. *Nutrition Today* 2005;40(6):253-6.

4. Popkin BM, Nielsen SJ. The sweetening of the world's diet. *Obesity Research* 2003;11(11):1325-32.

5. Welsh JA, Sharma AJ, Grellinger L, et al. Consumption of added sugars is decreasing in the United States. *American Journal of Clinical Nutrition* 2011;94(3):726-34.

6. Vartanian LR, Schwartz MB, Brownell KD. Effects of soft drink consumption on nutrition and health: a systematic review and meta-analysis. *American Journal of Public Health* 2007;97(4).

7. Stanhope KL, Havel PJ. Fructose consumption: considerations for future research on its effects on adipose distribution, lipid metabolism, and insulin sensitivity in humans. *The Journal of Nutrition* 2009;139(6):1236S-41S.

8. Stanhope KL, Schwarz JM, Keim NL, et al. Consuming fructose-sweetened, not glucose-sweetened, beverages increases visceral adiposity and lipids and decreases insulin sensitivity in overweight/obese humans. *The Journal of Clinical Investigation* 2009;119(5):1322.

9. Welsh JA, Sharma A, Abramson JL, et al. Caloric sweetener consumption and dyslipidemia among US adults. *JAMA: the Journal of the American Medical Association* 2010;303(15):1490-7.

10. Welsh JA, Sharma A, Cunningham SA, et al. Consumption of Added Sugars and Indicators of Cardiovascular Disease Risk Among US Adolescents. *Circulation* 2011;123(3):249-57.

11. Mann J, Truswell S. *Essentials of Human Nutrition*. Oxford: Oxford University Press; 2012.

12. Johnson RK, Appel LJ, Brands M, et al. Dietary sugars intake and cardiovascular health a scientific statement from the american heart association. *Circulation* 2009;120(11):1011-20.

13. Park YK, Yetley EA. Intakes and food sources of fructose in the United States. *American Journal of Clinical Nutrition* 1993;58(5):737S-47S.

14. Marriott BP, Cole N, Lee E. National estimates of dietary fructose intake increased from 1977 to 2004 in the United States. *The Journal of Nutrition* 2009;139(6):1228S-35S.

15. Wells HF, Buzby JC. Dietary Assessment of Major Trends in US Food Consumption, 1970-2005. *Economic Information Bulletin No 33*: Economic Research Service, U.S Dept of Agriculture, 2008.

16. Van Horn L, Johnson RK, Flickinger BD, et al. Translation and Implementation of Added Sugars Consumption Recommendations A Conference Report From the American Heart Association Added Sugars Conference 2010. *Circulation* 2010;122(23):2470-90.

17. Frayn KN, Kingman SM. Dietary sugars and lipid metabolism in humans. *American Journal of Clinical Nutrition* 1995;62(1):250S-61S.

18. Willett W. Dietary fat and obesity: an unconvincing relation. *The American Journal of Clinical Nutrition* 1998;68(6):1149.

19. Stanhope KL. Role of fructose-containing sugars in the epidemics of obesity and metabolic syndrome. *Annual Review of Medicine* 2012;63:329-43.

20. Stanhope KL, Havel PJ. Fructose consumption: recent results and their potential implications. *Annals of the New York Academy of Sciences* 2010;1190(1):15-24.

21. Delarue J, Normand S, Pachiaudi C, et al. The contribution of naturally labelled 13C fructose to glucose appearance in humans. *Diabetologia* 1993;36(4):338-45.

22. Schaefer EJ, Gleason JA, Dansinger ML. Dietary fructose and glucose differentially affect lipid and glucose homeostasis. *The Journal of Nutrition* 2009;139(6):1257S-62S.

23. Albrink MJ, Ullrich IH. Interaction of dietary sucrose and fiber on serum lipids in healthy young men fed high carbohydrate diets. *American Journal of Clinical Nutrition* 1986;43(3):419-28.

24. Black RNA, Spence M, McMahon RO, et al. Effect of Eucaloric High-and Low-Sucrose Diets With Identical Macronutrient Profile on Insulin Resistance and Vascular Risk A Randomized Controlled Trial. *Diabetes* 2006;55(12):3566-72.

25. Bossetti BM, Kocher LM, Moranz JF, et al. The effects of physiologic amounts of simple sugars on lipoprotein, glucose, and insulin levels in normal subjects. *Diabetes Care* 1984;7(4):309-12.

26. Slyper A, Jurva J, Pleuss J, et al. Influence of glycemic load on HDL cholesterol in youth. *American Journal of Clinical Nutrition* 2005;81(2):376-9.

27. Nicklas TA, O'Neil CE, Liu Y. Intake of added sugars is not associated with weight measures in children 6 to 18 years: National Health and Nutrition Examination Surveys 2003–2006. *Nutrition Research* 2011;31(5):338-46.

28. Morrison J, Biro F, Campaigne B. Obesity and cardiovascular disease risk factors in black and white girls: the NHLBI Growth and Health Study. *American Journal of Public Health* 1992;82(12):1613-20.

29. Mora S, Rifai N, Buring JE, et al. Fasting compared with nonfasting lipids and apolipoproteins for predicting incident cardiovascular events. *Circulation* 2008;118(10):993-1001.

30. Crawford PB, Obarzanek E, Morrison J, et al. Comparative advantage of 3-day food records over 24-hour recall and 5-day food frequency validated by observation of 9-and 10-year-old girls. *Journal of the American Dietetic Association* 1994;94(6):626-30.

31. Willett WC, Howe GR, Kushi LH. Adjustment for total energy intake in epidemiologic studies. *American Journal of Clinical Nutrition* 1997;65(4):1220S-8S.

32. Growth Charts: Percentile Data Files with LMS Values. Centers for Disease Control and Prevention. (http://www.cdc.gov/growthcharts/percentile_data_files.htm). (Accessed April 19, 2013).

33. About BMI for Children and Teens. Centers for Disease Control and Prevention. (http://www.cdc.gov/healthyweight/assessing/bmi/childrens_bmi/about_childrens_bmi.html). (Accessed April 19, 2013).

34. Obarzanek E, Schreiber GB, Crawford PB, et al. Energy intake and physical activity in relation to indexes of body fat: the National Heart, Lung, and Blood Institute Growth and Health Study. *American Journal of Clinical Nutrition* 1994;60(1):15-22.

35. Baranowski T, Dworkin RJ, Cieslik CJ, et al. Reliability and validity of self report of aerobic activity: Family Health Project. *Research Quarterly for Exercise and Sport* 1984;55(4):309-17.

36. Ku L, Shapiro L, Crawford P, et al. Body composition and physical activity in 8-year-old children. *American Journal of Clinical Nutrition* 1981;34(12):2770-5.

37. Van Stiphout W, Hofman A. Serum lipids in young women before, during, and after pregnancy. *American Journal of Epidemiology* 1987;126(5):922-8.

38. Lewis CE, Funkhouser E, Raczynski JM, et al. Adverse Effect of Pregnancy on High Density Lipoprotein (HDL) Cholesterol in Young Adult Women The CARDIA Study. *American Journal of Epidemiology* 1996;144(3):247-54.

