Nutrition and Genetics as Determinants of an Atherogenic Lipid Profile in African Americans: The Jackson Heart Study

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ABSTRACT

There is limited research on the relationship between genes, nutrients and cardiovascular disease (CVD) in African-Americans. Our objective was to investigate the relationship between omega-3 fatty acids, *trans* fatty acids (TFA) and lipid metabolism genes with serum lipid concentrations in the Jackson Heart Study (JHS). In addition, we aimed to determine the validity of a culturally specific food frequency questionnaire (FFQ) in estimating omega-3 and TFA intake.

Participants in JHS were between the ages of 25-85 y at first interview. A subset of participants was included in the Diet and Physical Activity Sub-Study (DPASS), a validation study of the diet and physical activity questionnaires used in the JHS. A second subset of JHS participants participated in a family study. We evaluated the correlation between dietary omega-3 and TFA from a short food frequency questionnaire (SFFQ), a long food frequency questionnaire (LFFQ), four, 24h recalls and plasma phospholipids (PL) in the JHS DPASS. General linear models were used to evaluate the association between omega-3, TFA and six SNPS in apolipoprotein-E (*APOE*), apolipoprotein-A5 (*APOA5*) and peroxisome proliferator-activated receptor gamma coactivator 1-alpha (*PPARGC1A*). Variance component analysis was used to assess heritability and Haseman-Elston regression analysis was used to assess linkage in the JHS family study. Covariates included age, BMI, sex, energy intake, smoking status, diabetes status and hypertension status.

Correlation analysis showed that dietary measures from the LFFQ were modestly correlated with plasma concentrations of total omega-3, docosahexaenoic (DHA), eicosapentaenoic acid (EPA) and total TFA. Measures from the SFFQ were significantly correlated with EPA, DHA and total omega-3, but not with total TFA. The LFFQ and the SFFQ both appear to be reasonably valid measurement tools for omega-3 intake in southern African Americans. The LFFQ also appears to be valid for estimation of TFA intake in this population. Total TFA estimated from the LFFQ was associated with fast food and southern dietary patterns. Dietary 16:1t was positively associated with high density lipoprotein (HDL-C) and total cholesterol. No other TFA were significantly associated with serum cholesterol. Total omega -3 intake was positively associated with HDL-C. Total omega-3 intake was negatively associated with total cholesterol.

HDL-C, low density lipoprotein (LDL-C), triglycerides (TG) and total cholesterol concentrations were significantly heritable. In a linkage analysis, we found 12 peaks with LOD >3.0. Regions of significant linkage were found for TG in 4 chromosomes; for LDL-C and HDL-C in 2 chromosomes. Associations with serum lipid concentrations were identified with the rs7412 and rs429358 SNPs (APO*E*); the rs3135506 SNP (*APOA5)* and the rs2970869 SNP (*PPARGC1A)*. Significant interactions were found between total dietary fat and rs662799 (*APOA5*) for HDL-C.

We found both nutritional and genetic associations with HDL-C, LDL-C, TG and total cholesterol. These results suggest that further research on the relationships between genes, nutrients and their interactions may prove beneficial to understanding the CVD burden in African Americans.

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They say it takes a village to raise a child, I'd say the same for a dissertation.

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Introduction

Cardiovascular disease (CVD) remains a leading cause of morbidity and mortality across all ethnicities in the United States despite recent declines [1]. However, the magnitude of the disease's effect is not equal across racial and ethnic groups [2, 3]. African-Americans have higher prevalence of total CVD than non-Hispanic white Americans or Mexican Americans [1, 4, 5]. Furthermore, whites and blacks living in the Southeastern U.S., particularly in Mississippi, share a higher disease burden than do their white and black counterparts residing elsewhere in the U.S. [6-8]. Cardiovascular disease is inclusive of many different pathophysiologic endpoints, such as coronary heart disease, angina, stroke, congestive heart failure and myocardial infarction, many of which disproportionately affect African-Americans [1, 4, 5, 9-11]. However, the reasons for this disproportionate disease burden are not well understood.

Differences between racial and ethnic groups also exist for CVD risk factors. Traditional risk factors, including hypertension, dyslipidemia, diabetes and obesity, contribute greatly to CVD risk in Americans [1, 5-7, 12]. Cardiovascular genetic research may provide some insight to cardiovascular health disparities. Although genetic risk has previously been thought of as non-modifiable, new understanding of gene-gene, gene-environment interactions have given rise to newer fields of research. These include gene expression modulation by environmental factors such as diet, smoking and alcohol consumption [13-17]. As a result of genetic research, we may obtain a better understanding of differences in disease burden between ethnic groups, and regional and cultural differences within ethnic groups. Nutrigenomics and nutritional genetics research show that the relationship between dietary intake of saturated fat, for example, and high density lipoprotein (HDL-C), differs by genotype for genes involved in lipid metabolism and

reverse cholesterol transport [14-16]. Diet and genetics are relevant to health disparity research and should be considered. Thus, we aim to investigate nutritional contributions of omega-3(ω-3) and *trans* fat (TFA) intake, the genetic contributions of lipid metabolism single nucleotide polymorphisms (SNPs) in the apolipoprotein-E (*APOE*), apolipoprotein-A5 (*APOA5*) and peroxisome proliferator activated receptor gamma coactivator 1-alpha (*PPARGC1A*) genes and the interaction between these SNPs and total fat intake on HDL-C, low density lipoprotein concentration (LDL-C), triglyceride (TG) and total cholesterol (TC) concentrations in the 5,302 African-American members of the Jackson Heart Study (JHS).

AIM 1. To assess the plasma phospholipid ω-3 and TFA profile in JHS participants and the correlation between plasma phospholipid ω-3 and TFA with dietary ω-3 and TFA, respectively, as assessed by three dietary assessment tools.

Hypothesis: Serum concentrations of **ω-3** and TFA in plasma phospholipid are correlated with dietary **ω-3** and TFA estimations from a regionally specific short food frequency questionnaire (SFFQ), a regionally specific long food frequency questionnaire (LFFQ) and the mean of four 24h recalls completed in the Diet and Physical Activity Sub-Study (DPASS) of JHS.

Aim 1a. To investigate the status of ω-3, dietary sources and the association between dietary ω-3 and serum lipid concentrations.

Hypothesis: Given significant correlation between dietary ω-3 with plasma phospholipid ω-3, dietary ω-3 is positively correlated with HDL-C and negatively correlated with LDL-C and TG in JHS participants.

Aim 1b. To investigate the status of TFA, dietary sources, patterns and association between TFA and serum lipid concentrations.

Hypothesis: Given significant correlation between dietary TFA and plasma phospholipid TFA, dietary TFA is positively correlated with LDL-C and TG and negatively correlated with HDL-C in JHS participants.

AIM 2. To identify genes and specific regions of the human genome associated with lipid phenotypes (HDL-C, LDL-C and TG).

Hypothesis: HDL-C, LDL-C and TG are significantly heritable. Furthermore, there are genomic regions significantly associated with lipid phenotypes.

AIM 3. To study the distribution of genetic variants in *APOE*, *APOA5* and *PPARGC1A* in African-Americans and to investigate the relationship between these genetic variants, dietary fat and baseline measures of HDL-C, LDL-C and TG in JHS participants.

Hypothesis: The frequency of specific polymorphisms in the candidate genes *APOE, APOA5* and *PPARGC1A* in this African American cohort will differ significantly from non-African American populations. Moreover, variants in selected candidate genes will be associated with serum lipid phenotypes and will show significant statistical interactions with dietary fat intake.

Literature Review

Cardiovascular Disease

Cardiovascular disease (CVD) remains a major cause of morbidity and mortality in developed or Western countries [10, 18-21]. In the U.S., CVD has been a public health problem since the 1940's [14]. Although research on CVD as a public health problem has been conducted for more than 70 years, much remains to be discovered. In the past 70 years, risk factors such as hypercholesterolemia, hypertension, diabetes, obesity, dyslipidemia, gender, age, and dietary patterns have been identified **[22-25].** One study that has contributed much to our knowledge about CVD is the Framingham Heart Study, a longitudinal epidemiologic study, funded by National Heart Lung and Blood Institute (NHLBI) [26]. Although, the Framingham Heart Study has contributed greatly to our knowledge of CVD in white Americans, it does not include many **African Americans.** The tools provided by the Human Genome Project, conducted by the U.S. Department of Energy and the National Institutes of Health and completed in 2003[27], also contribute to our growing understanding of CVD through the elucidation of genetic risk factors **[28, 29].** The understanding of genetic predisposition to disease in humans may provide some insight into CVD disparities [30]. Research in the area of nutritional genomics provides potential for understanding the relationship between genes, diet and health [15, 16]. This knowledge can be used to inform prevention and intervention of CVD and to alleviate CVD disparities in the American population.

Dyslipidemia and CVD

Research on lipid metabolism and homeostasis is important in the prevention of CVD [31]. Lipid metabolism is affected by a confluence of environmental, genetic and internal conditions. These conditions work synergistically and antagonistically to control cholesterol **[25, 32, 33].** Clinically, lipids include total cholesterol (TC), triglycerides (TG), very low density lipoprotein (VLDL-C), low-density lipoprotein (LDL-C) and high-density lipoprotein (HDL-C). Each of these lipid molecules influences risk of CVD according to the National Cholesterol Education Program (NCEP) **[34, 35].**

Dyslipidemia in African Americans

The importance of HDL-C and TG concentrations with CVD diagnoses in African Americans remains inconclusive [36]. In 2010, the American Heart Association reported the mean HDL-C for African Americans was 52.4 mg/dL in men and 61.3 mg/dL in women, while the mean HDL-C for non-Hispanic whites was 48.3 mg/dL in men and 60.1 mg/dL in women (Lloyd 2009) [37, 38]. Despite higher HDL-C, cardiovascular mortality statistics show the death rate as 306.6 for non-Hispanic white men, 215.5 for non-Hispanic white women, 422.8 for African American men and 298.2 for African-American women [1, 4, 36]. In a multiethnic study, African American ethnicity was positively associated with HDL-C and negatively with TG in unadjusted models ($p<0.001$) [39]. Previous research in the JHS showed that 15.2% of participants had high TC, 18.3% had high LDL-C, and 5.4% had high serum TG concentration. Age, obesity, type 2 diabetes (T2D), diagnosed CVD, chronic kidney disease, hypertension and physical activity were all significantly associated with hypercholesterolemia status in JHS participants [36]. A separate

study showed the prevalence of low HDL-C (defined as ≤40 mg/dL for men and ≤50 mg/dL for women) as 37.2% in JHS participants at baseline [40].

The Southern Diet

The Southern diet is characterized by foods prepared by deep frying, high intakes of fat, sugar, energy dense foods and few servings of fruit and vegetables [41]. Research on dietary quality in the Mississippi Delta found that Delta residents had a lower Healthy Eating Index (HEI) score than did the National Health and Nutrition Examination Survey (NHANES) participants (1990-2000) [42]. In the development of the Delta Nutrition Intervention Food Frequency Questionnaire (Delta NIRI FFQ), Tucker et al, found that soft drinks, white bread, ground meat, salty snacks and cakes were the top five contributors to energy intake in both black and white residents of the Lower Mississippi Delta [43]. The top contributors to fat intake included mayonnaise, ground beef and salty snacks. Foods like fried catfish, game meats, jambalaya, dirty rice, crawfish and sweet tea were added to the FFQ to improve cultural appropriateness [43].

Previous data from the JHS showed four dietary patterns derived from cluster analysis 1) Southern, 2) Prudent, 3) Fast Food and 4) Juice [44]. The Southern dietary pattern was characterized by corn products and bread; 2) the fast food cluster by soft drinks, snacks and fast food; 3) the prudent pattern by intakes of cereal, milk, fruit and vegetables, and 4) the juice pattern by high intakes of fruit juice [44]. The majority of JHS participants followed a fast food or southern pattern, both characterized by high intakes of saturated fat and *trans* fat (TFA), and low intakes of ω-3 and monounsaturated fats**.**

ω-3 Fatty Acids

ω-3 fatty acids have been associated with improvements in serum TG and CVD outcomes [45, 46]. Dietary patterns, like the Mediterranean diet highlight ω-3 fats as a key contributor to its atheroprotective affects [47]. Marine derived ω-3 fatty acids, docosapentaenoic acid (DHA) and eicosapentaenoic acid (EPA), are pervasive in nutrition research and food and supplement industries. Furthermore ω-3 supplements and foods fortified with ω-3 fatty acids are widely available [48-50]. The Institute of Medicine (IOM), the American Heart Association (AHA) and the dietary guidelines provided by the collaboration between the United States Department of Health and Human Service's Office of Disease Prevention and Health Promotion and the United States Department of Agriculture's Center for Nutrition Policy and Promotion and Agricultural Research Service provide recommendations for ω-3 intake. These recommendations include increasing intakes of DHA and EPA [23, 51-53]. These recommendations are in accord with several studies that suggest higher ω-3 intake is associated with higher HDL-C and lower TG [45, 47, 54, 55].

Trans Fatty Acids

TFA have been shown to be deleterious to health and associated with systemic inflammation, endothelial dysfunction, dyslipidemia and heart attack [56-60]. An increasing number of cities and food companies are banning TFA or eliminating it from their menus **[61, 62].** Studies report that mandatory labeling of trans fat has led to changes in fat content of U.S. snack foods **[63].** Further research suggests that governmental policies requiring TFA labeling have resulted in decreased use of partially hydrogenated vegetable oil, a source of TFA in chip and other snack products. [63].

The public health focus on TFA has coincided with an increase in research on physiologic effects [56, 58, 63-66]. New research highlights the different types of TFA, sources and metabolism. Recent studies show certain TFA are highly concentrated in ruminant foods (red meat, butter and fish), while others are primarily found in margarine, and are ubiquitously spread throughout the food supply [67-69]. New reports on individual TFA, like trans-palmitoleic acid, suggest that some TFAs may be associated with lower insulin resistance [66]. However, these associations have only been seen in observational studies.

Genetic Determinants of Dyslipidemia

Genetic predictors for heart disease are important. As new candidate genes are identified, we gain a greater understanding of the causes, the physiologic pathways and potential treatments for heart disease **[70-72].** Genetic markers for lipid metabolism have been a major focus of genetic CVD research. These genetic markers include an array of genes that code for various enzymes, lipoproteins, carrier proteins and transcription factors involved with endogenous and exogenous lipid metabolism. These markers include apolipoprotein-A1-5, (*APOA1-5*), apolipoprotein-E (*APOE*), and peroxisome proliferator-activated receptor-gamma coactivator 1-alpha (*PPARGC1A*) as well as many others [73-76]. These and other genetic markers have proven to be important contributors to CVD research.

The role of a single SNP in the context of complex chronic disease is not clear. Genes, such as *APOE*, have shown consistent relationships with TC, CVD and Alzheimer's disease [77-80]. However, associations with other SNPs have not been as consistent. Research suggests a

complex network of gene-gene, gene-environment and physiologic conditions underlying the relationship between a single SNP and a phenotype like TC [73, 81, 82].

Gene-Nutrient Interactions

Many nutrients are thought to interact with genes involved in CVD. One of the most studied gene-diet relationships is fatty-acids and lipoproteins. Although well-studied, the research is not complete. Previous studies suggest that saturated fat intake interacts significantly with the *APOE$_{\varepsilon}$2* allele [73, 79]. Such studies suggest that diet may be directly involved in modulating risk posed by genetic polymorphisms. Other genes that show significant interaction with nutrients and CVD include *APOA1, APOC3, APOA4, APOA5, PPARA, PPARGC1A, LIPC, MTHFR, ACE, PLIN, AGT, ANGPTL4* and *Il-6*. These genes code for proteins involved in inflammation, serum HDL-C, LDL-C and TG, blood pressure, obesity, insulin resistance, glucose homeostasis and homocysteine concentration. Nutrients found to interact with genes include total dietary fat, saturated fat, ω-3 fatty-acids, vitamin B12, monounsaturated fat, polyunsaturated fat, sodium and carbohydrate intake[15, 16, 83-85]. Some of these interactions have subsequently been associated with CVD outcomes, myocardial infarction (MI), and coronary heart disease (CHD) [81].

These interactions suggest that, given your genotype for a certain SNP, cholesterol molecules will have different responses to intake of certain nutrients. Although findings have been inconsistent, the field is promising, thus necessitating further research.