39. Singer JD, Willett JB. *Applied Longitudinal Data Analysis: Modeling Change and Event Occurrence*. 1st ed. New York City, New York: Oxford University Press; 2003.

40. Flegal KM, Keyl PM, Nieto FJ. Differential misclassification arising from nondifferential errors in exposure measurement. *American Journal of Epidemiology* 1991;134(10):1233-44.

41. Warburton DE, Nicol CW, Bredin SS. Health benefits of physical activity: the evidence. *Canadian Medical Association Journal* 2006;174(6):801-9.

42. Twisk J, Kemper H, Van Mechelen W. The relationship between physical fitness and physical activity during adolescence and cardiovascular disease risk factors at adult age. The Amsterdam Growth and Health Longitudinal Study. *International Journal of Sports Medicine* 2002;23(S1):8-14.

43. Twisk J, Van Mechelen W, Kemper H, et al. The relation between “long-term exposure” to lifestyle during youth and young adulthood and risk factors for cardiovascular disease at adult age. *Journal of Adolescent Health* 1997;20(4):309-19.

44. Raitakari OT, Taimela S, Porkka KV, et al. Associations between physical activity and risk factors for coronary heart disease: the Cardiovascular Risk in Young Finns Study. *Medicine and Science in Sports and Exercise* 1997;29(8):1055-61.

45. Strong WB, Malina RM, Blimkie CJ, et al. Evidence based physical activity for school-age youth. *The Journal of Pediatrics* 2005;146(6):732-7.

46. Cook S, Auinger P, Huang TT-K. Growth curves for cardio-metabolic risk factors in children and adolescents. *The Journal of Pediatrics* 2009;155(3):S6. e15.

47. Nishida C, Uauy R, Kumanyika S, et al. The joint WHO/FAO expert consultation on diet, nutrition and the prevention of chronic diseases: process, product and policy implications. *Public Health Nutrition* 2004;7(1A; SPI):245-50.

48. Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein, and amino acids. Washington, DC: Institute of Medicine, 2005.

49. Dubois L, Farmer A, Girard M, et al. Regular sugar-sweetened beverage consumption between meals increases risk of overweight among preschool-aged children. *Journal of the American Dietetic Association* 2007;107(6):924-34.

50. Duffey KJ, Gordon-Larsen P, Steffen LM, et al. Drinking caloric beverages increases the risk of adverse cardiometabolic outcomes in the Coronary Artery Risk Development in Young Adults (CARDIA) Study. *American Journal of Clinical Nutrition* 2010;92(4):954-9.

51. Montonen J, Järvinen R, Knekt P, et al. Consumption of sweetened beverages and intakes of fructose and glucose predict type 2 diabetes occurrence. *The Journal of Nutrition* 2007;137(6):1447-54.

52. Palmer JR, Boggs DA, Krishnan S, et al. Sugar-sweetened beverages and incidence of type 2 diabetes mellitus in African American women. *Archives of Internal Medicine* 2008;168(14):1487.

53. Schulze MB, Manson JE, Ludwig DS, et al. Sugar-sweetened beverages, weight gain, and incidence of type 2 diabetes in young and middle-aged women. *JAMA: Journal of the American Medical Association* 2004;292(8):927-34.

54. Berkey CS, Rockett HR, Field AE, et al. Sugar-added beverages and adolescent weight change. *Obesity Research* 2004;12(5):778-88.

Table 1: Descriptive of Statistics of NGHS Cohort by Year of Examination, n=2,223.

	Year 0 (n=1709)		Year 2 (n=1619)		Year 4 (n=1486)		Year 6 (n=1205)		Year 9 (n=818)		p-value for change over age
	Mean or number	S.D. or %	Mean or number	S.D. or %	Mean or number	S.D. or %	Mean or number	S.D. or %	Mean or number	S.D. or %	
Age at exam	10.0	0.6	12.0	0.6	14.0	0.6	16.0	0.6	18.9	0.6	--
Body Mass Index	18.6	3.8	20.5	4.5	22.5	5.2	23.7	5.5	25.3	6.8	<.0001
Underweight	59	3.5	50	3.1	20	1.3	17	1.4	19	2.3	
Normal	1175	68.8	1087	67.1	1001	67.4	844	70.0	552	67.5	
Overweight	257	15.0	246	15.2	231	15.5	172	14.3	108	13.2	
Obese	218	12.8	236	14.6	234	15.7	172	14.3	139	17.0	
Physical Activity Score	32.3	19.3	24.3	15.8	20.2	14.9	10.7	12.8	15.7	19.0	<.0001
Smoking											
Infrequent smoker	0	0	32	2.0	79	5.3	45	3.7	72	8.8	
Current smoker	8	0.5	1	0.1	20	1.3	73	6.1	133	16.3	
Maturation Stage											
1 (prepubescent)	861	50.4	60	3.7	3	0.2					
2	507	29.7	352	21.7	18	1.2					
3	216	12.6	374	23.1	53	3.6					
4	55	3.2	243	15.0	108	7.3					
5	66	3.9	525	32.4	893	60.1					
6 (physically mature)	4	0.2	65	4.0	411	27.7	1205	100	818	100	
Used Birth Control					26	1.7	114	9.5	179	21.9	
Average caloric intake	1826	488	1928	591	1854	598	1845	573	1793	537	0.03
Added sugar*	17.8	6.3	19.0	7.2	19.6	7.3	20.8	7.7	21.5	8.5	0.005
Fiber*	2.6	0.8	2.5	0.9	2.4	0.9	2.5	1.0	2.8	1.3	0.0001
Other carbohydrates*	31.5	5.7	31.0	6.3	31.0	6.9	32.0	8.1	33.6	9.3	<.0001
Saturated fat*	13.5	2.6	13.1	2.7	12.7	2.9	12.0	3.2	10.7	3.4	<.0001
Monounsaturated fat*	13.4	2.4	13.4	2.6	13.2	2.6	12.3	3.1	11.2	3.4	<.0001
Polyunsaturated fat*	6.2	2.0	6.3	2.1	6.5	2.2	6.6	2.4	6.3	2.5	0.0003
Protein*	14.3	2.8	14.4	3.3	14.0	3.2	14.0	3.3	13.9	3.4	0.0014
Non-fasting HDL-C (mg/dl)	54.3	12.6	54.4	11.9	55.8	11.4	53.6	10.8	54.0	11.8	0.2838

*Nutrients are expressed as % of daily caloric intake

Table 2. Descriptive Statistics of the NGHS Cohort by Race for Years 0, 4, and 9, n=2,086.

	Year 0				Year 4				Year 9			
	White (n=903)		Black (n=806)		White (n=732)		Black (n=754)		White (n=442)		Black (n=376)	
	Mean or number	S.D. or %	Mean or number	S.D. or %	Mean or number	S.D. or %	Mean or number	S.D. or %	Mean or number	S.D. or %	Mean or number	S.D. or %
Age at exam†	10.0	0.6	10.1	0.6	13.9	0.6	14.1	0.6	18.9	0.6	19.0	0.6
Body Mass Index†	17.8	3.2	19.4	4.3	21.3	3.9	23.8	5.9	23.7	5.1	27.2	8.0
Underweight†	44	4.9	15	1.9	14	1.9	6	0.8	9	2.0	10	2.7
Normal†	664	73.5	511	63.4	547	74.7	454	60.2	345	78.1	207	55.1
Overweight†	130	14.4	127	15.8	106	14.5	125	16.6	52	11.8	56	14.9
Obese†	65	7.2	153	19.0	65	8.9	169	22.4	36	8.1	103	27.4
Overall PA patterns score‡	33.5	19.1	30.9	19.4	22.8	15.6	17.6	13.7	21.3	21.0	9.2	13.6
Smoking												
Infrequent or former smoker	0	0.0	0	0.0	50	6.8	29	3.8	55	12.4	17	4.5
Current smoker	4	0.4	4	0.5	19	2.6	1	0.1	104	23.5	29	7.7
Maturation Stage†												
1 (no physical development)	603	66.8	258	32.0	3	0.4	0	0				
2	214	23.7	293	36.4	16	2.2	2	0.3				
3	53	5.9	163	20.2	41	5.6	12	1.6				
4	14	1.6	41	5.1	74	10.1	34	4.5				
5	19	2.1	47	5.8	455	62.2	438	58.1				
6 (fully physically developed)	0	0	4	0.5	143	19.5	268	35.5	442	100	376	100
Used Birth Control Pills					7	1.0	19	2.5	116	26.2	63	16.8
Non-fasting HDL-C (mg/dl)‡	53.3	11.6	55.5	13.5	53.9	10.5	57.6	12.0	52.4	10.7	55.9	12.7
Average caloric intake‡	1800	445.7	1855	530.2	1783	537.8	1922	643.3	1752	534.0	1842	536.7
Added sugar‡	17.5	6.2	18.0	6.4	18.4	7.1	20.8	7.3	20.7	8.6	22.5	8.3
Fiber†	2.6	0.8	2.5	0.9	2.6	0.9	2.3	0.8	3.1	1.4	2.4	0.9
Other carbohydrates†	32.4	5.4	30.5	5.8	33.4	6.8	28.6	6.1	36.6	9.8	30.1	7.2
Saturated fatty acids†	13.7	2.6	13.3	2.5	12.5	3.0	12.9	2.8	10.1	3.5	11.4	3.1
Monounsaturated fatty acids†	13.0	2.3	13.8	2.4	12.5	2.5	13.9	2.6	10.1	3.4	12.6	2.9
Polyunsaturated fatty acids	5.8	1.8	6.6	2.2	6.0	2.0	7.0	2.4	5.6	2.4	7.0	2.5
Protein	14.3	2.9	14.3	2.8	14.3	3.3	13.8	3.1	14.0	3.3	13.9	3.5

Nutrients are expressed as % of daily caloric intake

†Differences between races were significant at $p<0.001$ for all three years

‡Differences between races were significant at $p<0.05$ for all three years

Table 3. Baseline Demographic Characteristics of the NGHS Cohort by Added Sugar Consumption, n=1,709.

	<10% calories from added sugar n=158		**10 to <15% calories from added sugar n=454**		**15 to < 20% calories from added sugar n=511**		**20 to <25% calories from added sugar n=383**		**≥25% calories from added sugar n=203**		**p-value for trend test**
	Mean or number	S.D. or %	Mean or number	S.D. or %	Mean or number	S.D. or %	Mean or number	S.D. or %	Mean or number	S.D. or %	
Age at exam	10.04	0.58	9.99	0.57	10.04	0.56	9.99	0.55	10.18	0.55	0.0075
Race											0.03
African-American	72	44.72	205	43.90	252	47.82	199	50.64	108	52.17	
Caucasian	89	55.28	262	56.10	275	52.18	194	49.36	99	47.83	
BMI	18.27	3.64	18.75	3.79	18.77	3.95	18.41	3.61	18.25	3.85	0.3
Underweight	12	7.45	34	7.28	44	8.35	22	5.60	20	9.66	
Normal	107	66.46	293	62.74	323	61.29	272	69.21	134	64.73	0.12
Overweight	25	15.53	71	15.20	85	16.13	53	13.49	30	14.49	
Obese	17	10.56	67	14.35	71	13.47	43	10.94	22	10.63	
Physical Activity score	30.09	17.63	32.39	19.67	32.43	18.95	32.34	19.45	33.04	19.92	0.3
Smokes <7 cigarettes/week	1	0.62	3	0.64	3	0.57	1	0.25	0	0	0.16
Maturation stage											
1 (prepubescent)	85	52.80	242	51.82	253	48.01	191	48.60	99	47.83	
2	45	27.95	133	28.48	163	30.93	119	30.28	58	28.02	
3	19	11.80	60	12.85	68	12.90	50	12.72	25	12.08	0.09
4	4	2.48	11	2.36	15	2.85	17	4.33	9	4.35	
5	8	4.97	12	2.57	21	3.98	12	3.05	14	6.76	
6 (physically mature)	0	0	0	0	3	0.57	0	0	1	0.48	
Parental Income Category*											
0-$9,999	19	12.03	70	15.42	60	11.74	50	13.05	21	10.34	
$10,000-$19,999	18	11.39	63	13.88	65	12.72	43	11.23	18	8.87	0.03
$20,000-$39,999	51	32.28	133	29.30	165	32.29	118	30.81	61	30.05	
$40,000+	59	37.34	166	36.56	196	38.36	157	40.99	89	43.84	
Parental Education											
High School or Less	32	20.25	100	22.03	115	22.50	79	20.63	48	23.65	0.9
1-3 Years Post High School	72	45.57	173	38.11	188	36.79	156	40.73	81	39.90	
College Graduate +	54	34.18	181	39.87	208	40.70	148	38.64	74	36.45	
Non-fasting HDL	54.6	11.8	54.7	12.3	55.2	13.4	53.0	12.6	53.8	12.5	0.11

*87 participants were missing parents' income

Table 4. Baseline Nutrition Characteristics of the NGHS cohort by Added Sugar Consumption, n=1,709.

	<10% calories from added sugar n=158		**10 to <15% calories from added sugar n=454**		**15 to < 20% calories from added sugar n=511**		**20 to <25% calories from added sugar n=383**		**≥25% calories from added sugar n=203**		**p-value for trend test**
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	
Total caloric intake	1703	566	1789	465	1846	475	1889	497	1833	469	0.0009
All carbohydrates	44.2	7.2	47.1	5.8	51.2	5.09	54.1	5.1	58.6	5.16	<.0001
Added sugar	7.7	1.9	12.7	1.4	17.5	1.43	22.2	1.4	29.1	3.80	-
Fiber	2.8	1.0	2.6	0.9	2.6	0.8	2.5	0.8	2.3	0.8	<.0001
Other carbs	34.8	6.8	32.7	5.6	32.0	4.9	30.1	5.2	27.9	4.9	<.0001
All fats	39.4	6.3	38.1	5.0	35.7	4.6	34.2	4.8	31.4	4.5	<.0001
Saturated fat	14.6	3.0	14.4	2.5	13.5	2.4	13.0	2.4	12.0	2.1	<.0001
Monounsaturated fat	14.9	2.7	14.3	2.3	13.3	2.0	12.7	2.1	11.7	1.9	<.0001
Polyunsaturated fat	6.8	2.4	6.5	2.1	6.2	1.9	6.0	2.0	5.4	1.6	<.0001
Protein	17.0	3.0	15.6	2.5	14.2	2.2	13.1	2.4	11.6	2.0	<.0001

Nutrients are expressed as % of daily caloric intake

Table 5. Descriptive Statistics at Baseline of NGHS Cohort by Quartile of HDL, n=1,709.