Methods for analyzing genetic data

Research on genetic determinants of CVD and dyslipidemia continues to grow, along with methodologies for analysis of genetic data. Two existing methods include linkage analysis and association analysis. In 2006, the Lancet published a road map to genetic research and post human genome sequencing, highlighting the importance of understanding the biological mechanisms and modern statistical tools for analyzing genetic data in answering novel etiological questions[86]. Furthermore, the article suggested that inclusion of biological relationships in analyses potentially improves research outcomes. Burton et al. recommended that epidemiological investigation of genotype-phenotype relationships include 1) recurrence risk ratios, 2) heritability analysis, 3) segregation analysis, 4) linkage analysis, 5) association analysis, and 6) gene expression studies [86]. The authors suggest the synergy of multiple methods leads to a better understanding of the genetics of complex disease.

Health Disparities

Health disparity research focuses on understanding disease in populations with disproportionately higher burdens of disease **[87].** An understanding of risk factors and determinants of disease within different populations provides a basis for treatment and prevention of disease in these populations. Furthermore, the prevalence of genetic and environmental exposures may differ by ethnicity and geographic location. Different confounding factors and intermediates may become evident given cultural context. Thus, understanding of the relationship of diseases within different populations can provide a fuller understanding of disease pathophysiology.

The Jackson Heart Study

The JHS is a National Heart, Lung and Blood Institute (NHLBI) funded investigation into the risk factors for heart disease in African Americans age 25-85 y [88]. There are several reasons why this study population was selected. Perhaps the most important are the disparities in CVD among African Americans, in comparison to other American ethnic groups **[4, 6, 9, 10, 89].** In addition to the disproportionately high occurrence of CVD and related diseases in African Americans, there is a disproportionately high occurrence of these diseases in the Southeastern United States, particularly in Mississippi [5, 8, 11, 90]. Although use of an African American cohort will not allow examination of these hypotheses in direct comparison with other ethnic groups, investigation within the population are needed.

1. Lloyd-Jones, D., et al., *Heart disease and stroke statistics--2009 update: a report from the American Heart Association Statistics Committee and Stroke Statistics Subcommittee*. Circulation, 2009. **119**(3): p. e21-181.
2. Minino, A.M., *Death in the United States, 2009*. NCHS data brief, 2011(64): p. 1-8.
3. Minino, A.M., et al., *Deaths: final data for 2004*. National vital statistics reports : from the Centers for Disease Control and Prevention, National Center for Health Statistics, National Vital Statistics System, 2007. **55**(19): p. 1-119.
4. Lloyd-Jones, D., et al., *Heart disease and stroke statistics--2009 update: a report from the American Heart Association Statistics Committee and Stroke Statistics Subcommittee*. Circulation, 2009. **119**(3): p. 480-6.
5. Lloyd-Jones, D., et al., *Heart disease and stroke statistics--2010 update: a report from the American Heart Association*. Circulation, 2010. **121**(7): p. e46-e215.
6. Mokdad, A.H., D.F. Stroup, and W.H. Giles, *Public health surveillance for behavioral risk factors in a changing environment. Recommendations from the Behavioral Risk Factor Surveillance Team*. MMWR. Recommendations and reports : Morbidity and mortality weekly report. Recommendations and reports / Centers for Disease Control, 2003. **52**(RR-9): p. 1-12.
7. Akil, L. and H.A. Ahmad, *Relationships between obesity and cardiovascular diseases in four southern states and Colorado*. Journal of health care for the poor and underserved, 2011. **22**(4 Suppl): p. 61-72.
8. Ahluwalia, I.B., et al., *State-specific prevalence of selected chronic disease-related characteristics--Behavioral Risk Factor Surveillance System, 2001*. MMWR. Surveillance summaries : Morbidity and mortality weekly report. Surveillance summaries / CDC, 2003. **52**(8): p. 1-80.
9. Budoff, M.J., et al., *Ethnic differences of the presence and severity of coronary atherosclerosis*. Atherosclerosis, 2006. **187**(2): p. 343-50.
10. Roger, V.L., et al., *Heart disease and stroke statistics--2011 update: a report from the American Heart Association*. Circulation, 2011. **123**(4): p. e18-e209.
11. Roger, V.L., et al., *Heart disease and stroke statistics--2012 update: a report from the American Heart Association*. Circulation, 2012. **125**(1): p. e2-e220.
12. Harris-Hooker, S. and G.L. Sanford, *Lipids, lipoproteins and coronary heart disease in minority populations*. Atherosclerosis, 1994. **108 Suppl**: p. S83-104.
13. Hemminki, K., J. Lorenzo Bermejo, and A. Forsti, *The balance between heritable and environmental aetiology of human disease*. Nat Rev Genet, 2006. **7**(12): p. 958-965.
14. Ordovas, J.M., *The quest for cardiovascular health in the genomic era: nutrigenetics and plasma lipoproteins*. The Proceedings of the Nutrition Society, 2004. **63**(1): p. 145-52.
15. Ordovas, J.M. and D. Corella, *Nutritional genomics*. Annual review of genomics and human genetics, 2004. **5**: p. 71-118.
16. Ordovas, J.M. and V. Mooser, *Nutrigenomics and nutrigenetics*. Current opinion in lipidology, 2004. **15**(2): p. 101-8.
17. Paoloni-Giacobino, A., R. Grimble, and C. Pichard, *Genetics and nutrition*. Clinical nutrition, 2003. **22**(5): p. 429-35.
18. Roger, V.L., et al., *Executive summary: heart disease and stroke statistics--2012 update: a report from the American Heart Association*. Circulation, 2012. **125**(1): p. 188-97.
19. Egan, B.M., Y. Zhao, and R.N. Axon, *US trends in prevalence, awareness, treatment, and control of hypertension, 1988-2008*. JAMA : the journal of the American Medical Association, 2010. **303**(20): p. 2043-50.
20. Health, N.H.L.a.B.I.P.S., *2012 NHLBI Morbidity and Mortality Chart Book*. 2012.

21. MMWR, *Prevalence of Coronary Heart Disease---United States, 2006-2010*. Morbidity and Mortality Weekly Report, 2011. **60**(40): p. 1377-1381.
22. *Diet, nutrition and the prevention of chronic diseases*. World Health Organization technical report series, 2003. **916**: p. i-viii, 1-149, backcover.
23. Lichtenstein, A.H., et al., *Summary of American Heart Association Diet and Lifestyle Recommendations revision 2006*. Arteriosclerosis, thrombosis, and vascular biology, 2006. **26**(10): p. 2186-91.
24. Sempos, C.T., et al., *Prevalence of high blood cholesterol among US adults. An update based on guidelines from the second report of the National Cholesterol Education Program Adult Treatment Panel*. JAMA : the journal of the American Medical Association, 1993. **269**(23): p. 3009-14.
25. Talayero, B.G. and F.M. Sacks, *The role of triglycerides in atherosclerosis*. Current cardiology reports, 2011. **13**(6): p. 544-52.
26. Yang, B., *A Change of Heart- How the people of Framingham, Massachusetts, Helped Unravel the Mysteries of Cardiovascular Disease*. Discovery Medicine, 2009. **5**(25): p. 104-1111.
27. Health, U.S.D.o.E.a.N.I.o. *Human Genome Project Information*. 2011 July 25, 2011 [cited 2012 5/30/2012]; Available from: www.ornl.gov/hgmis.
28. Collins, F.S., M. Morgan, and A. Patrinos, *The Human Genome Project: lessons from large-scale biology*. Science, 2003. **300**(5617): p. 286-90.
29. Collins, F.S., et al., *New goals for the U.S. Human Genome Project: 1998-2003*. Science, 1998. **282**(5389): p. 682-9.
30. Kaput, J., et al., *The case for strategic international alliances to harness nutritional genomics for public and personal health*. The British journal of nutrition, 2005. **94**(5): p. 623-32.
31. *Summary of the second report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel II)*. JAMA : the journal of the American Medical Association, 1993. **269**(23): p. 3015-23.
32. Lewington, S., et al., *Blood cholesterol and vascular mortality by age, sex, and blood pressure: a meta-analysis of individual data from 61 prospective studies with 55,000 vascular deaths*. Lancet, 2007. **370**(9602): p. 1829-39.
33. Yang, Q., et al., *Trends in cardiovascular health metrics and associations with all-cause and CVD mortality among US adults*. JAMA : the journal of the American Medical Association, 2012. **307**(12): p. 1273-83.
34. *Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report*. Circulation, 2002. **106**(25): p. 3143-421.
35. Lepor, N.E. and R.E. Vogel, *Summary of the third report of the National Cholesterol Education Program Adult Treatment Panel III*. Reviews in cardiovascular medicine, 2001. **2**(3): p. 160-5.
36. Taylor, H.A., Jr., et al., *Dyslipidemia and the treatment of lipid disorders in African Americans*. The American journal of medicine, 2009. **122**(5): p. 454-63.
37. Morrison, J.A., et al., *Black-white differences in plasma lipids and lipoproteins in adults: The Cincinnati lipid research clinic population study*. Preventive medicine, 1979. **8**(1): p. 34-39.
38. Tyroler, H.A., et al., *Black-white differences in serum lipids and lipoproteins in Evans County*. Preventive medicine, 1975. **4**(4): p. 541-549.
39. Rodriguez, C., et al., *Comparison of modifiable determinants of lipids and lipoprotein levels among African-Americans, Hispanics, and Non-Hispanic Caucasians > or =65 years of age living in New York City*. The American journal of cardiology, 2002. **89**(2): p. 178-83.

40. Taylor, H., et al., *Distinct component profiles and high risk among African Americans with metabolic syndrome: the Jackson Heart Study*. Diabetes care, 2008. **31**(6): p. 1248-53.
41. Thomson, J.L., et al., *Food and beverage choices contributing to dietary guidelines adherence in the Lower Mississippi Delta*. Public health nutrition, 2011: p. 1-11.
42. McCabe-Sellers, B.J., et al., *Assessment of the diet quality of US adults in the Lower Mississippi Delta*. The American journal of clinical nutrition, 2007. **86**(3): p. 697-706.
43. Tucker, K.L., et al., *A regional food-frequency questionnaire for the US Mississippi Delta*. Public health nutrition, 2005. **8**(1): p. 87-96.
44. Talegawkar, S.A., et al., *Serum carotenoid and tocopherol concentrations vary by dietary pattern among African Americans*. Journal of the American Dietetic Association, 2008. **108**(12): p. 2013-20.
45. Hoenselaar, R., *Saturated fat and cardiovascular disease: the discrepancy between the scientific literature and dietary advice*. Nutrition, 2012. **28**(2): p. 118-23.
46. Newby, P.K., et al., *Race and region are associated with nutrient intakes among black and white men in the United States*. The Journal of nutrition, 2011. **141**(2): p. 296-303.
47. Vafeiadou, K., et al., *A review of the evidence for the effects of total dietary fat, saturated, monounsaturated and n-6 polyunsaturated fatty acids on vascular function, endothelial progenitor cells and microparticles*. The British journal of nutrition, 2012. **107**(3): p. 303-24.
48. Artaud-Wild, S.M., et al., *Differences in coronary mortality can be explained by differences in cholesterol and saturated fat intakes in 40 countries but not in France and Finland. A paradox.* Circulation, 1993. **88**(6): p. 2771-9.
49. Balk, E., et al., *Effects of omega-3 fatty acids on cardiovascular risk factors and intermediate markers of cardiovascular disease*. Evidence report/technology assessment, 2004(93): p. 1-6.
50. Bernstein, A.M., et al., *A meta-analysis shows that docosahexaenoic acid from algal oil reduces serum triglycerides and increases HDL-cholesterol and LDL-cholesterol in persons without coronary heart disease*. The Journal of nutrition, 2012. **142**(1): p. 99-104.
51. Esposito, K., et al., *Effect of a mediterranean-style diet on endothelial dysfunction and markers of vascular inflammation in the metabolic syndrome: a randomized trial*. JAMA : the journal of the American Medical Association, 2004. **292**(12): p. 1440-6.
52. Harris, W.S., *n-3 Fatty acid fortification: opportunities and obstacles*. The British journal of nutrition, 2007. **97**(4): p. 593-5.
53. Hu, F.B. and J.E. Manson, *Omega-3 Fatty Acids and Secondary Prevention of Cardiovascular Disease--Is It Just a Fish Tale?: Comment on "Efficacy of Omega-3 Fatty Acid Supplements (Eicosapentaenoic Acid and Docosahexaenoic Acid) in the Secondary Prevention of Cardiovascular Disease"*. Archives of internal medicine, 2012.
54. Kwak, S.M., et al., *Efficacy of Omega-3 Fatty Acid Supplements (Eicosapentaenoic Acid and Docosahexaenoic Acid) in the Secondary Prevention of Cardiovascular Disease: A Meta-analysis of Randomized, Double-blind, Placebo-Controlled Trials*. Archives of internal medicine, 2012.
55. Institute of Medicine (U.S.). Panel on Macronutrients. and Institute of Medicine (U.S.). Standing Committee on the Scientific Evaluation of Dietary Reference Intakes., *Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein, and amino acids*2005, Washington, DC: National Academies Press. xxv, 1331 p.
56. Thompson, T.G. and National Institutes of Health (U.S.), *HHS Secretary Thompson and USDA Secretary Veneman announce Dietary guidelines for Americans, 2005 edition*, 2005, National Institutes of Health,: Bethesda, Md.
57. Lichtenstein, A.H., et al., *Diet and lifestyle recommendations revision 2006: a scientific statement from the American Heart Association Nutrition Committee*. Circulation, 2006. **114**(1): p. 82-96.

58. Finnegan, Y.E., et al., *Plant and marine derived (n-3) polyunsaturated fatty acids do not affect blood coagulation and fibrinolytic factors in moderately hyperlipidemic humans*. The Journal of nutrition, 2003. **133**(7): p. 2210-3.
59. Hu, F.B., et al., *Fish and omega-3 fatty acid intake and risk of coronary heart disease in women*. JAMA : the journal of the American Medical Association, 2002. **287**(14): p. 1815-21.
60. Jacobson, T.A., et al., *Effects of eicosapentaenoic acid and docosahexaenoic acid on low-density lipoprotein cholesterol and other lipids: a review*. Journal of clinical lipidology, 2012. **6**(1): p. 5-18.
61. Clifton, P.M., J.B. Keogh, and M. Noakes, *Trans fatty acids in adipose tissue and the food supply are associated with myocardial infarction*. The Journal of nutrition, 2004. **134**(4): p. 874-9.
62. Lopez-Garcia, E., et al., *Consumption of trans fatty acids is related to plasma biomarkers of inflammation and endothelial dysfunction*. The Journal of nutrition, 2005. **135**(3): p. 562-6.
63. Mozaffarian, D., *Trans fatty acids - effects on systemic inflammation and endothelial function*. Atherosclerosis. Supplements, 2006. **7**(2): p. 29-32.
64. Mozaffarian, D., et al., *Trans fatty acids and cardiovascular disease*. The New England journal of medicine, 2006. **354**(15): p. 1601-13.
65. Mensink, R.P., et al., *Effects of dietary fatty acids and carbohydrates on the ratio of serum total to HDL cholesterol and on serum lipids and apolipoproteins: a meta-analysis of 60 controlled trials*. The American journal of clinical nutrition, 2003. **77**(5): p. 1146-55.
66. Coombes, R., *Trans fats: chasing a global ban*. BMJ, 2011. **343**: p. d5567.
67. Mello, M.M., *New York City's war on fat*. The New England journal of medicine, 2009. **360**(19): p. 2015-20.
68. Van Camp, D., N.H. Hooker, and C.T. Lin, *Changes in fat contents of US snack foods in response to mandatory trans fat labelling*. Public health nutrition, 2012: p. 1-8.
69. Booker, C.S. and J.I. Mann, *Trans fatty acids and cardiovascular health: translation of the evidence base*. Nutrition, metabolism, and cardiovascular diseases : NMCD, 2008. **18**(6): p. 448-56.
70. Micha, R. and D. Mozaffarian, *Trans fatty acids: effects on metabolic syndrome, heart disease and diabetes*. Nature reviews. Endocrinology, 2009. **5**(6): p. 335-44.
71. Mozaffarian, D., et al., *Trans-palmitoleic acid, metabolic risk factors, and new-onset diabetes in U.S. adults: a cohort study*. Annals of internal medicine, 2010. **153**(12): p. 790-9.
72. Jakobsen, M.U., et al., *Intake of ruminant trans fatty acids and risk of coronary heart disease-an overview*. Atherosclerosis. Supplements, 2006. **7**(2): p. 9-11.
73. Jakobsen, M.U., et al., *Intake of ruminant trans fatty acids in the Danish population aged 1-80 years*. European journal of clinical nutrition, 2006. **60**(3): p. 312-8.
74. Jakobsen, M.U., et al., *Intake of ruminant trans fatty acids and risk of coronary heart disease*. International journal of epidemiology, 2008. **37**(1): p. 173-82.
75. Arnett, D.K., et al., *Relevance of genetics and genomics for prevention and treatment of cardiovascular disease: a scientific statement from the American Heart Association Council on Epidemiology and Prevention, the Stroke Council, and the Functional Genomics and Translational Biology Interdisciplinary Working Group*. Circulation, 2007. **115**(22): p. 2878-901.
76. Bentley, P., et al., *Causal relationship of susceptibility genes to ischemic stroke: comparison to ischemic heart disease and biochemical determinants*. PloS one, 2010. **5**(2): p. e9136.
77. Fan, A.Z., et al., *Gene polymorphisms in association with emerging cardiovascular risk markers in adult women*. BMC medical genetics, 2010. **11**: p. 6.
78. Campos, H., M. D'Agostino, and J.M. Ordovas, *Gene-diet interactions and plasma lipoproteins: role of apolipoprotein E and habitual saturated fat intake*. Genetic epidemiology, 2001. **20**(1): p. 117-128.

79. Chang, M.H., et al., *Genetic variants associated with fasting blood lipids in the U.S. population: Third National Health and Nutrition Examination Survey*. BMC medical genetics, 2010. **11**: p. 62.
80. Dong, C., et al., *Genetic loci for blood lipid levels identified by linkage and association analyses in Caribbean Hispanics*. Journal of lipid research, 2011. **52**(7): p. 1411-9.
81. Waterworth, D.M., et al., *Genetic variants influencing circulating lipid levels and risk of coronary artery disease*. Arteriosclerosis, thrombosis, and vascular biology, 2010. **30**(11): p. 2264-76.
82. Erkkila, A.T., et al., *APOE polymorphism and the hypertriglyceridemic effect of dietary sucrose*. The American journal of clinical nutrition, 2001. **73**(4): p. 746-52.
83. Moreno, J.A., et al., *Apolipoprotein E gene promoter -219G->T polymorphism increases LDL-cholesterol concentrations and susceptibility to oxidation in response to a diet rich in saturated fat*. The American journal of clinical nutrition, 2004. **80**(5): p. 1404-9.
84. Rubin, J. and L. Berglund, *Apolipoprotein E and diets: a case of gene-nutrient interaction?* Current opinion in lipidology, 2002. **13**(1): p. 25-32.
85. Seripa, D., et al., *The genetics of the human APOE polymorphism*. Rejuvenation research, 2011. **14**(5): p. 491-500.
86. Corella, D. and J.M. Ordovas, *Nutrigenomics in cardiovascular medicine*. Circulation. Cardiovascular genetics, 2009. **2**(6): p. 637-51.
87. Ordovas, J.M., *Nutrigenetics, plasma lipids, and cardiovascular risk*. Journal of the American Dietetic Association, 2006. **106**(7): p. 1074-81; quiz 1083.
88. Paoloni-Giacobino, A., R. Grimble, and C. Pichard, *Genomic interactions with disease and nutrition*. Clinical nutrition, 2003. **22**(6): p. 507-14.
89. Santos, J.L., et al., *Genotype-by-nutrient interactions assessed in European obese women. A case-only study*. European journal of nutrition, 2006. **45**(8): p. 454-62.
90. Talmud, P.J. and D.M. Waterworth, *In-vivo and in-vitro nutrient-gene interactions*. Current opinion in lipidology, 2000. **11**(1): p. 31-6.
91. Burton, P.R., M.D. Tobin, and J.L. Hopper, *Key concepts in genetic epidemiology*. Lancet, 2005. **366**(9489): p. 941-51.
92. Braveman, P., *Health disparities and health equity: concepts and measurement*. Annual review of public health, 2006. **27**: p. 167-94.
93. Taylor, H.A., Jr., *The Jackson Heart Study: an overview*. Ethnicity & disease, 2005. **15**(4 Suppl 6): p. S6-1-3.
94. Metcalf, P.A., et al., *African American-white differences in lipids, lipoproteins, and apolipoproteins, by educational attainment, among middle-aged adults: the Atherosclerosis Risk in Communities Study*. American journal of epidemiology, 1998. **148**(8): p. 750-60.

The association between self-reported omega-3 and *trans* fat intake and plasma phospholipid fatty acid concentrations in African Americans: The Jackson Heart Study (JHS)

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ABSTRACT

Background: A culturally specific food frequency questionnaire (FFQ) was created and used to capture diet in the Jackson Heart Study (JHS). Given the variability of *trans* fat (TFA) and omega-3 fat (ω-3) intakes, it is important to validate this culturally specific FFQ with ω-3and TFA biomarkers.

Objective: Examine correlations between self-reported dietary intake from two culturally derived food frequency questionnaires, a long (LFFQ) and short (SFFQ) and the average of four, 24h recalls with plasma phospholipid (PL) ω-3 and TFA concentrations.

Design: Cross-sectional analysis of individual and total ω-3 and TFA intake was completed in 271 participants from the Diet and Physical Activity Sub-study of the JHS. Pearson's correlation analysis was used to compare dietary intake estimates from LFFQ, SFFQ and the average of four, 24h recalls with PL ω-3 and TFA. Covariates included energy, age, sex, smoking status, BMI, and total serum cholesterol. Multivariable regression analysis was used to assess cross-sectional associations between dietary intake of ω-3 and TFA with PL concentrations.

Results: The LFFQ showed significant positive correlation with PL ω-3 and TFA. The SFFQ showed significant positive correlation with ω-3, but not with TFA. Seafood intake was positively correlated with PL ω-3 concentration, as well as ω-3 intake reported from the SFFQ, LFFQ and 24h recalls.

Conclusions: Data from the LFFQ which included summary questions on cooking fats resulted in the strongest correlations with PL fatty acids. Revisions of the SFFQ to include summary questions on cooking oils could prove beneficial in capturing TFA fat intake in this population.

Introduction

Given the prevalence of obesity and related diseases in the United States, there has been an increasing focus on diet. Dietary fat and individual fatty acids have been a key focus of nutrition research. Individual fatty acids have differing effects on health. Saturated fat (SFA) and *trans* fat (TFA) are thought to be atherogenic [60, 91]. Omega-3 (ω-3) fats are considered preventive of cardiovascular and other chronic diseases. Thus, the relationship between dietary fat and health status depends on the type of fat.

Assessing Fat in the Diet

Investigating the relationship between fat intake and health requires accurate quantification of fat intake. The gold standard in dietary assessment is comparison with a biomarker, a biological quantification of the amount of a nutrient in blood [92, 93]. However, use of biomarkers is complicated by the lack of a good biological representative for most nutrients, metabolic break down of compounds, endogenous production of some nutrients, absorption, bioavailability, and known or unknown physiologic interactions affecting compounds, as well as cost. Therefore, many researchers use dietary assessment tools, which rely on self report to estimate the intake of nutrients. Instruments like 24h recalls and dietary records rely on participants to remember or record what they've actually eaten over a given period of time. Tools such as the food frequency questionnaire (FFQ) rely on an individual's ability to estimate patterns of intake over an extended period of time, and are intended to capture usual intake [92, 93].

The FFQ uses preformed questions to prompt a participant's recall of intake over an extended period of time. In doing so, they combine foods for efficiency. Therefore, general questions

regarding fatty or white fish intake may be confusing for a population for which 90% of fish intake is catfish. Furthermore, follow-up questions specifying preparation of fish, i.e. deep fried with batter, pan fried with batter, pan fried without batter, broiled and baked, all of which will affect the nutrient content of the fish are important. Failure to include detailed questions about preparation of foods (fried, types of oils, etc.) may provide a source of error for nutrient intake estimation. As portion sizes, food types, sources and cooking styles can be culturally and geographically driven, culturally derived FFQs provide a means for reducing estimate error in populations with distinct dietary characteristics[43].

African Americans are a culturally diverse group, comprised of blacks with ancestry inclusive of U.S. slavery, mixed race ancestry, Caribbean origin, African immigrants and their children, as well as other persons who self-identify as African Americans. The diets of African Americans are as diverse as the numerous ethnicities to which they may subscribe. Traditionally, southern blacks are associated with a southern dietary pattern, characterized by fried foods and high intake of animal products, fat and carbohydrates. The foods characterized by a southern dietary pattern and the way they are prepared may differ from those highlighted in other descriptions of the American or Western diet. Thus, it is important to include these foods in a dietary instrument intended to capture the southern diet [94].

To ensure the validity of a dietary instrument, it is important to compare it to other measures, preferably a gold standard. Popular instruments such as the Willett and Block FFQ have been previously validated in non-Hispanic white American populations [95, 96]. The United States Department of Agriculture (USDA) Lower Mississippi Delta Nutrition Intervention (LMD NIRI)

FFQ, a 283-item FFQ developed to capture the southern diet was validated in African American Mississippians using 24h recalls, plasma total antioxidant capacity, carotenoids and tocopherols [43, 94, 97-99]. Given the ubiquity of fat in diet and the contribution of fat to energy intake, it is important to validate the culturally specific instrument with biomarkers for fat intake. Thus, in this study, we aim to determine the association between dietary ω-3and TFA assessed by a long food frequency questionnaire (LFFQ) and short food frequency questionnaire (SFFQ) with ω-3 and TFA PL concentrations in African American participants in the JHS.

Materials and Methods

Source Population

The JHS is a single-site prospective epidemiologic study investigating the nature of cardiovascular disease in African Americans ages 25-85 y in Jackson, MS. Specifics of the study design, recruitment and data collection have been described elsewhere [88, 100]. Briefly, participants were recruited from Jackson, MS and surrounding counties. Participants included existing Atherosclerosis Risk In Communities (ARIC) study participants, plus a random sample of volunteers and a convenience based sampling of volunteers. At baseline, participants completed questionnaires on socio-economic status, perceived racism, cognition, depression, diet, physical activity, employment, medical history and emotional scales. In addition, blood was drawn by trained nurses and cardiovascular screening tests were completed by trained individuals during the baseline clinic visit. The JHS was approved by the University of Mississippi Medical Center Review Board.

The Diet and Physical Activity Sub Study (DPASS) of JHS is a diet and physical activity validation study in a subset of the JHS cohort. DPASS included 499 participants. The DPASS population was designed to include equal numbers of men and women, equal numbers of younger (34-64 year olds) and older (65+) participants and equal representation of socio-economic class and physical activity groups [94].

The data presented in this study include a subset of DPASS participants for whom plasma phospholipid fatty acids were extracted (n=271). Tufts Medical Center Review Board provided IRB approval for the current study.

Dietary Assessment

DPASS included data from three different dietary instruments (four, 24h recalls, a 283-item Delta FFQ (LFFQ) and a 158-item FFQ (SFFQ)). A series of four, 24h recalls two of which were completed on week days and two on weekend days were administered over telephone by a trained interviewer at one month intervals. Recalls were completed using the Nutrient Data System (NDS) developed by the University of Minnesota. Details regarding the development and validation of the full Delta FFQ (LFFQ) have been previously described [43, 97-99]. Briefly, a 283-item questionnaire was developed using 24h recall interviews of individuals residing in the Mississippi Delta. This FFQ containing regionally specific foods, portion sizes and weighting of food items was shortened to a 158-item FFQ (SFFQ) to reduce JHS participant burden. Recall and FFQ databases were created with NDS (version 5.0-35, 2004)[99]. The TFA values in NDS are based on the USDA report by Exler et al [101].

Laboratory Analysis

As a part of the JHS protocol, 12 hour fasting blood samples were collected from all participants during the baseline study visit. Blood samples were collected in vacutainer tubes and centrifuged at 3000x g for 10 min at 4°C. Serum was then separated and frozen at -70◦C until analyzed for plasma phospholipid (PL) fatty acid analysis.

Plasma Phospholipid Fatty Acid Composition

Serum samples used for fatty acid extraction were collected at baseline (2000-2004). PL fatty acid analysis began in June 2007 and was completed in August 2009. Storage of serum samples ranged from three to nine years. It has been previously established that the fatty acid composition of samples stored at -80C is stable for at least 10 years[102]. Plasma lipids were extracted using a modification of the Folch, et al. method [103]. A solid-phase extraction using aminopropyl columns was used to isolate the phospholipid subfraction [104]. Samples were then saponified and methylated [105] allowing methyl esters to be analyzed using an Autosystem XL gas chromatograph (Perkin, Elmer, Boston, MA) with a 30m x 0.25mm i.d. (film thickness 0.25µm) capillary column (HP INNOWAX, Agilent Technologies, DE). Authentic fatty acid standards (Nu-Check Prep, Inc. MN) were used to identify fatty acid peaks. Following peak comparison, all data were imported into Excel spreadsheets and evaluated for errors. Data are expressed as molar percentage (mol %).

Covariates

At baseline, data for self-reported age, sex, and smoking status were provided. Body Mass Index (BMI) was calculated from height and weight obtained by clinic trained nursing staff. Total physical activity score was ascertained from the validated 30-item JHS Physical Activity

Questionnaire [106-108]. The score is based on responses to four categories of activity (home and family life, work, sports and active living). Computation of the total physical activity score was based on the methods described by Richardson et al. and Ainsworth et al. [109, 110].Total cholesterol values resulted from blood samples that were collected during baseline clinic interviews. Participants were requested to fast for 12 hour prior to the clinic interview. Cholesterol concentrations were determined at the University of Minnesota Medical Center, Fairview. Lipid assays were standardized through the Lipid Standardization Program of the Centers for Disease Control and Prevention, Atlanta, Ga. [31]. Details regarding the methods and storage of lipid assays were previously described [36, 111]. Total cholesterol (TC), high density lipoprotein (HDL-C), low density lipoprotein (LDL-C) and triglyceride (TG) concentrations were assessed using fasting serum samples[103].

Statistical Analysis

SAS statistical software (version 9.2, SAS Institute, Cary, NC) was used for data analysis. Eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), docosahexaenoic acid (DHA), alpha- linolenic acid (ALA), total ω-3, 18:1t isomers, 18:2t isomers and total TFA were the focus of the analysis. Nutrients were expressed as percent of total energy intake. Study characteristics, including dietary intake variables, were stratified by sex and compared using the GLM procedure. Energy adjustment for comparison of nutrients by sex was completed using the residual method [93]. Pearson partial correlation coefficients were used to assess associations between PL fatty acids with intake variables assessed by the SFFQ, LFFQ and average of the 24h recalls and selected foods. The association between PL ω-3 and TFA and measures from

SFFQ, LFFQ and 24h recalls were completed using PROC GLM. Models were adjusted for age, sex, and total energy intake from the appropriate assessment tool, BMI, TC, and smoking status. A p-value < 0.05 was considered statistically significant.

Results

Characteristics of JHS-DPASS Participants

The mean age and physical activity score did not differ significantly between men and women. The mean BMI of women (32±7) was higher than that of men (29±4), $p<0.05$.
The mean HDL-C in women (56 mg/dL) was higher than men (46 mg/dL), $p<0.05$. Differences in LDL-C and TG concentrations did not differ statistically **(Table1)**.

Plasma phospholipid (mol %) of Fatty Acids of JHS DPASS Participants

Men had higher PL levels of total polyunsaturated fatty acids (PUFA), linoleic acid (LA) and docosapentaenoic acid (DPA) than women ($p<0.05$). PL levels of alpha linolenic acid (ALA) were higher in women than men ($p<0.05$). Total saturated fat (SFA), monounsaturated fat (MUFA), total ω-3, eicosapentaenoic acid (EPA), docosapentaenoic acid (DHA), total TFA,18:1t and 18:2t were not significantly different between men and women ($p>0.05$) **(Table2)**.