	Lowest Quartile HDL ≤ 46 n = 473		Second Quartile HDL ≤ 54 n = 429		Third Quartile HDL ≤ 62 n = 395		Top Quartile HDL > 62 n= 412		p-value for linear trend
	Mean or n	S.D. or %	Mean or n	S.D. or %	Mean or n	S.D. or %	Mean or n	S.D. or %	
Age at exam	10.08	0.58	10.01	0.56	10.01	0.58	10.02	0.54	0.07
Race									0.007
African-American	211	44.6	186	43.4	191	48.4	218	52.9	
Caucasian	262	55.4	243	56.6	204	51.7	194	47.1	
Body Mass Index	20.08	4.46	18.40	3.56	18.03	3.40	17.63	3.06	<.0001
Underweight	12	2.5	12	2.8	13	3.3	22	5.3	
Normal	257	54.3	308	71.8	296	74.9	314	76.2	<.0001
Overweight	97	20.5	59	13.8	50	12.7	51	12.4	
Obese	107	22.6	50	11.7	36	9.1	25	6.1	
Physical Activity score	33.2	19.8	30.0	18.1	34.1	19.1	31.9	19.7	0.8
Smokes <7 cigarettes/week	2	0.42	3	0.70	1	0.25	2	0.49	0.9
Parents' Income Category*									
0-$9,999	48	10.6	52	12.6	55	14.8	65	16.8	
$10,000-$19,999	57	12.6	58	14.0	39	10.5	53	13.7	0.12
$20,000-$39,999	149	33.0	146	35.4	123	33.2	110	28.4	
$40,000+	197	43.7	157	38.0	154	41.5	159	41.1	
Parents' Education									
High school or less	116	24.5	87	20.3	79	20.0	92	22.3	0.6
1-3 years post high school	193	40.8	151	35.2	154	39.0	172	41.8	
College graduate +	164	34.7	191	44.5	162	41.0	148	35.9	
Maturation stage									
1 (prepubescent)	220	50.2	218	49.5	218	49.8	214	48.7	
2	128	29.2	132	30.0	138	31.5	120	27.3	
3	59	13.5	58	13.2	49	11.2	56	12.8	0.3
4	11	2.5	11	2.5	12	2.7	22	5.0	
5	14	3.2	15	3.4	16	3.7	22	5.0	
6 (physically mature)	14	3.2	15	3.4	16	3.7	22	5.0	
Total caloric intake	1806	478	1832	466	1862	508	1807	501	0.8
All carbohydrates**	52	7	51	7	51	7	51	7	0.2
Added sugar**	18.3	6.4	17.4	6.1	17.7	6.3	17.6	6.2	0.2
Fiber**	2.6	0.8	2.6	0.8	2.5	0.9	2.6	0.8	0.6
Other carbohydrates**	31.6	5.4	31.6	5.9	31.2	5.6	31.7	5.8	0.98
Saturated fat**	13.4	2.5	13.6	2.6	13.6	2.6	13.5	2.5	0.6
Monounsaturated fat**	13.2	2.4	13.5	2.4	13.5	2.4	13.3	2.3	0.3
Polyunsaturated fat**	6.1	2.1	6.2	2.0	6.2	2.0	6.3	2.2	0.13
Protein**	14.3	2.7	14.3	2.8	14.3	3.0	14.3	2.8	0.99
Non-fasting HDL (mg/dl)	39.9	5.3	50.5	2.3	58.1	2.2	71.1	8.1	-

*87 participants were missing parents' income

**Nutrients are expressed as % of daily caloric intake

Table 6. Baseline Descriptive Statistics Comparing NGHS Participants Who Had At Least Three or Less than Three Visits Used in Analysis, n=2,379.

	At least 3 visits n=1,452		Less than 3 visits n=927		P-value
	Mean or number	S.D. or %	Mean or number	S.D. or %	
Age at exam*	10.01	0.56	10.06	0.55	0.04
Race					
African-American	703	48.42	510	55.02	0.002
Caucasian	749	51.58	417	44.98	
BMI*	18.54	3.85	18.61	3.78	0.64
Underweight	54	3.72	38	4.10	
Normal	995	68.53	610	65.80	0.83
Overweight	215	14.81	143	15.43	
Obese	184	12.67	120	12.94	
Physical Activity score*	32.03	19.27	32.06	19.41	0.97
Smokes <7 cigarettes/week	1	0.07	8	0.86	0.003
Parents' Income Category (Missing)	72	4.96	63	6.80	
0-$9,999	152	10.47	252	27.18	
$10,000-$19,999	168	11.57	155	16.72	<.0001
$20,000-$39,999	447	30.79	247	26.65	
$40,000+	613	42.22	210	22.65	
Parental Education (Missing)	0	0.00	3	0.32	
High School or Less	279	19.21	338	36.46	<.0001
1-3 Years Post High School	570	39.26	355	38.30	
College Graduate +	603	41.53	231	24.92	
Maturation stage (Missing)	10	0.69	28	3.02	
1 (prepubescent)	735	50.62	452	48.76	
2	417	28.72	294	31.72	
3	190	13.09	95	10.25	0.10
4	51	3.51	21	2.27	
5	46	3.17	34	3.67	
6 (physically mature)	3	0.21	3	0.32	
Missing Nutrition information	70	4.82	189	20.39	
Total caloric intake	1822	487	1832	594	0.69
% calories from all carbohydrates	51.4	6.8	50.3	6.8	0.0002
% calories from added sugar	18.0	6.3	17.4	6.3	0.0497
% calories from fiber	2.6	0.8	2.5	0.8	0.07
% calories from other carbs	31.7	5.7	31.2	5.7	0.06
% calories from all fats	35.5	5.4	36.5	5.4	<.0001
% calories from saturated fat	13.3	2.4	13.6	2.3	0.0001
% calories from monounsaturated fat	14.3	2.8	14.3	2.7	0.0006
% calories from polyunsaturated fat	6.2	2.0	6.3	2.0	0.15
% calories from protein	13.4	2.5	13.8	2.6	0.92
Category of Added Sugar Consumption					
<10%	190.0	13.1	263.0	28.4	
10-15%	343.0	23.6	213.0	23.0	<.0001
15-20%	433.0	29.8	216.0	23.3	
20-25%	310.0	21.3	157.0	16.9	
>25%	176.0	12.1	78.0	8.4	
Non-fasting HDL*	54.0	12.7	55.1	12.6	0.08

*Age was missing for 1 participant, BMI was missing for 20 participants, Physical Activity Score was missing for 92 participants, and HDL was missing for 423 participants.

Table 7. Pearson's Correlation Coefficients of Continuous Variables[a] in the NGHS Cohort, N=6,387.

	Fiber[b]	Carbohydrates excluding sugar and fiber[b]	Saturated fat[b]	Monounsaturated fat[b]	Polyunsaturated fat[b]	Average Caloric Intake	Age	Physical Activity Score
Fiber[b]	1	0.54*	-0.33*	-0.32*	-0.03*	0.00	-0.003	0.11*
Carbohydrates excluding sugar and fiber[b]	0.54	1	-0.44*	-0.52*	-0.26*	0.00	-0.01	0.12*
Saturated fat[b]	-0.33	-0.44	1	0.57*	-0.08*	0.00	-0.004	-0.07*
Monounsaturated fat[b]	-0.32	-0.52	0.57	1	0.41*	0.00	0.002	-0.10*
Polyunsaturated fat[b]	-0.03	-0.26	-0.08	0.41	1	0.00	0.01	-0.05*
Average Caloric Intake	0.00	0.00	0.00	0.00	0.00	1	-0.02	0.02
Age	-0.003	-0.01	-0.004	0.002	0.01	-0.02	1	-0.36*
Physical Activity Score	0.11	0.12	-0.07	-0.10	-0.05	0.02	-0.36	1

[a]All years of data were evaluated at once; correlations by age were similar in magnitude and significance level.

[b]Nutrient residuals were used for correlations; this will a priori create no association with average caloric intake

* indicates significant at a Bonferroni-corrected alpha of 0.022 (for 23 tests, excluding caloric intake and nutrient residuals)

Table 8. Distribution of Sugar Consumption Categories by Age, NGHS cohort, N=6,387.

Age	N	<10% calories from added sugar		10 to <15% calories from added sugar		15 to < 20% calories from added sugar		20 to <25% calories from added sugar		≥25% calories from added sugar	
		n	%	n	%	n	%	n	%	n	%
9	779	72	9.2	226	29.0	221	28.4	186	23.9	74	9.5
10	879	80	9.1	215	24.5	277	31.5	188	21.4	119	13.5
11	806	77	9.6	177	22.0	243	30.2	172	21.3	137	17.0
12	799	75	9.4	178	22.3	216	27.0	165	20.7	165	20.7
13	787	70	8.9	138	17.5	230	29.2	190	24.1	159	20.2
14	711	59	8.3	139	19.6	171	24.1	176	24.8	166	23.4
15	635	34	5.4	116	18.3	159	25.0	156	24.6	170	26.8
16	577	42	7.3	99	17.2	142	24.6	130	22.5	164	28.4
17	67	7	10.5	11	16.4	19	28.4	14	20.9	16	23.9
18	412	35	8.5	51	12.4	108	26.2	99	24.0	119	28.9
19	357	28	7.8	48	13.5	73	20.5	98	27.5	110	30.8
20	28	2	7.1	3	10.7	8	28.6	7	25.0	8	28.6
Total	**6837**	**581**	**8.5**	**1401**	**20.5**	**1867**	**27.3**	**1581**	**23.1**	**1407**	**20.6**

Table 9. Parameter Estimates for Categories of Sugar Consumption from Mixed Models using Reduced Maximum Likelihood in the NGHS cohort, N=6,387.

	Model 1: Unadjusted		**Model 2: Demographics only**		**Model 3: Full model**	
	β	p-value	β	p-value	β	p-value
<10% (referent)						
10-15%	0.345	0.6	0.328	0.6	0.553	0.41
15-20%	0.908	0.17	0.857	0.19	1.318	0.05
20-25%	0.0786	0.9	-0.146	0.8	0.543	0.45
≥25%	0.296	0.7	0.251	0.7	1.173	0.15
Age*(<10%) (referent)						
age*(10-15%)	-0.209	0.15	-0.190	0.18	-0.219	0.12
age*(15-20%)	-0.238	0.08	-0.239	0.08	-0.295	0.03
age*(20-25%)	-0.130	0.35	-0.114	0.41	-0.182	0.19
age*(≥25%)	-0.213	0.14	-0.219	0.12	-0.269	0.06

Model 1 does not adjust for any covariates aside from age.
Model 2 adjusts for race, smoking, physical activity, BMI category, and BMI*age.
Model 3 adjusts for the variables in model 2 as well as residuals for fiber, other carbohydrates, saturated fats, monounsaturated fats, polyunsaturated fats, average caloric intake, and average caloric intake times age.
All models contained a random intercept to account for within-subject correlation.

Table 10. Parameter Estimates for Mixed Models Containing Interaction Terms, Estimated using Full Maximum Likelihood in the NGHS cohort, N=6,387.

	Full Model (Model 3)		Model 4		Model 5	
	β	SE	β	SE	β	SE
10-15% sugar	0.55	(0.67)	0.26	(0.87)	0.38	(0.79)
15-20% sugar	1.32*	(0.67)	0.85	(0.88)	1.42˜	(0.79)
20-25% sugar	0.54	(0.72)	-0.18	(0.94)	0.02	(0.84)
>25% sugar	1.17	(0.81)	1.91˜	(1.06)	1.01	(0.93)
Age	-0.23	(0.17)	-0.34˜	(0.20)	-0.23	(0.18)
Black race	4.24**	(0.43)	3.17**	(1.22)	4.21**	(0.43)
Overweight	-4.74**	(0.55)	-4.74**	(0.55)	-6.58**	(1.51)
Obese	-8.46**	(0.64)	-8.38**	(0.65)	-6.91**	(1.80)
Age*10-15% sugar	-0.22	(0.14)	-0.14	(0.18)	-0.27	(0.17)
Age*15-20% sugar	-0.29*	(0.14)	-0.18	(0.18)	-0.32*	(0.16)
Age*20-25% sugar	-0.18	(0.14)	-0.0015	(0.18)	-0.10	(0.16)
Age*>25% sugar	-0.26˜	(0.14)	-0.38*	(0.19)	-0.32˜	(0.17)
Age*obese	0.20*	(0.10)	0.18˜	(0.10)	-0.08	(0.36)
Age*overweight	0.32**	(0.10)	0.31**	(0.10)	0.48	(0.32)
Age*black race			0.29	(0.24)		
Black race *10-15% sugar			0.65	(1.35)		
Black race*15-20% sugar			1.02	(1.32)		
Black race*20-25% sugar			1.55	(1.37)		
Black race*>25% sugar			-1.20	(1.46)		
Age*black race*10-15% sugar			-0.19	(0.29)		
Age*black race*15-20% sugar			-0.27	(0.27)		
Age*black race*20-25% sugar			-0.40	(0.28)		
Age*black race*>25% sugar			0.15	(0.28)		
Overweight*10-15% sugar					1.75	(1.78)
Overweight*15-20% sugar					1.36	(1.74)
Overweight*20-25% sugar					2.93	(1.83)
Overweight*>25% sugar					2.76	(1.94)
Obese*10-15% sugar					-1.60	(2.00)
Obese*15-20% sugar					-2.72	(1.97)
Obese*20-25% sugar					0.12	(2.07)
Obese*>25% sugar					-2.41	(2.18)
Age*overweight*10-15% sugar					0.06	(0.40)
Age*overweight*15-20% sugar					-0.19	(0.37)
Age*overweight*20-25% sugar					-0.57	(0.38)
Age*overweight*>25% sugar					-0.07	(0.39)
Age*obese*10-15% sugar					0.43	(0.41)
Age*obese*15-20% sugar					0.38	(0.41)
Age*obese*20-25% sugar					0.02	(0.41)
Age*obese*>25% sugar					0.42	(0.42)
P-value for test that all βs=0 compared to Model 3			0.57		0.10	

˜Indicates p<0.1, *Indicates p<0.05, **Indicates p<0.01

All models contained a random intercept term to account for within-subject correlation.