Intakes of fatty acids by JHS DPASS Participants

Pairwise comparisons of pecent ω-3 and TFA intake by sex were completed using a general linear model approach. In the SFFQ, men reported higher percent energy intakes of DHA and 16:1t than women ($p<0.05$). In the LFFQ, energy-adjusted intakes of PUFA, LA, ALA, total trans, 16:1t and 18:1t were all significantly greater in men than women ($p<0.05$). Dietary recall

data showed significantly higher percent intakes of, ALA, DPA, and DHA in men, compared to women (**Table3**).

Pearson's Correlations between Plasma PL and Dietary Fatty Acid Intake

In crude models, plasma PL 18:2t was correlated with 24h recalls ($p<0.05$), SFFQ ($p<0.05$) and LFFQ ($p<0.05$) intakes. In models adjusted for age, sex, energy intake, BMI, serum TC and smoking status, plasma PL concentrations of total ω-3, DHA and EPA were significantly correlated with corresponding estimates from dietary intakes measured using 24h recalls, LFFQ and SFFQ ($p<0.05$). DHA in PL was significantly correlated ($p<0.001$) with SFFQ and LFFQ. EPA in PL showed a positive correlation with the LFFQ ($p<0.05$). Total TFA, 18:1t and 18:2t in PL were significantly correlated with dietary intakes from the LFFQ and 24h recalls ($p<0.05$ and $p<0.001$ respectively), but not with the SFFQ. From 24h recalls, only 18:2t intake was positively correlated with PL in both crude and adjusted models. DHA, EPA, TFA, 18:1t and 18:2t from the LFFQ were all positively correlated ($p<0.05$) with plasma in both crude and adjusted models **(Table 4)**.

Associations between Plasma Phospholipid Fatty Acids and Dietary Fatty Acids by Different Assessment Instruments

PL ω-3 concentrations were positively associated with intakes from SFFQ ($p<0.05$), LFFQ ($p<0.05$) and 24h recall ($p<0.05$). PL TFA was positively associated with dietary TFA in LFFQ ($p<0.001$) and 24 h recall ($p<0.05$), but not SFFQ ($p>0.05$) **(Table5)**.

Correlations between Plasma Phospholipid, Diet (24h recalls, SFFQ and LFFQ) and selected foods from LFFQ

In models adjusted for age, sex, BMI and energy intake, as well as unadjusted analyses, seafood intake was positively correlated with PL ω- 3 ($p<0.05$), SFFQ ($p<0.05$), LFFQ ($p<0.05$) and 24h recall ($p<0.05$). PL total TFA was not correlated with expected foods, including margarine, oils, fast food, fried chicken fried fish or miscellaneous fat. However, margarine was positively correlated with TFA intake from the SFFQ ($p<0.05$), LFFQ ($p<0.05$) and 24h recalls ($p<0.05$) in both crude and adjusted models. Intakes of these foods are based on a combined grouping of foods prepared at home and commercially prepared foods. Percent energy from oils, fast food, fried chicken and fried fish were positively associated with total TFA intake from the SFFQ in the crude model, but not in adjusted models. Energy from vegetable oils was only associated with TFA intake from the LFFQ ($p<0.05$). Miscellaneous fat ($p<0.05$), fast food ($p<0.05$), fried chicken ($p<0.05$) and fried fish ($p<0.05$) were all positively correlated with TFA intake from the SFFQ, LFFQ and 24h recall in the crude models, but only with the LFFQ and 24h recalls in adjusted models ($p<0.05$). Fast food was positively correlated with TFA intake from the LFFQ ($p <0.05$) for both crude and adjusted models **(Table6)**.

Discussion

The LFFQ showed the strongest correlation with PL ω-3 and TFA in comparison with the SFFQ and 24h recalls. Significant associations were seen in both the crude and adjusted models. The LFFQ contains summary questions regarding use of fats for cooking. Given the relationship between cooking fats (margarine, lard, vegetable oil, olive oil, butter etc) and fat intake, these

summary questions appear to be important to the accurate capture of fat intake in JHS participants. The 24h recall also performed well, showing significant correlations with plasma PL ω-3 and TFA. 24h recalls have the ability to capture cooking preparation through prompts provided by interviewers. The SFFQ correlated the least with PL fatty acids. The SFFQ captured frequency of intake of individual food items without additional questions on fats used to prepare foods. A major source of TFA in the American diet is partially hydrogenated fats used in cooking oils. This may be the reason that TFA from the SFFQ failed to correlate with PL biomarkers. Adjustment for energy, age, sex, BMI, plasma cholesterol and smoking improved the correlations between the LFFQ and fatty acid plasma concentrations.

SFFQ ω-3 was positively correlated with PL ω-3 intake. Total ω-3 intakes captured by all three dietary assessment tools correlated with PL ω-3. The SFFQ is the dietary instrument used to capture diet in the entire JHS cohort. These findings suggest that total ω-3 intake, as captured by the SFFQ in the JHS, is reasonably valid. This will be important to future ω-3 research in JHS.

The correlations between PL and dietary ω-3 and TFA are similar to those found by Sun et al in the Nurses' Health study [112]. Additionally, these findings are consistent with previous findings from JHS, which reported a correlation of (r=1.18-0.33 for different demographic groups) for dietary polyunsaturated fat between SFFQ and LFFQ with 24h recalls.

DPA was the only ω-3 fatty acids which do not show significant correlation between PL and reported intake from SFFQ, LFFQ or 24h recalls. DPA is often not included in ω-3 research. There are some reports that the prevalence of DPA in the food supply is negligible in relation to DHA and EPA [45]. In nutritional databases the DPA content of catfish, the most commonly

eaten fish in the JHS, ranged from 0.02g to 0.09g for fried catfish; and 0.02g to 0.11g for steamed, baked, broiled or raw catfish [113]. The DPA content of catfish depends on the type of catfish and the cooking method. In the food science literature, DPA has been repeatedly omitted in reports of the ω-3 content of fish. The paucity of literature on DPA content of foods makes it difficult to resolve discrepancies in nutrient databases. Most ω-3 and health research focuses on DHA and EPA. In this light, it possible that database estimates of DPA lack validity due to less research and interest in DPA.

Overall, we found that ω-3 fatty acids had strong positive correlations with all three dietary assessment tools. The correlation of PL total ω-3, DHA and EPA with the SFFQ suggests that estimated intakes from the SFFQ are valid markers for ω-3. However, PL TFA was not significantly correlated with SFFQ estimated intake. A possible solution is post hoc calibration of the SFFQ, using information from the LFFQ and 24h recalls to weight food selections from the database. In addition, the SFFQ could be amended to include summary questions regarding cooking fats and implemented in future cycles of JHS. Additional cycles of dietary intake data may be needed to establish consistency of diet, patterns and temporal relationships between diet and disease.

Table 1. Characteristics of JHS-DPASS participants

	Men(n=133)	**Women(n=143)**
	Mean ±SD	Mean ±SD
Age (years)	59.6 ±10.7	60.9±9.5
Body Mass Index, kg/m2 *	29.4±4.9	32.0±7.1
Physical Activity Score	8.2±2.9	8.1±2.6
LDL-C mg/dL	127±36	123±39
HDL-C mg/dL *	46.0±11.1	56.4±16.2
Triglycerides mg/dL	112±65	121±140
Total Cholesterol mg/dL	194±39	203±5

JHS, Jackson Heart Study; DPASS, Diet and Physical Activity Sub-Study; SD, Standard Deviation; LDL-C, low density lipoprotein concentration; HDL-C, high density lipoprotein concentration
Sex groups were compared by GLM, * Indicates $p<0.05$.

Table 2. Plasma phospholipid concentrations (mol %) of fatty acids in JHS-DPASS participants

	Men (n=133)	**Women (n=143)**
	Mean ± SD	Mean ± SD
Saturated Fat	45.9±1.7	46.2±2.02
Monounsaturated Fat	12.8±1.8	13.2±2.0
Polyunsaturated Fat *	41.2±2.0	40.7±1.8
Linoleic Acid *	20.1±2.6	19.5±2.7
Total omega-3	4.41±1.1	4.41±1.0
Alpha Linolenic Acid*	0.13±0.05	0.17±0.1
Eicosapentaenoic acid (EPA)	0.57±0.4	0.59±0.4
Docosapentaenoic acid (DPA) *	0.79±0.2	0.73±0.2
Docosahexaenoic acid (DHA)	2.92±0.8	2.92±0.8
Total *trans*	2.40±1.3	2.43±1.1
18:1t	1.90±1.1	1.89±1.0
18:2t	0.50±0.2	0.54±0.2

JHS, Jackson Heart Study; DPASS, Diet and Physical Activity Sub-Study; SD, Standard Deviation
Sex groups were compared by GLM, * Indicates $p<0.05$.

Table 3. Mean percent energy intakes of fatty acids in men and women JHS-DPASS participants

	Short FFQ	**Long FFQ**	**Average of four, 24 h recalls**
Dietary Nutrient (Mean±SD)			
Men (n=133)			
Energy (kcal)	2113±805	2351±946	2066.9±638
Fat (%)	34.8 ±6.2	35.8±6.0	35.9±6.8
Saturated Fat (%)	10.6±2.3	11.1±2.2	10.8±2.4
Monounsaturated fat (%)	13.3±2.6	13.9±2.6	14.8±3.7
Polyunsaturated fat (%)	8.1±2.0	7.9±1.9	7.1±1.7
Linoleic Acid (%)	7.0±1.8	6.9±1.6	6.2±1.6
Alpha Linolenic Acid (ALA)(%)	1.7±0.9	1.7±0.8	1.4±0.58
Eicosapentaenoic Acid (EPA)(%)	0.07±0.06	0.06±0.06	0.05±0.06
Docosapentaenoic Acid (DPA)(%)	0.02±0.02	0.02±0.01	0.02±0.03
Docosahexaenoic acid (DHA) (%)	0.06±0.05	0.06±0.04	0.07±0.07
Total trans fat(%)	1.9 ±0.6	2.3±0.7	2.6±1.0
16:1t (%)	0.03±0.01	0.03±0.01	0.02±0.01
18:1t (%)	1.6±0.6	2.0±0.7	2.3±0.9
18:2t (%)	0.2 ±0.06	0.3±0.08	0.3±0.1
Women (n=143)			
Energy (kcal)	1761±647*	1928±863*	1669±556*
Fat (%)	33.1±6.7	35.5±6.4	34.7±6.5
Saturated Fat (%)	10.0±2.7	10.4±2.3	10.4±2.4
Monounsaturated fat (%)	12.5±2.8	14.0±2.9	14.1±3.0
Polyunsaturated fat (%)	7.9±2.1	8.3±2.0*	7.2±2.0
Linoleic Acid (%)	7.0±2.0	7.3±1.9*	6.3±1.9
Alpha Linolenic Acid (ALA)(%)	0.7±0.0.2	0.7±0.2*	0.6±0.2*
Eicosapentaenoic Acid (EPA)(%)	0.06±0.05	0.06±0.05	0.04±0.05
Docosapentaenoic Acid (DPA)(%)	0.02±0.01	0.02±0.01	0.02±0.02*
Docosahexaenoic acid (DHA)(%)	0.05±0.03*	0.05±0.04	0.05±0.06*
Total trans fat(%)	1.8±0.6	2.5 ±0.9*	2.5 ±0.9
16:1t (%)	0.02±0.01*	0.02 ±0.01*	0.02±0.01
18:1t (%)	1.5 ±0.6	2.2 ±0.8*	2.2 ±0.8
18:2t (%)	0.2±0.07	0.3 ±0.08	0.3 ±0.1

JHS, Jackson Heart Study; DPASS, Diet and Physical Activity Sub-Study; SD, Standard Deviation; FFQ, food-frequency questionnaire.

Values are means ±SD. Sex groups were compared by GLM after adjusting for age; * indicates women are different from men P<0.05

Table 4. Crude and Adjusted Pearson's Correlations between plasma phospholipid fatty acid biomarkers and fatty acid intakes in the JHS-DPASS

	Average of four 24 h recalls	Short FFQ	Long FFQ
Crude			
Total ω-3	0.1*	-0.004	0.04
DHA	0.09	0.1*	0.2**
DPA	0.1**	0.04	0.1*
EPA	0.1*	0.1*	0.2**
ALA	0.002	0.01	-0.05
Total *Trans*	0.1**	0.1*	0.2***
18:1t	0.06	0.10	0.2**
18:2t	0.2***	0.2**	0.1**
Adjusted			
Total ω-3	0.2**	0.1**	0.2**
DHA	0.1**	0.2***	0.2***
DPA	0.1	0.08	0.10*
EPA	0.2***	0.1**	0.1**
ALA[1]	0.06	0.1**	0.2***
Total *Trans*	0.1*	0.09	0.1**
18:1t	0.1**	0.07	0.2***
18:2t	0.2***	0.1*	0.2**

JHS, Jackson Heart Study; DPASS, Diet and Physical Activity Sub-Study;
FFQ, food-frequency questionnaire.
Adjusted for age, sex, energy intake from appropriate assessment tool, BMI, plasma cholesterol and smoking status.
[1]also adjusted for fat intake
*p<0.10, **p<0.05, ***p<0.001

Table 5: Association between plasma phospholipid concentration of omega-3 and *trans* fat with dietary intake for the JHS-DPASS participants

	Average of four 24h recalls	**Short Food Frequency Questionnaire (SFFQ)**	**Long Food Frequency Questionnaire (LFFQ)**
Plasma phospholipid Fatty Acids			
Total ω-3 (mol%)			
β-estimate	0.08*	0.1**	0.1**
Model R^2	0.05	0.06	0.06
Total *trans* Fat (mol%)			
β-estimate	0.03**	0.04*	0.05***
Model R^2	0.08	0.06	0.09

plasma phospholipid values are log transformed.
JHS, Jackson Heart Study; DPASS, Diet and Physical Activity Sub-Study; FFQ, food-frequency questionnaire.
Adjusted for age, sex, energy intake from appropriate assessment tool, BMI, plasma total cholesterol and smoking status.
*P<0.1, **p<0.05,***p<0.001

Table 6. Correlation between plasma phospholipid omega-3 and *trans* fat with specific foods in JHS-DPASS participants.