Figure 1: Flow Chart of Exclusion Criteria

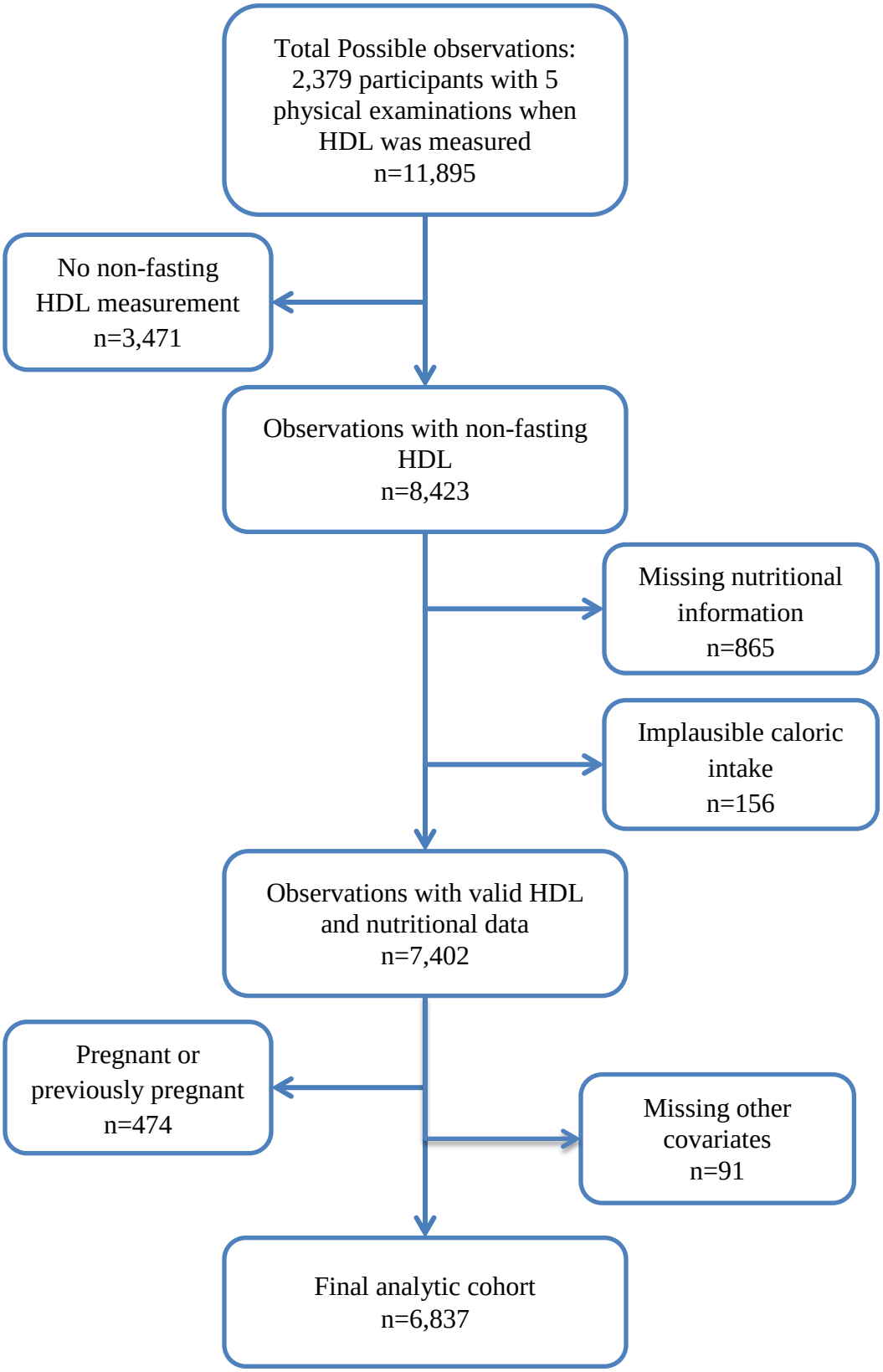


Figure 2: A Random Sample of 20 Individual Scatter Plots of Non-Fasting HDL and Age from the NGHS cohort.

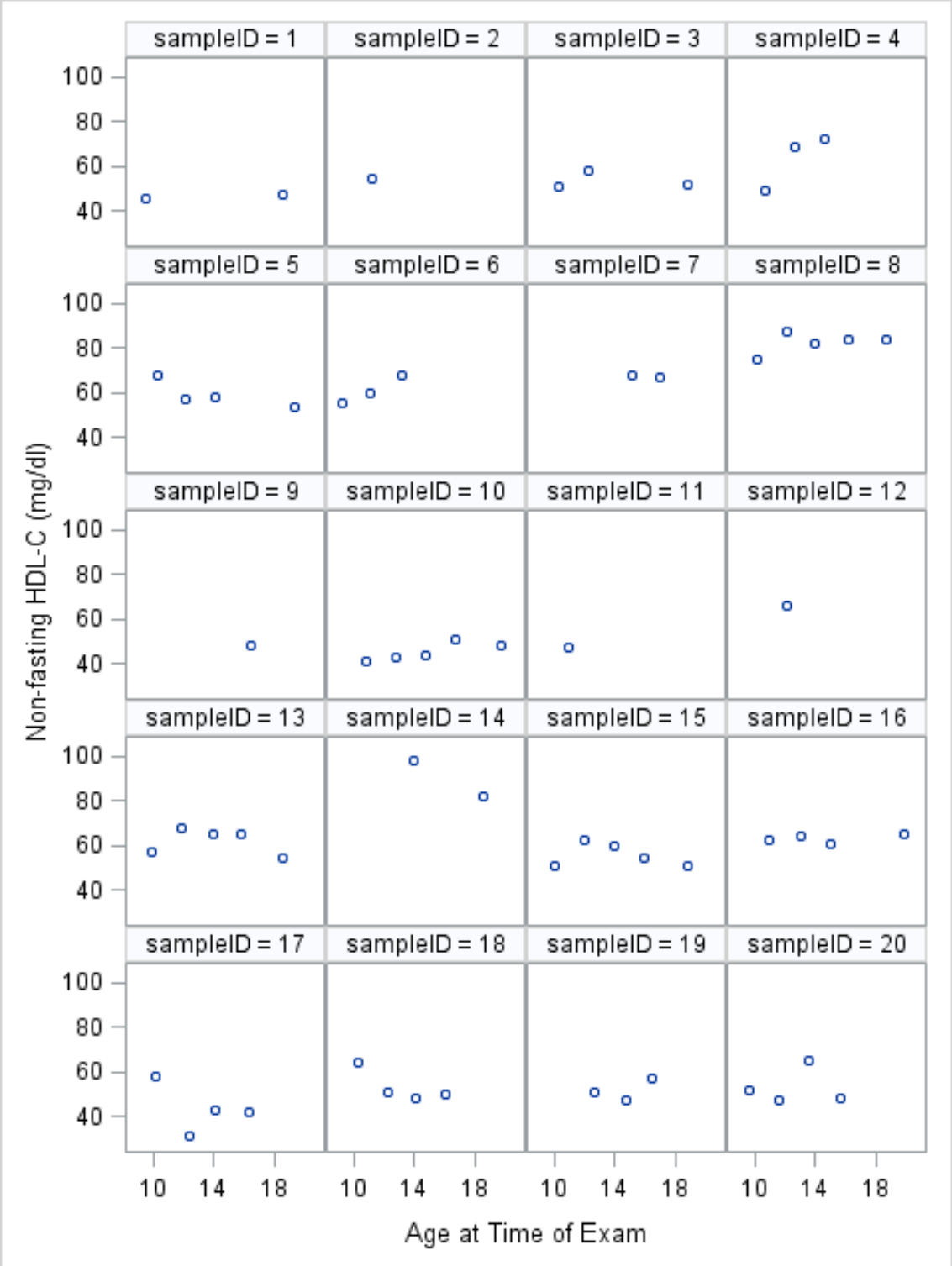


Figure 3a: Predicted HDL by Category of Sugar Consumption for Caucasian Adolescents from the Fully Adjusted Mixed Model from NGHS.

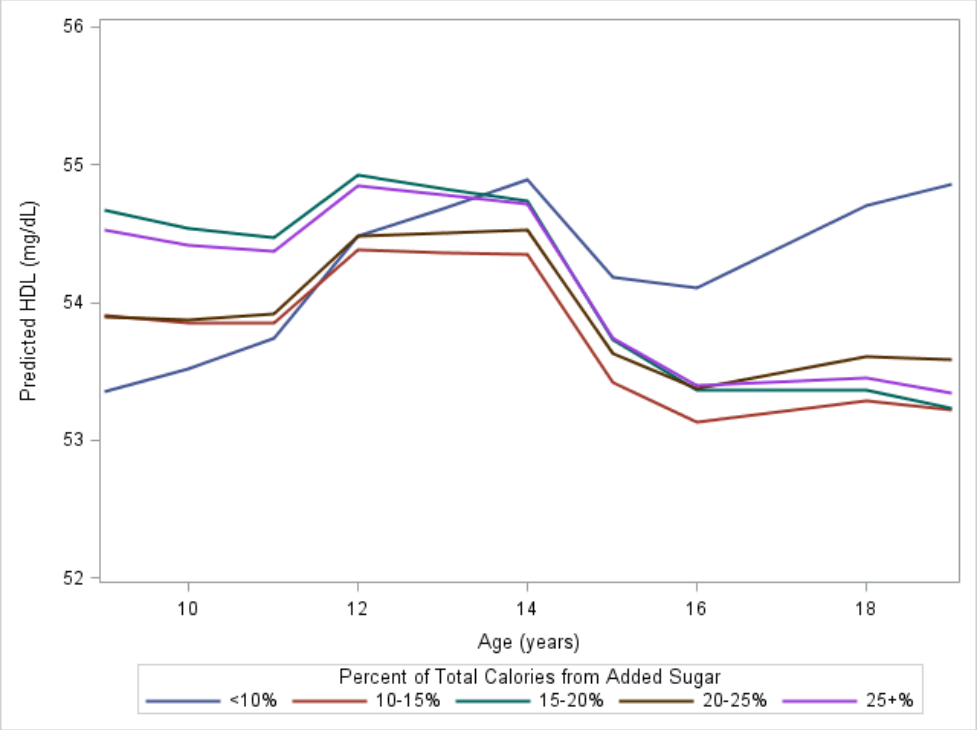


Figure 3b: Predicted HDL by Category of Sugar Consumption for African-American Adolescents from the Fully Adjusted Mixed Model from NGHS.

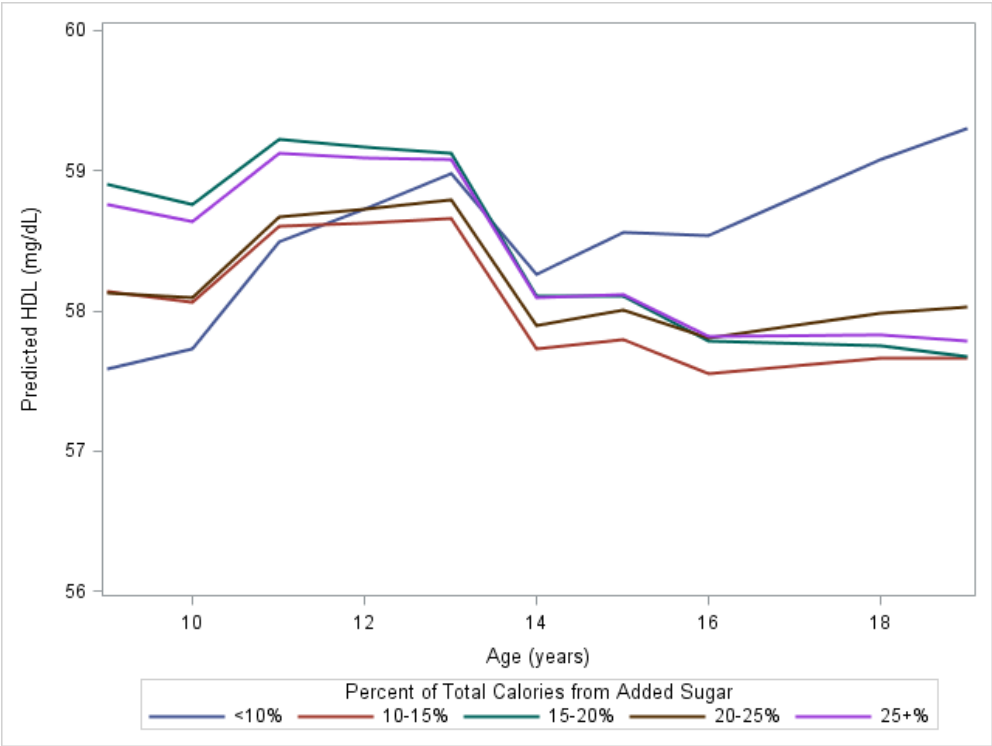


Figure 4a: Predicted HDL and 95% Confidence Bands for the Lowest and Highest Categories of Sugar Consumption for Caucasian Adolescents from the Fully Adjusted Mixed Model from the NGHS cohort.