Food	Plasma phospholipid Fatty acids	Average of four 24 h recalls	Short FFQ	Long FFQ
Crude				
Total ω-3				
Seafood	0.2**	0.2***	0.4***	0.6***
Fried fish	-0.003	0.2	0.2**	0.2***
Nuts and seeds	-0.05	0.1*	0.2**	0.2***
Oils	-0.06	0.08	0.3***	0.6***
Total *trans* fat				
Margarine	-0.01	0.2**	0.3***	0.5***
Miscellaneous fat	0.05	0.2**	0.2**	0.2**
Oils	-0.03	0.1**	0.2**	0.3***
Fast food	0.07	0.3***	0.4***	0.4***
Fried Chicken	-0.008	0.3***	0.2**	0.3***
Fried Fish	0.007	0.2***	0.2**	0.2***
Adjusted				
Total ω-3				
Seafood	0.2**	0.2**	0.3***	0.5***
Fried fish	-0.009	0.07	-0.05	0.1*
Nuts and seeds	0.008	0.1	0.09	0.2**
Oils	0.00002	0.09	0.2**	0.6***
Total *trans* fat				
Margarine	-0.02	0.2**	0.2**	0.5***
Miscellaneous fat	0.04	0.2**	0.1*	0.2**
Oils	-0.07	0.1	-0.1	0.3***
Fast food	-0.01	0.2**	0.03*	0.3***
Fried Chicken	-0.05	0.2**	0.01	0.2**
Fried Fish	0.02	0.1**	-0.07	0.1**

Total trans for 24h recall, SFFQ and LFFQ includes 16:1t.
16:1t is not included in plasma phospholipid total trans.
JHS, Jackson Heart Study; DPASS, Diet and Physical Activity Sub-Study; SD, Standard Deviation; FFQ, food-frequency questionnaire; PL, phospholipid
Adjusted for age, sex, energy intake from appropriate assessment tool, body mass index, plasma cholesterol and smoking status.
*p<0.10, **p<0.05, ***p<0.001

1. Mensink, R.P., et al., *Effects of dietary fatty acids and carbohydrates on the ratio of serum total to HDL cholesterol and on serum lipids and apolipoproteins: a meta-analysis of 60 controlled trials*. The American journal of clinical nutrition, 2003. **77**(5): p. 1146-55.
2. Vafeiadou, K., et al., *A review of the evidence for the effects of total dietary fat, saturated, monounsaturated and n-6 polyunsaturated fatty acids on vascular function, endothelial progenitor cells and microparticles*. The British journal of nutrition, 2012. **107**(3): p. 303-24.
3. Willett, W., *Nutritional epidemiology*. Monographs in epidemiology and biostatistics1990, New York: Oxford University Press. vii, 396 p.
4. Willett, W., *Nutritional epidemiology*. 2nd ed. Monographs in epidemiology and biostatistics1998, New York: Oxford University Press. xiv, 514 p.
5. Tucker, K.L., et al., *A regional food-frequency questionnaire for the US Mississippi Delta*. Public health nutrition, 2005. **8**(1): p. 87-96.
6. Carithers, T., et al., *Dietary assessment in African Americans: methods used in the Jackson Heart Study*. Ethnicity & disease, 2005. **15**(4 Suppl 6): p. S6-49-55.
7. Boucher, B., et al., *Validity and reliability of the Block98 food-frequency questionnaire in a sample of Canadian women*. Public health nutrition, 2006. **9**(01): p. 84-93.
8. Willett, W.C., et al., *Reproducibility and validity of a semiquantitative food frequency questionnaire*. American journal of epidemiology, 1985. **122**(1): p. 51-65.
9. Talegawkar, S.A., et al., *Total antioxidant performance is associated with diet and serum antioxidants in participants of the diet and physical activity substudy of the Jackson Heart Study*. The Journal of nutrition, 2009. **139**(10): p. 1964-71.
10. Talegawkar, S.A., et al., *Carotenoid intakes, assessed by food-frequency questionnaires (FFQs), are associated with serum carotenoid concentrations in the Jackson Heart Study: validation of the Jackson Heart Study Delta NIRI Adult FFQs*. Public health nutrition, 2008. **11**(10): p. 989-97.
11. Carithers, T.C., et al., *Validity and calibration of food frequency questionnaires used with African-American adults in the Jackson Heart Study*. Journal of the American Dietetic Association, 2009. **109**(7): p. 1184-1193.
12. Fuqua, S.R., et al., *Recruiting African-American research participation in the Jackson Heart Study: methods, response rates, and sample description*. Ethnicity & disease, 2005. **15**(4 Suppl 6): p. S6-18-29.
13. Taylor, H.A., Jr., *The Jackson Heart Study: an overview*. Ethnicity & disease, 2005. **15**(4 Suppl 6): p. S6-1-3.
14. Exler, J.L., Linda; Smith, Julie, *Fat and Fatty Acid Content of Selected Foods Containing Trans-Fatty Acids*, A.R.S.N.D. Laboratory, Editor 1989, 1993: Beltsville, MD 20705. p. 38.
15. Matthan, N.R., et al., *Long-term fatty acid stability in human serum cholesteryl ester, triglyceride, and phospholipid fractions*. Journal of lipid research, 2010. **51**(9): p. 2826-32.
16. Folch, J., M. Lees, and G.H. Sloane Stanley, *A simple method for the isolation and purification of total lipides from animal tissues*. The Journal of biological chemistry, 1957. **226**(1): p. 497-509.
17. Agren, J.J., A. Julkunen, and I. Penttila, *Rapid separation of serum lipids for fatty acid analysis by a single aminopropyl column*. Journal of lipid research, 1992. **33**(12): p. 1871-6.
18. Morrison, W.R. and L.M. Smith, *Preparation of Fatty Acid Methyl Esters and Dimethylacetals from Lipids with Boron Fluoride--Methanol*. Journal of lipid research, 1964. **5**: p. 600-8.
19. Hertogh, E.M., et al., *Validity of the modified Baecke questionnaire: comparison with energy expenditure according to the doubly labeled water method*. The international journal of behavioral nutrition and physical activity, 2008. **5**: p. 30.
20. Dubbert, P.M., et al., *Physical activity assessment methods in the Jackson Heart Study*. Ethnicity & disease, 2005. **15**(4 Suppl 6): p. S6-56-61.
21. Smitherman, T.A., et al., *Validation of the Jackson Heart Study Physical Activity Survey in African Americans*. Journal of physical activity & health, 2009. **6 Suppl 1**: p. S124-32.

22. Ainsworth, B.E., et al., *Evaluation of the kaiser physical activity survey in women*. Medicine and science in sports and exercise, 2000. **32**(7): p. 1327-38.
23. Richardson, M.T., et al., *Ability of the Atherosclerosis Risk in Communities (ARIC)/Baecke Questionnaire to assess leisure-time physical activity*. International journal of epidemiology, 1995. **24**(4): p. 685-93.
24. *Summary of the second report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel II)*. JAMA : the journal of the American Medical Association, 1993. **269**(23): p. 3015-23.
25. Carpenter, M.A., et al., *Laboratory, reading center, and coordinating center data management methods in the Jackson Heart Study*. The American journal of the medical sciences, 2004. **328**(3): p. 131-44.
26. Taylor, H.A., Jr., et al., *Dyslipidemia and the treatment of lipid disorders in African Americans*. The American journal of medicine, 2009. **122**(5): p. 454-63.
27. Sun, Q., et al., *Comparison between plasma and erythrocyte fatty acid content as biomarkers of fatty acid intake in US women*. The American journal of clinical nutrition, 2007. **86**(1): p. 74-81.
28. Balk, E., et al., *Effects of omega-3 fatty acids on cardiovascular risk factors and intermediate markers of cardiovascular disease*. Evidence report/technology assessment, 2004(93): p. 1-6.
29. (ARS), A.R.S., *USDA National Nutrient Database for Standard Reference*, 2011, USDA: Nutrient Data Lab.

Omega-3 Fatty-Acid Intake, Sources and Relationship to Serum Lipid Concentrations in African Americans: Jackson Heart Study (JHS)

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ABSTRACT

Background: There is limited data on the relationship between omega-3 (ω-3) intake and serum lipids concentrations in African-Americans.

Objective: We examined the cross-sectional relationship between ω-3 intake and serum lipids in a cohort of African-Americans, the Jackson Heart Study (JHS). In addition, we described the sources and status of ω-3 intake in this population.

Design: Dietary ω-3 and serum lipid concentrations were measured in 4,771 members of the Jackson Heart Study. Rankings of the ω-3 sources in JHS were compiled from food frequency data. Multivariable regression analysis was used to assess the cross-sectional association between dietary ω-3 with serum lipids.

Results: Catfish was the main contributor to ω-3 intake in the JHS. Alpha-linolenic (ALA) intake was positively associated with high density lipoprotein (HDL-C). We found no significant associations between dietary eicosapentaenoic acid (EPA) and docosapentaenoic (DHA) with serum lipids. There were no associations between dietary ω-3 and low density lipoprotein (LDL-C) or total cholesterol (TC).

Conclusions: Main sources of eicosapentaenoic acid (EPA) and docosapentaenoic acid (DHA) differ in this population of older African American subjects than those included in dietary recommendations. We found no association between marine fatty acids DHA and EPA with serum lipids. The failure to see an association with marine fatty-acids may be a result of the lower intake, sources of marine fatty-acids or the predominance of frying as a cooking method for fish.

Introduction

Diet is an important modifier of cardiovascular disease (CVD) risk. Recent recommendations focus on increasing intakes of "good" fats [22, 23]. "Good" fats include omega 3 (ω-3) polyunsaturated fatty- acids (PUFA). Both plant and marine derived ω-3 are suggested to be protective against CVD and related diseases [45, 114-116]. Research suggests that ω-3 can improve endothelial function, decrease blood pressure, improve glucose intolerance, decrease risk for arrhythmias [117-120]. Higher intakes of ω-3 are also suggested to improve lipid profiles by not only decreasing serum triglyceride (TG) concentrations, but also by increasing serum high density lipoprotein (HDL-C); however, these results have not been consistent across studies [120-123].

African Americans suffer a disproportionate burden of CVD mortality and morbidity in comparison to their non-Hispanic white counterparts [54]. However, data suggest that African Americans have lipid profiles that are protective against heart disease (lower proportion of individuals with low HDL-C and high low density lipoprotein (LDL-C)) when compared to other ethnic groups [124]. This paradox between the disproportionate burden of CVD morbidity and mortality in African Americans and the cardioprotective lipid profiles is not well understood. Further research on dyslipidemia and CVD is needed to further understand this relationship in African Americans. The literature suggests educational attainment, socioeconomic status, blood pressure, insulin resistance, body mass index (BMI), and age are predictors of dyslipidemia in African Americans [12, 89, 125].

Previous data from the Jackson Heart Study (JHS) [36, 40, 126] classified 33% of the baseline cohort as having hypercholesterolemia. Thirty percent of those with the condition lacked

awareness of their illness, while only 43% received treatment. Taylor et al [36] showed that co-morbid conditions such as diabetes, hypertension, chronic kidney disease and diagnosed CVD were significant predictors of hypercholesterolemia (defined as self report of lipid lowering medications use in the previous 2 weeks, a fasting LDL-C ≥ 160mg/dL or a total cholesterol (TC) concentration ≥ 240 mg/dL) in JHS participants. In a simple model (adjusting for only age and sex), physical activity, younger age, normal weight, and preventive care were significantly associated with lower likelihood of hypercholesterolemia. However, diet was not examined. Therefore, in this study, we examine the association of ω-3 and serum lipid concentrations in JHS participants.

Methods

Study Population

We evaluated data from the JHS cohort. Between 2000 and 2004 5,302 African Americans from Jackson, MS were recruited to participate in the single site prospective cohort study. Participant recruitment included random selection, volunteer participation, recruitment of family members and enrollment of current participants of the Jackson, MS site of the Atherosclerosis Risk in Communities study (ARIC)[100]. ARIC participants comprise 31% of the total JHS cohort. Participant ages ranged from 25 to 85 years of age. Detailed descriptions of the design and recruitment for the JHS are described elsewhere [100, 127]. Institutional Review Board (IRB) approval for the JHS cohort was provided by the University of Mississippi IRB. Approval for this study was provided by the IRB at Tufts Medical Center.

Dietary Intake Assessment

All dietary data was obtained from an interviewer administered food frequency questionnaire (FFQ). The 158-item short JHS FFQ (SFFQ) is a shortened version of a 283-item long FFQ (LFFQ), originally developed for use in the United States Mississippi Delta with 24h recall data from the Mississippi Delta region. The SFFQ was developed specifically for JHS to alleviate overall participant burden during the baseline study visit. The development and validation of this regional SFFQ is described elsewhere [43, 97-99, 128].

Lipids

Blood samples were collected during baseline clinic interviews. Participants were requested to fast for 12h prior to the clinic interview. Serum lipid concentrations were determined at the University of Minnesota Medical Center, Fairview. Lipid assays were standardized through the Lipid Standardization Program of the Centers for Disease Control and Prevention, Atlanta, Ga. [31]. Details regarding the methods and storage of lipid assays were previously described [36, 111].

Covariates

The total physical activity score was ascertained from the validated 30-item JHS Physical Activity Questionnaire [106-108]. The score is based on responses to four categories of activity (home and family life, work, sports and active living). Computation of the total physical activity score was based on the methods described by Richardson et al. and Ainsworth et al. [109, 110]. Smoking status was obtained through questionnaires completed during in-person interviews. Pack years were computed by multiplying years smoked by the result of the number of cigarettes smoked per day divided by 20. For persons classified as having never smoked, a pack year value of zero was assigned. Body Mass Index (BMI) was calculated from weight and height obtained

by trained JHS clinic staff during the baseline study visit. Anthropometric measurements were obtained from trained clinic staff with participants in study administered hospital gowns without their shoes. Age was determined from self-reported date-of-birth. Detailed information regarding the anthropometric measurements and other study covariates is presented elsewhere [36, 100, 127].

Disease Status

Using JHS study criteria, hypercholesterolemia was defined as a fasting LDL-C ≥ 160mg/dL or TC concentration ≥ 240 mg/dL or self-report of lipid lowering medications use in the previous 2 weeks, [129]. The hypercholesterolemia classification is based on the third report of the Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults, Adult Treatment Panel III [31, 36]. Based on the 7th Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC7) [36, 130-133], hypertension was defined as use of antihypertensive medication, or systolic blood pressure ≥ 140mm Hg or diastolic blood pressure ≥ 90mm Hg. Type 2 diabetes was defined using the 2004 American Diabetes Association Guidelines [134] of fasting plasma glucose ≥126 mg/dL or current use of insulin or oral hypoglycemic medications [36, 40].

Data Analysis

SAS statistical software (version 9.2, SAS Institute, Cary, NC) was used for data analysis. Serum lipid concentrations were evaluated for normality and measures of central tendency. Because serum TG was not normally distributed, a logarithmic transformation was applied. All other lipid values were normally distributed. Both alcohol intake and pack years lacked normality. The logarithm of the sum of alcohol +10 was used to transform alcohol intake. Similarly, a

logarithm of the sum of pack years + 100 was used to transform the pack years variable. Transformed values of pack years and alcohol intake were used as covariates. In sensitivity analyses, smoking status (yes/no) from baseline self-report was also used as a covariate in separate models. Both variables, smoking status and pack years, performed similarly. Serum lipid concentrations and dietary predictors were analyzed as continuous variables. Dietary intake is expressed as percent of total energy intake. Study characteristics were stratified by sex. The PROC GLM procedure was used to evaluate differences by sex. Differences in hypertension, diabetes and hypercholesterolemia status by sex were examined with the chi-square test. Variance inflation factor (VIF) and tolerance, were used to assess collinearity within dietary covariates and lipid outcomes. Outliers were identified by examining the distribution of residuals.

We used the GLM procedure to test the linear association between ω-3 intake and serum lipids after adjustment for age, sex, energy, smoking status, alcohol (g), diabetes status, and BMI. ω-3s were examined individually and collectively, resulting in two separate models; 1. DHA +EPA and ALA in separate models with previously mentioned covariates, and 2. DHA+EPA and ALA in the same model adjusting with the aforementioned covariates. FFQ data were used to compile rankings of percent contribution to ω-3 intake. A p-value < 0.05 was considered statistically significant.

Results

Descriptive Characteristics

Mean HDL-C and TC concentrations were significantly different between men and women ($P < 0.001$) **(Table 1).** According to the NCEP III criterion, 76% of men in the JHS had high LDL-C,

34% had low HDL-C, and 42% had high TC concentration. Similarly, 73% of women had high LDL-C, 13% had low HDL-C and 47% had high TC. Approximately 35% of women and 45% of men had high serum TG concentration.

Dietary Intake

Men had higher total energy, percent DHA, percent AA and percent protein intake. Women had higher percent carbohydrate, percent polyunsaturated fatty acid and linoleic acid intakes **(Table 2).**

Food Sources of ω-3 Fatty-acids

Catfish provided the largest percent contribution to ω-3 intake **(Table 3).** Of the top 10 ranked contributions to ω-3 intake, three were from fish (catfish, sardines and tuna fish). Together, these three comprised 22 % of ω-3 intake in this population. Canola oil ranked among the top 5 contributors to total ω-3 intake. However, it contributed less than 4% to the total ω-3 intake.

Association of ω-3 Fatty-acid Intake with Serum Lipid Concentrations

In multivariable regression analysis **(Table 4.),** Dietary ALA was positively associated with HDL-C. This association was consistent across models (adjusting/not adjusting for DHA+EPA). ALA was not significantly associated with LDL-C, TG or total cholesterol. We found no associations between intakes of marine ω-3 (DHA and EPA) and serum lipids.

Discussion

The prevalence of dyslipidemia is notable in this cohort of African American men and women. Mean concentrations for LDL-C and TC in JHS were above normal for women (according to the

NCEP classifications) [31, 34]. Mean LDL-C in men was 'borderline' according to guidelines, while mean TC concentrations were in the upper limit of normal. Mean TG concentrations were within normal limits, but were not normally distributed. These results demonstrate high prevalence of dyslipidemia in this population.

The Institute of Medicine's (IOM) Food and Nutrition Board recommends an ω-3 intake of 1.6 g/day for men and 1.1 g/day for women [51]. Mean intakes for men and women were higher than the current recommended dietary allowance (RDA). Our study found 55.4% of men and 71.6% of women in the JHS met the current recommended dietary allowance (RDA) for ω-3 intake. However, despite these ω-3 intake levels, the JHS cohort reports a high prevalence of CVD risk factors, hypertension, diabetes and hypercholesterolemia [36, 40].

Research from the Agency for Healthcare Research and Quality (AHRQ) suggests a strong and consistent inverse relationship between ω-3 and TG [45, 120]. This conclusion is based on evidence presented in 19 studies. However, we did not find an association between ω-3 intake and TG in our analysis. Studies show the association between ω-3 and TG is more likely to be seen with very high levels of ω-3 from fish oil supplements [120, 135, 136]. Furthermore, our data suggests a positive association between HDL-C and ALA. We did not detect any significant associations between ω-3 and serum LDL-C and TC. Neither did we find significant associations between marine derived ω-3, DHA and EPA, with serum lipid concentrations.

The sources of ω-3 in this population deserve further investigation. The predominant sources include catfish, dressing with mayonnaise, cornbread, sardines, canola oil, potato salad, ice cream, tuna fish, soft margarine, and processed meat. Of these, only three are fish sources. Though catfish provides the largest contribution to ω-3 intake, it contains insignificant amounts

of DHA and EPA [120]. The majority of the contribution from catfish is from fried catfish which presents additional issues. The type of fat used for frying could affect the nutrient content of catfish. The process of deep frying may result in lower than estimated levels of DHA and EPA, leading to misclassification of intake exposure. Tuna also contains low amounts of EPA and DHA, depending on the type. White tuna canned in water contains higher amounts of ω-3s than other types of tuna. The FFQ did not have questions on the specific type of tuna. Sardines are reported to contain 0.5g per 100g of food serving of EPA, and DHA [120]. Of the three fish sources of ω-3 fatty acids, sardines have the highest ω-3 content. Overall, the ω-3 fatty acid content of the main fish sources in the JHS is low. It is important to note, the ω-3 intake in this study is based on nutrient data system estimations and FFQ averages across foods.

In conclusion, our study did not identify an association between ω-3 intake and TG. The low intake of marine ω-3 fat and the limited number of participants with high triglycerides may explain these findings. We found a significant association between HDL-C and ALA intake. The mean intake of ω-3 in this population is in accordance with the RDA for ω-3. However, further investigation into sources of ω-3, the relationship between ω-3 intake and dyslipidemia with subsequent CVD in African Americans is required.

Table 1. Descriptive Characteristics of JHS participants

	Men (n=1678)		Women (n=2930)	
	Mean	**SD**	**Mean**	**SD**
Age (yrs)	54.6	12.8	55.8	12.6
Body Mass Index (BMI)*	29.8	6.1	32.8	7.6
Physical Activity Score	8.6	2.6	8.2	2.6
Alcohol (g)	6.5	14.2	1.3	5.6
Low Density Lipoprotein (LDL-C) mg/dL	126.9	36.9	125.2	14.7
High Density Lipoprotein (HDL-C) mg/dL*	45.9	12.9	55.2	69.7
Triglyceride mg/dL	120.3	120.4	103.3	7.3
Total Cholesterol mg/dL*	195.8	40.3	200.8	39.7

JHS, Jackson Heart Study; SD, standard deviation
Differences between sexes were computed using general linear models procedure.
*indicates statistically significant differences between sexes; $p<0.05$

Table 2. Dietary Characteristics of JHS Participants

	Men (n=1480)		**Women (n=2783)**	
	Mean	**SD**	**Mean**	**SD**
Energy (kcal)*	2162	807	1812	761
PUFA (%)*	7.9	2.0	8.2	2.2
MUFA (%)	13.2	2.7	13.1	2.9
Saturated Fat (%)	10.7	2.5	10.7	2.7
Total Fat (%)	34.7	6.5	34.8	7.1
Carbohydrate (%)*	49.8	9.1	51.9	9.7
Protein (%)*	25.7	5.6	25.2	6.1
Selected Fatty Acids				
ALA (%)	0.7	0.2	0.7	0.2
EPA (%)	0.07	0.06	0.06	0.06
DPA (%)	0.02	0.02	0.02	0.02
DHA (%)*	0.06	0.04	0.05	0.04
Total Omega-3 (%)	0.9	0.3	0.9	0.3
LA (%)*	6.9	1.8	7.2	2.0
AA (%)*	0.09	0.04	0.08	0.04

JHS, Jackson Heart Study; SD, standard deviation; PUFA, polyunsaturated fatty acid; MUFA, monounsaturated fatty acid; ALA, alpha linolenic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; PUFA, polyunsaturated fatty acid; LA, linoleic acid;AA, arachidonic acid

Differences between sexes were computed using general linear models procedure.

*indicates statistically significant differences between sexes after adjusting for energy intake; $p<0.05$

Table 3. Top 10 FFQ Ranked food items contributing to Omega 3 intake in JHS participants

Food Group	**% of energy contribution to ω-3 intake [1]**
Catfish	15.0
Dressing w/mayonnaise	12.3
Cornbread	11.4
Sardines	4.4
Canola Oil	3.8
Potato Salad	3.5
Ice Cream	3.1
Tuna fish from oil/can made with Mayonnaise	2.8
Soft Margarine	1.8
Processed meat, pork bacon	1.3

[1]Energy contributions do not total 100% because only top 10 items are included. Data are based on the whole cohort, FFQ data. Nutrient composition of foods is provided from completed FFQ's using the Nutrient Data System (NDS, version 5.0-35, 2004, Nutrition Coordinating Center) developed by the University of Minnesota. Data are based on food composition data corresponding to the JHS baseline (200-2004).

Table 4. Association between dietary ω-3 and serum lipid concentrations in JHS participants

	Predictor Variables	
Outcome Variable	**DHA+EPA**	**ALA**
HDL-C	β (SE)	β (SE)
Model 1	0.5(1.0)	1.0(0.5)**
Model 2	-0.3(1.0)	1.1(0.5)**
LDL-C		
Model 1	2.6(2.6)	0.6(1.3)
Model 2	2.4(2.8)	0.2(1.3)
Triglycerides		
Model 1	-3.9(6.6)	-3.4(3.2)
Model 2	-1.7(0.8)	-0.06(3.4)
Total Cholesterol		
Model 1	2.6(2.8)	0.9(1.4)
Model 2	2.2(3.0)	0.6(1.4)

JHS, Jackson Heart Study; SE, standard error; HDL-C, high density lipoprotein LDL-C, low density lipoprotein; DHA, docosapentaenoic acid; EPA, eicosapentaenoic acid; ALA, alpha linolenic acid
Both models were adjusted for age, sex, BMI, Energy, smoking status and alcohol intake
In model 1 DHA+EPA and ALA are not included in the same model
In model 2 DHA+EPA and ALA are included in the same model
*p<0.10, **p<0.05, ***p<0.001

1. *Diet, nutrition and the prevention of chronic diseases*. World Health Organization technical report series, 2003. **916**: p. i-viii, 1-149, backcover.
2. Lichtenstein, A.H., et al., *Summary of American Heart Association Diet and Lifestyle Recommendations revision 2006*. Arteriosclerosis, thrombosis, and vascular biology, 2006. **26**(10): p. 2186-91.
3. Hu, F.B. and W.C. Willett, *Optimal diets for prevention of coronary heart disease*. JAMA : the journal of the American Medical Association, 2002. **288**(20): p. 2569-78.
4. Balk, E., et al., *Effects of omega-3 fatty acids on cardiovascular risk factors and intermediate markers of cardiovascular disease*. Evidence report/technology assessment, 2004(93): p. 1-6.
5. Yokoyama, M., et al., *Effects of eicosapentaenoic acid on major coronary events in hypercholesterolaemic patients (JELIS): a randomised open-label, blinded endpoint analysis*. Lancet, 2007. **369**(9567): p. 1090-8.
6. Daviglus, M.L., et al., *Fish consumption and the 30-year risk of fatal myocardial infarction*. The New England journal of medicine, 1997. **336**(15): p. 1046-53.
7. Kaushik, M., et al., *Long-chain omega-3 fatty acids, fish intake, and the risk of type 2 diabetes mellitus*. The American journal of clinical nutrition, 2009. **90**(3): p. 613-20.
8. Carpentier, Y.A., L. Portois, and W.J. Malaisse, *n-3 fatty acids and the metabolic syndrome*. The American journal of clinical nutrition, 2006. **83**(6 Suppl): p. 1499S-1504S.
9. MacLean, C.H., et al., *Effects of omega-3 fatty acids on lipids and glycemic control in type II diabetes and the metabolic syndrome and on inflammatory bowel disease, rheumatoid arthritis, renal disease, systemic lupus erythematosus, and osteoporosis*. Evidence report/technology assessment, 2004(89): p. 1-4.
10. Wang, C., et al., *Effects of omega-3 fatty acids on cardiovascular disease*. Evidence report/technology assessment, 2004(94): p. 1-8.
11. Sirtori, C.R., et al., *One-year treatment with ethyl esters of n-3 fatty acids in patients with hypertriglyceridemia and glucose intolerance: reduced triglyceridemia, total cholesterol and increased HDL-C without glycemic alterations*. Atherosclerosis, 1998. **137**(2): p. 419-27.
12. Woodman, R.J., et al., *Effects of purified eicosapentaenoic and docosahexaenoic acids on glycemic control, blood pressure, and serum lipids in type 2 diabetic patients with treated hypertension*. The American journal of clinical nutrition, 2002. **76**(5): p. 1007-15.
13. Dunstan, D.W., et al., *The independent and combined effects of aerobic exercise and dietary fish intake on serum lipids and glycemic control in NIDDM. A randomized controlled study*. Diabetes care, 1997. **20**(6): p. 913-21.
14. Finnegan, Y.E., et al., *Plant and marine derived (n-3) polyunsaturated fatty acids do not affect blood coagulation and fibrinolytic factors in moderately hyperlipidemic humans*. The Journal of nutrition, 2003. **133**(7): p. 2210-3.
15. Roger, V.G., AS; Lloyd-Jones, DM; Benjamin, EJ; Berry, JD; Borden, WB; Bravata, DM; Dai, S; Ford, ES; Fox, CS; Fullerton, HJ; Gillespie, C; Halipern, SM; Heit, JA; Howard, VJ; Kissela, BM; Kittner, SJ; Lackland, DT; Lichtman, JH, Lisabeth, LD; Makuc, DM; Marcus, GM; Marelli, A; Matchar, DB; Moy, CS; Mozaffarian, D; Mussolino, ME; Nichol, G; Paynter, NP; Soliman, EZ; Sorlie, PD; Sotoodehnia, N; Turan, TN, Virani, SS; Wong, ND; Woo, D; Turner, MB; on behalf of the American Heart Association Statistics Committee and STroke Statistics Subcommitee. , *Heart disease and stroke statistics--2012 update: a report from the American Heart Association*. Circulation 2012.
16. Harris-Hooker, S. and G.L. Sanford, *Lipids, lipoproteins and coronary heart disease in minority populations*. Atherosclerosis, 1994. **108 Suppl**: p. S83-104.
17. Benton, J.L., et al., *Predictors of low-density lipoprotein particle size in a high-risk African-American population*. The American journal of cardiology, 2005. **95**(11): p. 1320-3.

18. Metcalf, P.A., et al., *African American-white differences in lipids, lipoproteins, and apolipoproteins, by educational attainment, among middle-aged adults: the Atherosclerosis Risk in Communities Study*. American journal of epidemiology, 1998. **148**(8): p. 750-60.
19. Liu, J., et al., *Impact of abdominal visceral and subcutaneous adipose tissue on cardiometabolic risk factors: the Jackson Heart Study*. The Journal of clinical endocrinology and metabolism, 2010. **95**(12): p. 5419-26.
20. Taylor, H., et al., *Distinct component profiles and high risk among African Americans with metabolic syndrome: the Jackson Heart Study*. Diabetes care, 2008. **31**(6): p. 1248-53.
21. Taylor, H.A., Jr., et al., *Dyslipidemia and the treatment of lipid disorders in African Americans*. The American journal of medicine, 2009. **122**(5): p. 454-63.
22. Fuqua, S.R., et al., *Recruiting African-American research participation in the Jackson Heart Study: methods, response rates, and sample description*. Ethnicity & disease, 2005. **15**(4 Suppl 6): p. S6-18-29.
23. Taylor, H.A., Jr., et al., *Toward resolution of cardiovascular health disparities in African Americans: design and methods of the Jackson Heart Study*. Ethnicity & disease, 2005. **15**(4 Suppl 6): p. S6-4-17.
24. Carithers, T.C., et al., *Validity and calibration of food frequency questionnaires used with African-American adults in the Jackson Heart Study*. Journal of the American Dietetic Association, 2009. **109**(7): p. 1184-1193.
25. Talegawkar, S.A., et al., *Total antioxidant performance is associated with diet and serum antioxidants in participants of the diet and physical activity substudy of the Jackson Heart Study*. The Journal of nutrition, 2009. **139**(10): p. 1964-71.
26. Talegawkar, S.A., et al., *Carotenoid intakes, assessed by food-frequency questionnaires (FFQs), are associated with serum carotenoid concentrations in the Jackson Heart Study: validation of the Jackson Heart Study Delta NIRI Adult FFQs*. Public health nutrition, 2008. **11**(10): p. 989-97.
27. Tucker, K.L., et al., *A regional food-frequency questionnaire for the US Mississippi Delta*. Public health nutrition, 2005. **8**(1): p. 87-96.
28. Champagne, C.M., et al., *Dietary intake in the lower Mississippi delta region: results from the Foods of our Delta Study*. Journal of the American Dietetic Association, 2004. **104**(2): p. 199-207.
29. *Summary of the second report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel II)*. JAMA : the journal of the American Medical Association, 1993. **269**(23): p. 3015-23.
30. Carpenter, M.A., et al., *Laboratory, reading center, and coordinating center data management methods in the Jackson Heart Study*. The American journal of the medical sciences, 2004. **328**(3): p. 131-44.
31. Hertogh, E.M., et al., *Validity of the modified Baecke questionnaire: comparison with energy expenditure according to the doubly labeled water method*. The international journal of behavioral nutrition and physical activity, 2008. **5**: p. 30.
32. Dubbert, P.M., et al., *Physical activity assessment methods in the Jackson Heart Study*. Ethnicity & disease, 2005. **15**(4 Suppl 6): p. S6-56-61.
33. Smitherman, T.A., et al., *Validation of the Jackson Heart Study Physical Activity Survey in African Americans*. Journal of physical activity & health, 2009. **6 Suppl 1**: p. S124-32.
34. Ainsworth, B.E., et al., *Evaluation of the kaiser physical activity survey in women*. Medicine and science in sports and exercise, 2000. **32**(7): p. 1327-38.
35. Richardson, M.T., et al., *Ability of the Atherosclerosis Risk in Communities (ARIC)/Baecke Questionnaire to assess leisure-time physical activity*. International journal of epidemiology, 1995. **24**(4): p. 685-93.

36. Gillum, R.F., M.E. Mussolino, and J.H. Madans, *Coronary heart disease risk factors and attributable risks in African-American women and men: NHANES I epidemiologic follow-up study*. American journal of public health, 1998. **88**(6): p. 913-7.
37. National High Blood Pressure Education Program. and National Heart Lung and Blood Institute., *Primary prevention of hypertension : clinical and public health advisory from the National High Blood Pressure Education Program*. NIH publication2002, Bethesda, Md.: National Institutes of Health, National Heart, Lung, and Blood Institute. vi, 17 p.
38. National High Blood Pressure Education Program. and National High Blood Pressure Education Program. Working Group on Hypertension and Chronic Renal Failure., *National High Blood Pressure Education Program (NHBPEP) working group report on hypertension and chronic renal*. NIH publication1990, Bethesda, Md.: U.S. Dept. of Health and Human Services, Public Health Service, National Institutes of Health, National Heart, Lung, and Blood Institute. viii, 20 p.
39. National High Blood Pressure Education Program. Working Group on Ambulatory Blood Pressure Monitoring. and National Heart Lung and Blood Institute., *National High Blood Pressure Education Program (NHBPEP) Working Group report on ambulatory blood pressure monitoring*. NIH publication1990, Bethesda, Md.: U.S. Dept. of Health and Human Services, Public Health Service, National Institutes of Health, National Heart, Lung, and Blood Institute. vi, 21 p.
40. National High Blood Pressure Education Program. Working Group on Hypertension in Diabetes., *National High Blood Pressure Education Program Working Group report on hypertension in diabetes*, in *NIH publication no 94-3530*1994, U.S. Dept. of Health and Human Services, Public Health Service, National Institutes of Health, National Heart, Lung, and Blood Institute,: Bethesda, Md.
41. *Standards of medical care in diabetes*. Diabetes care, 2004. **27 Suppl 1**: p. S15-35.
42. Willett, W., *Nutritional epidemiology*. 2nd ed. Monographs in epidemiology and biostatistics1998, New York: Oxford University Press. xiv, 514 p.
43. *Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report*. Circulation, 2002. **106**(25): p. 3143-421.
44. Institute of Medicine (U.S.). Panel on Macronutrients. and Institute of Medicine (U.S.). Standing Committee on the Scientific Evaluation of Dietary Reference Intakes., *Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein, and amino acids*2005, Washington, DC: National Academies Press. xxv, 1331 p.
45. Jacobson, T.A., et al., *Effects of eicosapentaenoic acid and docosahexaenoic acid on low-density lipoprotein cholesterol and other lipids: a review*. Journal of clinical lipidology, 2012. **6**(1): p. 5-18.
46. Wei, M.Y. and T.A. Jacobson, *Effects of eicosapentaenoic acid versus docosahexaenoic acid on serum lipids: a systematic review and meta-analysis*. Current atherosclerosis reports, 2011. **13**(6): p. 474-83.