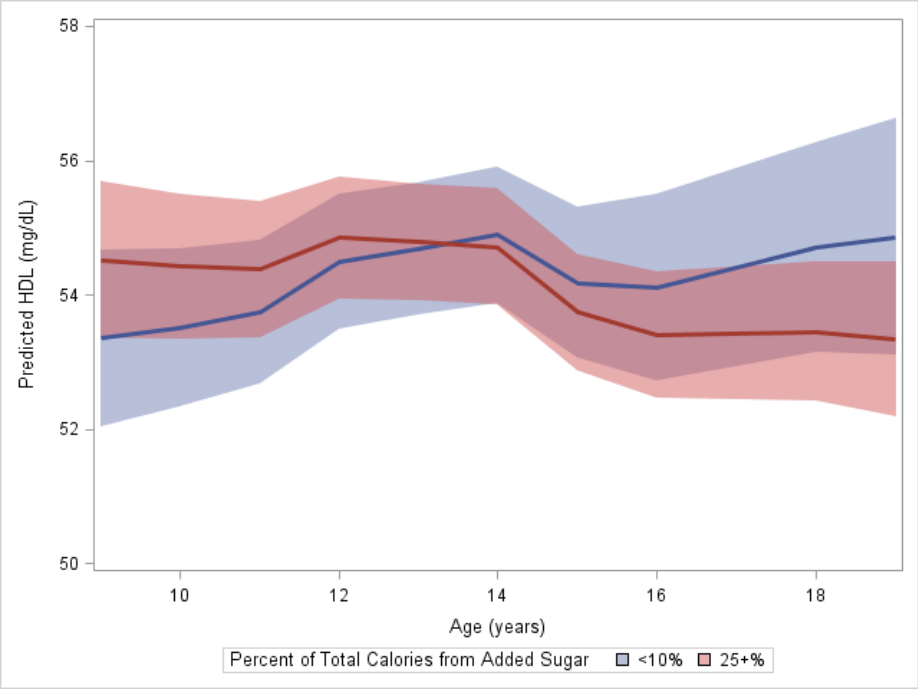
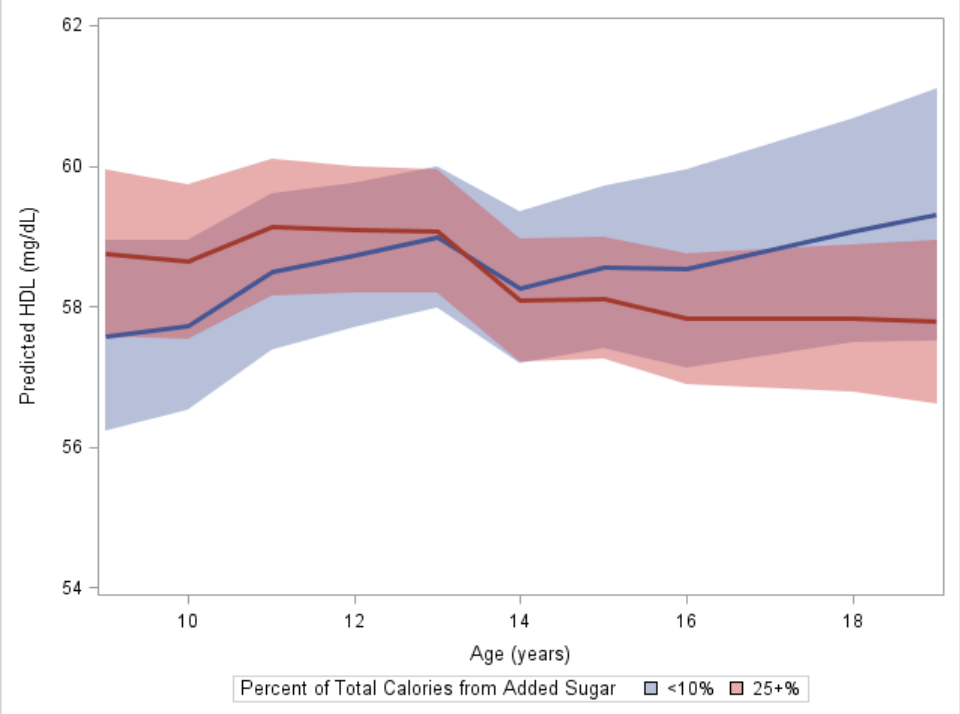


Figure 4b: Predicted HDL and 95% Confidence Bands for the Lowest and Highest Categories of Sugar Consumption for African-American Adolescents from the Fully Adjusted Mixed Model from the NGHS cohort.



APPENDIX

Date: April 21, 2013 7:34:28 PM EDT
View: SF - IRB AM Cover Sheet

Print Close

Amendment Request

Parent Study ID: IRB00035010
Parent Study name: Added Sugar Consumption and Cardiometabolic Risk

- An amendment request can include two parts: the Amendment form and changes to the Study form.
- *After you complete the Amendment request, you will be provided a link to modify the existing study submission form. Edit to reflect how the complete study is to be approved.*
- Only one amendment request is allowed at any given time, i.e: amendment 1 must be approved, denied or withdrawn before amendment 2 can be created

---

1.0 * **Type of change this amendment is making** (check all that apply):
Amendment Type

Changes to Study Team members *[1]*

[1] Please check to see if your Clinical Research Key Points Summary needs to be updated, if applicable for this study, in the Office of Quality section of the eIRB application.
[2] Please consider whether this requires an update to the "Conflict of Interest" section of the eIRB application.
[3] Please select this if you are adding any Conflict of Interest disclosures, any use of radiation, or any use of biohazardous substances/recombinant DNA.

2.0 * **Description of Changes** - briefly summarize the changes:
I would like to clarify my status as the P.I. on this study and to add Alexandra Lee as a co-investigator. She has a current CITI certification through Emory but if you need a copy of her certificate let me know.

Thanks.

Jean Welsh
Department of Pediatrics
School of Medicine

3.0 * **Current Subjects** - are any subjects currently enrolled in the study?:
○ Yes ◉ **No**

4.0 **Subject Notification** - how will subjects be notified of changes (if necessary):

5.0 **If the sponsor has requested any special review or handling of this amendment, please describe:**

6.0 **Which study site(s) will be affected by this amendment?**

Emory Sites
There are no items to display

EMORY UNIVERSITY | Institutional Review Board

TO: Jean Welsh, PhD, MPH, RN
Principal Investigator
GRS: GDBBS NHS

DATE: June 15, 2012

RE: **Notification of Amendment Approval**
AM1_IRB00035010
IRB00035010
Added Sugar Consumption and Cardiometabolic Risk among Adolescent Girls

Thank you for submitting an amendment request. The Emory IRB reviewed and approved this amendment under the expedited review process on 06/15/2012. This amendment includes the following:

Changes to Study Team members - Change in PI - Jean Welsh has been deemed eligible by the School of Medicine to be eligible to assume the role of PI as a Post-Doctoral Research Fellow.

In future correspondence with the IRB about this study, please include the IRB file ID, the name of the Principal Investigator and the study title. Thank you.

Sincerely,

Carol Corkran, MPH, CIP
Senior Research Protocol Analyst
This letter has been digitally signed

CC	Raviele	Nicholas	Ctr for Transplant'n
	Lee	Alexandra	Public Health
	Vos	Miriam	Gastroente

Emory University
1599 Clifton Road, 5th Floor - Atlanta, Georgia 30322
Tel: 404.712.0720 - Fax: 404.727.1358 - Email: irb@emory.edu - Web: http://www.irb.emory.edu/
An equal opportunity, affirmative action university