Trans fat intake is associated with dietary patterns and serum lipid concentration in the Diet and Physical Activity Sub-Study of the Jackson Heart Study (JHS)

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ABSTRACT

Background: Americans living in the southern states have been characterized as consuming a diet rich in fried foods, large portion sizes and high fat content. However, there is limited research on the dietary intake of *trans* fat (TFA) in this population and the association between dietary patterns, TFA and serum lipid concentrations in African Americans living in the South.

Objective: This study examines the cross-sectional relationship between TFA intake, dietary patterns and serum lipids in Jackson Heart Study (JHS) participants.

Design: Dietary TFA, previously derived dietary patterns and serum cholesterol were available in 499 participants of the Diet and Physical Activity Sub-study (DPASS) in JHS. Rankings of TFA food sources in JHS were compiled from food frequency (FFQ) data. The GLM procedure in SAS v 9.2 was used to assess the cross-sectional association between dietary TFA with dietary patterns and serum cholesterol.

Results: Margarine contributed the most to TFA intake among JHS participants. Dietary TFA was significantly associated with southern and fast food dietary patterns when compared with the prudent pattern, but not with the fruit juice pattern. TFA, 16:1t was positively associated with high density lipoprotein (HDL-C) and total cholesterol (TC) concentrations.

Conclusions: No associations were identified between TFA and low density lipoprotein (LDL-C) or triglycerides (TG). There was a positive association between 16:1t and HDL-C. Dietary sources of TFA were similar to those reported by other studies.

Introduction

There has been increasing interest in the relationship between *trans* fatty acids (TFA) and health. In 2002, the American Academy of Sciences made a recommendation to consume as little TFA as possible. Since then, sentiment against the use of TFA in commercially prepared foods has risen. Restaurants like McDonalds, Burger King, Wendy's, Jack in the Box and Dairy Queen have significantly reduced their use of TFA in food preparation. In addition, cities like New York City, NY have banned the commercial use of partially-hydrogenated fat, the major source of TFA in the US diet. These industry and governmental changes are a result of growing literature on the deleterious effects of TFA to human health [58, 59, 64]).

Two types of TFA exist, 1) from natural bacterial hydrogenation of fatty acids in ruminants and 2) an industry derived product from the partial hydrogenation of vegetable oils. Research on the effect of ruminant TFA is growing. However, observational studies show a mostly neutral effect of the natural form of TFA on CVD risk, lipids, and glucose metabolism[64, 66-69, 137, 138]. Some investigators have suggested that deleterious effects of TFA are mostly the result of exposure to partially hydrogenated vegetable oils (PHVO) [138, 139]. However, a review by Brouwer et al suggests that "all fatty acids with a double bond in the trans configuration raise the ratio of plasma LDL-C to HDL-C cholesterol [140]".

Research on TFA from PHVO has shown predominantly negative effects on health. TFA has been associated with increases in atherosclerosis, low density lipoprotein concentration (LDL-C), systemic inflammation, endothelial dysfunction, thrombogenesis and glucose intolerance[57, 58, 141]. Similarly, TFA from PHVO has been associated with lower high density lipoprotein concentration (HDL-C) and cellular nitric oxide availability. TFA are believed to enter cells such

as monocytes, adipocytes and endothelial cells and affect signaling pathways [56, 57, 141]. Cumulatively, these occurrences provide for an atherogenic environment, resulting in accumulation of lipids in vasculature, atherosclerotic plaques and CVD events [56, 64, 65, 142].

PHVO TFAs are provided in the diet by commercially prepared foods. Margarine and foods such as popcorn, processed cookies and other snack foods and convenience foods like instant soups, grain-based items and fast food which uses partially hydrogenated oils are all sources of TFA in the diet[63, 143]. Data on TFA intake trends are limited as labeling of TFA in commercially prepared food and food products was not required until 2006 [63, 143, 144].

The USDA SR database began including TFAs in 2002. At the time, it included the total TFA values for 2 items. Since then, inclusion of TFA in the USDA database has increased exponentially, with a total of 1213 items in the database as of 2007 [145]. The major contributors to USDA database values of dietary TFA are fast-food and grain based items with a combined contribution of over 40%. Fats, oils, snacks and sweets contribute less than 15% to the available TFA data. Individual TFAs are included to a lesser extent in the USDA database. This provides a discrepancy not only in the number of foods with available TFA data for nutrition research, but also the types of foods.

Industry and government regulations regarding TFA have caused changes to the U.S. food supply. This complicates interpretation, as changes in TFA content of foods occurs simultaneously with attempts to add and update values[145]. Furthermore, U.S. regulations allow the use of 0.5g of TFA to be labeled as “trans fat free”. This can lead to un-captured TFA in the diet. All of these issues are potential problems for dietary assessment of TFA.

One study showed that intakes of TFA were 2.7-3.0% of total energy intake in men residing in the Southern U.S. [90]. This study also found that intakes differed significantly by region and ethnicity, with higher intakes in the southeastern U.S. than in other regions, and among African Americans than among non-Hispanic whites [90]. Much of the data regarding the relationship between TFA and health in Americans has been conducted in white populations. Given the high intake of TFA in African Americans, together with the high morbidity and mortality of CVD in this population, it is important to investigate this relationship in African Americans. In this study, we investigated the relationship between dietary TFA, dietary patterns and serum cholesterol in the Jackson Heart Study (JHS), a Mississippi based African American Cohort.

Materials and Methods

Study Participants:

The JHS is a longitudinal investigation of CVD risk factors and etiology in an African American cohort based in Jackson, MS. Baseline data collection for the study commenced in 2000 and was completed in 2004. The study includes 5,302 self-identified African Americans residing in Hinds, Rankin and Madison counties in and near Jackson, MS. Participant ages ranged from 35to 85 years of age. A detailed description of the study design and recruitment is provided elsewhere [127].

Diet and Physical Activity Sub-study

A subset of participants (n=499) from the JHS were included in the Diet and Physical Activity Sub-study (DPASS). Participants in DPASS completed additional questionnaires on physical activity, four, 24h recalls, a short (20 minute) interview–administered food frequency questionnaire (SFFQ), as well as a longer (40 minute) interview-based food frequency

questionnaire (LFFQ). Multiple dietary instruments in DPASS were used for the purpose of validating the SFFQ for use in the entire cohort. In a separate study, the SFFQ failed to correlate with plasma phospholipid (PL) TFA (unpublished data). Dietary TFA from the LFFQ, which included summary questions on fats and oils used for cooking, did correlate with PL TFA. Data from the LFFQ is only available in DPASS. Therefore, the focus of this analysis is the DPASS population. Institutional Review Board (IRB) approval for the JHS cohort was provided by the University of Mississippi IRB. Approval for this study was provided by the IRB at Tufts Medical Center.

Assessment of Diet

The FFQ used in the JHS was a regionally specific FFQ developed with the Lower Mississippi Delta Nutrition Intervention Research Initiative (LMD Delta NIRI) [43]. This FFQ, funded by the USDA Agricultural Research Service, was based on a series of telephone based 24h recalls surveys of people living in the Mississippi Delta. In short, the FFQ includes regionally specific foods, portion sizes and regionally appropriate weighting of ingredients. The FFQ contains 283 items. Details regarding the development of this FFQ are described elsewhere [43, 94]. Data from the shorter, 158-item 20 minute FFQ, developed to ease participant burden, were not used in this study.

Dietary Patterns

The creation of dietary patterns is described elsewhere [97]. In short, the 283 items of the LFFQ were sorted into 33 food groups defined by similarity or difference in nutrient content and general usage. Calculation of percent energy contribution from each food group was entered for

cluster analysis using the FASTCLUS procedure in SAS. Four patterns were selected as the most meaningful, southern, fast food, juice and prudent

Lipids

Blood samples were collected during baseline clinic interviews. Participants were requested to fast for 12h prior to the clinic interview. Serum cholesterol concentrations were determined at the University of Minnesota Medical Center, Fairview [36, 88]. Lipid assays were standardized through the Lipid Standardization Program of the Centers for Disease Control and Prevention, Atlanta, Ga. [31]. Details regarding the methods and storage of lipid assays were previously described [36, 111].

Covariates

Survey questionnaires administered during the JHS baseline clinic visit were used to ascertain covariate data. Data on self-reported age, diabetes status, hypercholesterolemia, hypertension, myocardial infarction, and physical activity were collected by interviewer-administered questionnaires. Body Mass Index (BMI) was calculated as weight in kilograms divided by height in meter squared. All anthropometric measures were obtained by trained nurses during the baseline clinic visit. Physical activity score was based on a validated physical activity questionnaire given at baseline. Details on the development and validation of the physical activity questionnaire, as well as the other baseline data collection are provided elsewhere [106-108].

Disease status

Hypercholesterolemia was determined by participant's report of lipid lowering medication use in the previous 2 weeks, a fasting LDL-C ≥ 160mg/dL or a TC concentration ≥ 240 mg/dL [129]. The hypercholesterolemia classification is based on the third report of the Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults, Adult Treatment Panel III [31, 36]. Hypertension was determined by use of antihypertensive medication, or systolic blood pressure ≥ 140mm Hg or diastolic blood pressure ≥ 90mm Hg. Criteria for hypertension status are based on the 7th Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC7) [36, 130-133]. Type 2 diabetes was defined by fasting plasma glucose ≥126 mg/dL or current use of insulin or oral hypoglycemic medications [36, 40]. These criteria for type 2 diabetes are in accordance with the American Diabetic Association 2004 Guidelines [134].

Statistical Analysis

Cross sectional statistical analysis was done using SAS 9.2 (SAS Inc, Cary, NC). All variables were evaluated for normality and measures of central tendency. We used the GLM procedure to assess mean differences study characteristics and dietary characteristics, expressed as percent of energy, between men and women; and to assess differences in TFA intake and energy from select foods by dietary pattern. Serum triglyceride (TG) concentration was transformed using a logarithm based 10. All other lipid values were normally distributed. Alcohol intake lacked normality. The logarithm of the sum of alcohol +10 was used to transform alcohol intake. Log-transformed alcohol intake was used as a covariate. Examination of outliers was completed using the residual method. Frequency statistics were used to compare categorical variables. Collinearity diagnostic tools, variance inflation factor (VIF) and tolerance, were used to assess collinearity within dietary covariates and lipid outcomes.

Correlation analysis of TFA with selected food items were adjusted for age, BMI, sex, and energy intake. GLM was used to assess the association of TFA with dietary patterns and serum cholesterol. Covariates included age, BMI, serum TC, alcohol intake (g), energy intake (kcal) and diabetes status. FFQ data were used to compile rankings of percent contribution to total TFA intake.

Results

Descriptive Characteristics

Men had higher LDL-C and lower HDL-C than women. According to the NCEP III criterion, 76% of men in the JHS had high LDL-C, 34% had low HDL-C, and 42% had high TC concentration. Similarly, 73% of women had high LDL-C, 13% had low HDL-C and 47% had high TC. Approximately 45% of men and 35% of women had high serum TG concentration.

Men had significantly higher intakes of energy, carbohydrates and protein in comparison to women ($p<0.05$) **Table 1**.

Relationship Between Selected foods and TFA

There were positive correlations between fast food and margarine intakes with all TFA **(Table 2).** Baked goods, salty snacks and miscellaneous fats had positive correlations with 18:1t, 18:2t and total TFA. In addition to fast food and margarine, 16:1t was positively correlated with meat, and18:2t with seafood. Vegetable oils and dairy showed no significant correlations with selected food items.

TFA Percent Contributions

We identified and ranked foods contributing to total TFA intake (Table 3). Top contributing food sources to TFA intake included margarine (20%), popcorn (11%) and commercial French fries (7%).

Association between TFA and serum lipids

We found a significant, positive association between 16:1t and HDL-C and TC **(Table 4).** No significant associations were found between 18:1t or 18:2t with serum TC. Additionally, no associations between serum TG or LDL-C with TFA were observed.

Dietary characteristics by dietary cluster pattern

There were statistically significant differences between mean percent carbohydrate, protein and energy by dietary pattern ($P<0.05$) **Table 5.** There were significant differences in mean percent of energy intake of all TFA by dietary pattern cluster **(Table 6).** The fast food and southern patterns had the highest proportion of energy from 16:1t, 18:1t, 18:2t and total TFA, followed by the southern food pattern, the juice pattern and prudent pattern. The fast food dietary pattern represented the largest group, with a sample size of 141, the prudent and juice patterns had the lowest number of participants, with sample sizes of 60 and 56 respectively.

Intake of Selected Foods by Dietary Pattern

Participants in the fast food dietary pattern consumed the largest proportion of energy from oils, nuts and seeds, miscellaneous fat, fast food, meat, snacks and baked goods **(Table 7).** Persons reporting a southern dietary pattern had the largest consumption of energy from margarine. Participants in the prudent pattern consumed more energy from seafood than participants in the fast food, juice and southern patterns.

Association between dietary** patterns **and TFA

The southern and fast food dietary patterns both contained more TFA, 16:1t, 18:1t and 18:2t intake in comparison to the prudent pattern (p-value < 0.001) **(Table 8**). The juice pattern did not differ from the prudent pattern in its TFA content.

Discussion

TFA is an increasingly important nutrient in health. Individual TFAs are present in different foods and are thought to have differential effects on the body. We investigated self-reported intake of three individual TFA (16:1t. 18:1t. and 18:2t), and found higher consumption of each in men. However, we did not look at the different isomers within each of these TFA. The fast food dietary pattern had the highest mean percent contribution to energy from TFA. The prudent dietary pattern had the lowest intake of TFA, consistent with its connotation as the "healthier" dietary pattern in JHS.

In contrast to expectation, mean serum cholesterol concentrations were highest in the prudent pattern. This finding was consistent across cholesterol types. However, the prudent pattern was associated with older age. Higher serum LDL-C and TC in older persons is consistent with current knowledge. In addition, other studies in JHS have shown a positive association between age and HDL-C [40]. These findings have formerly been attributed to more physically demanding occupations in older African Americans [36]. Dietary patterns may also contribute to the differences in HDL-C between older and younger African Americans. Previous work in the JHS, showed higher consumption of fast food dietary patterns in younger persons, which further supports a relationship between age, fast food dietary pattern and lower HDL-C [97].

These findings support those of Micha et al, which suggest that 16:1t is present in margarine; 18:2t in baked goods; and that18:1t is present in all foods containing hydrogenated oil (ex: chips, baked goods and fried foods) [65, 143]. We found correlations between 16:1t and meat. Although, there are ruminant forms of TFA (mainly the 9cis 16:1t) in meats, this correlation may be driven by the popularity of breading and frying meats with margarine. Similarly, we found a correlation between seafood and 18:2t which may be a result of hydrogenated oils used to fry fish and shellfish.

We did not find a significant association between TFA and LDL-C. Previous studies have shown that TFA increases serum LDL-C concentration. Given our limited sample, post-hoc calibration of the JHS short food frequency questionnaire to allow for these analyses to be replicated in the full JHS cohort may prove beneficial. Further investigation of the relationship between TFA with other fatty acids, including interactions and confounding factors is needed. Szabo et al. found interference in metabolism between TFA and ω-3 [146]. We saw a positive association between 16:1t and HDL-C. Other studies have shown beneficial relationships between 16:1t and CVD risk factors [66], however the biologic mechanism underlying this association is not known.

These generally support findings in other populations, and provide insight into the intake, sources and associations of TFA in the JHS cohort. Future research should include ruminant TFA and other cardiovascular risk factors.

Table 1: Study characteristics of JHS DPASS participants

	Men (n=155)	**Women(n=212)**
	Mean ±SD	Mean ±SD
Age (years)	60.0 ±10.2	61.7±9.0
BMI, kg/m^2 *	29.7±6.2	31.0±7.2
Physical Activity Score	8.1±2.5	8.1±2.6
LDL-C mg/dL	125±35	121±36
HDL-C mg/dL *	46±11	57±16
Triglycerides mg/dL	112±67	119±119
Total Cholesterol mg/dL *	193±37	204±43
Energy (kcal/day)	2173±747	1794±592*
PUFA (%)	7.7±1.6	7.9±1.7
MUFA (%)	13.5±2.4	13.2±2.5
Saturated Fat (%)	10.9±2.0	10.3±2.2
Total Fat (%)	35.0±5.5	34.0±5.8
Total Trans fat(%)	2.1±0.6	2.3±0.8
16:1t (%)	0.03±0.01	0.02±0.009
18:1t (%)	1.8±0.6	2.0±0.7
18: 2t (%)	0.3±0.07	0.3±0.08
Carbohydrate (%)	49.8±7.2	52.5±7.0 *
Protein (%))	15.1±2.4	14.8±2.6*

JHS, Jackson Heart Study; DPASS, Diet and Physical Activity Sub-study; BMI, body mass index; LDL-C, low density lipoprotein; HDL-C, high density lipoprotein; PUFA, polyunsaturated fatty acid; MUFA, monounsaturated fatty acid
Mean presented for triglycerides is untransformed value. Triglycerides were log transformed for comparison between sexes.
*significant differences between men and women alpha=0.05

Table 2. Correlation between intake of *trans* fat and selected foods in JHS-DPASS participants

	16:1t	18:1t	18:2t	Total TFA
Seafood	-0.1*	-0.06	-0.1**	-0.078
Fast food	0.2***	0.2***	0.2***	0.2***
Oils	-0.04	0.05	0.05	0.05
Margarine	0.2***	0.3***	0.3***	0.3***
Miscellaneous fat	0.05	0.1**	0.1**	0.01***
Meat	0.2***	-0.01	-0.03	-0.01
Salty snacks	-0.02	0.4***	0.2***	0.4***
Dairy	-0.04	-0.02	0.05	-0.01
Baked desserts	0.05	0.2***	0.4***	0.3***

JHS, Jackson Heart Study; DPASS, Diet and Physical Activity Sub-study;
Values presented are correlation coefficients; Statistically significant correlations are indicated by
*P<0.10; **P<0.05; ***P<0.001

Table 3. Top 10 FFQ ranked food items contributing to *trans* fat intake in JHS participants

Food	**% energy contribution to TFA intake** [1]
Margarine	19.9
Microwave popcorn	10.7
French Fries	6.8
Fried Shellfish	3.7
Biscuits	3.3
Rice and Beans	2.4
Hush Puppy	2.2
Apple Turnover	2.1
Ice Cream	2.1
Doughnuts	2.0

[1] Energy contributions do not total 100% because only top 10 items are included
JHS, Jackson Heart Study
Foods presented comprise the top 10 food items contributing to trans fat intake in JHS (Jackson Heart Study) participants. Data are based on the whole cohort, FFQ data. Nutrient composition of foods is provided from completed using the Nutrient Data System (NDS, version 5.0-35, 2004, Nutrition Coordinating Center) developed by the University of Minnesota. Data are based on food composition data corresponding to the JHS baseline (200-2004).

Table 4. Associations between *trans* fat intake with serum lipid concentrations in JHS-DPASS participants.

	HDL-C	**LDL-C**	**TG**
	β (SE)	**β (SE)**	**β (SE)**
Dietary TFA			
16:1t (g/day)	75.1 (31.0) **	127(80.3)	0.4 (1.2)
18:1t (g/day)	0.3 (0.5)	-0.9(1.3)	-0.005 (0.02)
18:2t (g/day)	5.8(4.3)	-4.4(11.1)	-0.1(0.2)
Total TFA (g/day)	0.3(0.6)	-0.8(1.2)	-0.006 (0.02)

JHS, Jackson Heart Study; DPASS, Diet and Physical Activity Sub-study; TFA, *trans* fat LDL-C, low density lipoprotein concentration; HDL-C, high density lipoprotein concentration; TG, log-transformed triglyceride concentration;
Multivariable regression models were adjusted for age, BMI, sex, total energy intake from LFFQ, alcohol (g) and diabetes status.
*P<0.10; **P<0.05; ***P<0.001

Table 5. Selected dietary characteristics by dietary patterns in JHS DPASS

	Fast Food	Southern	Prudent	Juice
	n=150	n=97	n=62	n=58
	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD
Energy (kcal/day)*	2161±755	1900±574	1660±508	1826±697
PUFA (g/day) *	8.2±1.6	8.2±1.6	7.1±1.5	6.9±1.7
MUFA (g/day) *	14.3±2.0	13.7±2.3	11.6±2.4	12.0±2.4
Saturated Fat (g/day) *	11.6±1.8	10.9±1.8	9.0±2.0	9.2±1.8
Total Fat (g/day) *	36.9±4.5	35.6±5.1	30.3±5.4	30.7±5.3
Carbohydrate (g/day)*	48.6±6.2	50.7±6.6	55.4±2.4	55.3±7.5
Protein (g/day) *	14.8±2.6	14.4±2.7	15.9±2.4	14.9±2.9

JHS, Jackson Heart Study; DPASS, Diet and Physical Activity Sub-study; PUFA, polyunsaturated fatty acid; MUFA, monounsaturated fatty acid

Differences between dietary patterns were computed using general linear models procedure.

*significant difference between dietary patterns after adjusting for energy, alpha=0.05

Table 6. Mean *trans* fat intake by dietary pattern in JHS-DPASS participants

	Southern (n=95)	**Prudent** (n=60)	**Fast food** (n=141)	**Juice** (n=56)
TFA	Mean±SD	Mean±SD	Mean±SD	Mean±SD
16:1t (%)*	0.03±0.01	0.02±0.01	0.03±0.01	0.02±0.009
18:1t (%)*	2.1±0.6	1.6±0.5	2.1±0.7	1.7±0.6
18:2t (%)*	0.3±0.07	0.2±0.07	0.3±0.07	0.2±0.06
Total TFA (%)	2.4±0.6	1.9±0.6	2.4±0.7	2.0±0.7

TFA, *trans* fat; JHS, Jackson Heart Study; DPASS, Diet and Physical Activity Sub-study;
Differences between dietary patterns were computed using general linear models procedure.
*Indicates statistically significant differences b/w cluster means $p<0.05$.

Table 7. Mean energy intake from selected foods by dietary pattern in JHS-DPASS participants

	Southern(n=97)	**Prudent (n=62)**	**Fast food(n=150)**	**Juice (n=58)**
Mean energy intake ± SD				
Seafood (%)*	0.03±0.04	0.04±0.03	0.05±0.02	0.05±0.04
Oils (%)	0.02±0.02	0.03±0.03	0.02±0.02	0.02±0.03
Nuts and Seeds (%)	0.02±0.04	0.02±0.03	0.03±0.03	0.02±0.04
Margarine (%)*	0.02±0.01	0.02±0.01	0.02±0.02	0.01±0.01
Miscellaneous fat (%)	0.01±0.02	0.01±0.02	0.02±0.02	0.01±0.02
Fast food (%)*	0.03±0.04	0.02±0.02	0.05±0.02	0.03±0.02
Meat (%)*	0.03±0.02	0.02±0.02	0.04±0.02	0.03±0.02
Snacks (%)*	0.02±0.05	0.02±0.03	0.05±0.03	0.02±0.04
Dairy (%)*	0.05±0.04	0.07±0.05	0.05±0.04	0.04±0.03
Baked Goods (%)*	0.05±0.04	0.03±0.03	0.06±0.04	0.04±0.02

JHS, Jackson Heart Study; DPASS, Diet and Physical Activity Sub-study;
Differences between dietary patterns were computed using general linear models procedure.
* significant difference between dietary pattern groups after adjustment for age, sex and energy (for total intake), alpha = 0.05

Table 8. Association between dietary patterns and *trans* fat intake in JHS-DPASS participants

	Total TFA	**16:1t**	**18:1t**	**18:2t**
Juice				
β	0.4	0.007	0.3	0.04
SE	0.4	0.006	0.4	0.04
Southern				
β	1.2**	0.02***	1.0**	0.2***
SE	0.4	0.005	0.3	0.04
Fast Food				
β	1.8***	0.03***	1.6***	0.2***
SE	0.3	0.005	0.3	0.04

JHS, Jackson Heart Study; DPASS, Diet and Physical Activity Sub-study; TFA, *trans* fat; SE, Standard Error
Dietary patterns are compared to the prudent pattern; All models were adjusted for age, BMI, serum cholesterol, alcohol Intake (g), and total energy (kcal)
*P<0.10; **P<0.05; ***P<0.001

1. Booker, C.S. and J.I. Mann, *Trans fatty acids and cardiovascular health: translation of the evidence base.* Nutrition, metabolism, and cardiovascular diseases : NMCD, 2008. **18**(6): p. 448-56.
2. Mozaffarian, D., *Trans fatty acids - effects on systemic inflammation and endothelial function.* Atherosclerosis. Supplements, 2006. **7**(2): p. 29-32.
3. Mozaffarian, D., et al., *Trans fatty acids and cardiovascular disease.* The New England journal of medicine, 2006. **354**(15): p. 1601-13.
4. Jakobsen, M.U., et al., *Intake of ruminant trans fatty acids and risk of coronary heart disease-an overview.* Atherosclerosis. Supplements, 2006. **7**(2): p. 9-11.
5. Jakobsen, M.U., et al., *Intake of ruminant trans fatty acids in the Danish population aged 1-80 years.* European journal of clinical nutrition, 2006. **60**(3): p. 312-8.
6. Jakobsen, M.U., et al., *Intake of ruminant trans fatty acids and risk of coronary heart disease.* International journal of epidemiology, 2008. **37**(1): p. 173-82.
7. Mozaffarian, D., et al., *Trans-palmitoleic acid, metabolic risk factors, and new-onset diabetes in U.S. adults: a cohort study.* Annals of internal medicine, 2010. **153**(12): p. 790-9.
8. Tardy, A.L., et al., *Dairy and industrial sources of trans fat do not impair peripheral insulin sensitivity in overweight women.* The American journal of clinical nutrition, 2009. **90**(1): p. 88-94.
9. Chardigny, J.M., et al., *Do trans fatty acids from industrially produced sources and from natural sources have the same effect on cardiovascular disease risk factors in healthy subjects? Results of the trans Fatty Acids Collaboration (TRANSFACT) study.* The American journal of clinical nutrition, 2008. **87**(3): p. 558-66.
10. Motard-Belanger, A., et al., *Study of the effect of trans fatty acids from ruminants on blood lipids and other risk factors for cardiovascular disease.* The American journal of clinical nutrition, 2008. **87**(3): p. 593-9.
11. Brouwer, I.A., A.J. Wanders, and M.B. Katan, *Effect of animal and industrial trans fatty acids on HDL and LDL cholesterol levels in humans--a quantitative review.* PloS one, 2010. **5**(3): p. e9434.
12. Lemaitre, R.N., et al., *Plasma phospholipid trans fatty acids, fatal ischemic heart disease, and sudden cardiac death in older adults: the cardiovascular health study.* Circulation, 2006. **114**(3): p. 209-15.
13. Lopez-Garcia, E., et al., *Consumption of trans fatty acids is related to plasma biomarkers of inflammation and endothelial dysfunction.* The Journal of nutrition, 2005. **135**(3): p. 562-6.
14. Clifton, P.M., J.B. Keogh, and M. Noakes, *Trans fatty acids in adipose tissue and the food supply are associated with myocardial infarction.* The Journal of nutrition, 2004. **134**(4): p. 874-9.
15. Kuhnt, K., et al., *Trans fatty acid isomers and the trans-9/trans-11 index in fat containing foods.* European journal of lipid science and technology : EJLST, 2011. **113**(10): p. 1281-1292.
16. Micha, R. and D. Mozaffarian, *Trans fatty acids: effects on metabolic syndrome, heart disease and diabetes.* Nature reviews. Endocrinology, 2009. **5**(6): p. 335-44.
17. Micha, R., et al., *Food sources of individual plasma phospholipid trans fatty acid isomers: the Cardiovascular Health Study.* The American journal of clinical nutrition, 2010. **91**(4): p. 883-93.
18. Van Camp, D., N.H. Hooker, and C.T. Lin, *Changes in fat contents of US snack foods in response to mandatory trans fat labelling.* Public health nutrition, 2012: p. 1-8.

19. Mozaffarian, D., M.F. Jacobson, and J.S. Greenstein, *Food reformulations to reduce trans fatty acids.* The New England journal of medicine, 2010. **362**(21): p. 2037-9.
20. Lemar, L. *Fatty Acid Data in the USDA National Nutrient Databank: Data handling and currency issues*. in *32nd Nutrient Databank Conference*. 2008. USDA.
21. Newby, P.K., et al., *Race and region are associated with nutrient intakes among black and white men in the United States.* The Journal of nutrition, 2011. **141**(2): p. 296-303.
22. Taylor, H.A., Jr., et al., *Toward resolution of cardiovascular health disparities in African Americans: design and methods of the Jackson Heart Study.* Ethnicity & disease, 2005. **15**(4 Suppl 6): p. S6-4-17.
23. Tucker, K.L., et al., *A regional food-frequency questionnaire for the US Mississippi Delta.* Public health nutrition, 2005. **8**(1): p. 87-96.
24. Carithers, T., et al., *Dietary assessment in African Americans: methods used in the Jackson Heart Study.* Ethnicity & disease, 2005. **15**(4 Suppl 6): p. S6-49-55.
25. Talegawkar, S.A., et al., *Total antioxidant performance is associated with diet and serum antioxidants in participants of the diet and physical activity substudy of the Jackson Heart Study.* The Journal of nutrition, 2009. **139**(10): p. 1964-71.
26. Taylor, H.A., Jr., *The Jackson Heart Study: an overview.* Ethnicity & disease, 2005. **15**(4 Suppl 6): p. S6-1-3.
27. Taylor, H.A., Jr., et al., *Dyslipidemia and the treatment of lipid disorders in African Americans.* The American journal of medicine, 2009. **122**(5): p. 454-63.
28. *Summary of the second report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel II).* JAMA : the journal of the American Medical Association, 1993. **269**(23): p. 3015-23.
29. Carpenter, M.A., et al., *Laboratory, reading center, and coordinating center data management methods in the Jackson Heart Study.* The American journal of the medical sciences, 2004. **328**(3): p. 131-44.
30. Dubbert, P.M., et al., *Physical activity assessment methods in the Jackson Heart Study.* Ethnicity & disease, 2005. **15**(4 Suppl 6): p. S6-56-61.
31. Smitherman, T.A., et al., *Validation of the Jackson Heart Study Physical Activity Survey in African Americans.* Journal of physical activity & health, 2009. **6 Suppl 1**: p. S124-32.
32. Hertogh, E.M., et al., *Validity of the modified Baecke questionnaire: comparison with energy expenditure according to the doubly labeled water method.* The international journal of behavioral nutrition and physical activity, 2008. **5**: p. 30.
33. Gillum, R.F., M.E. Mussolino, and J.H. Madans, *Coronary heart disease risk factors and attributable risks in African-American women and men: NHANES I epidemiologic follow-up study.* American journal of public health, 1998. **88**(6): p. 913-7.
34. National High Blood Pressure Education Program. and National Heart Lung and Blood Institute., *Primary prevention of hypertension : clinical and public health advisory from the National High Blood Pressure Education Program*. NIH publication2002, Bethesda, Md.: National Institutes of Health, National Heart, Lung, and Blood Institute. vi, 17 p.
35. National High Blood Pressure Education Program. and National High Blood Pressure Education Program. Working Group on Hypertension and Chronic Renal Failure., *National High Blood Pressure Education Program (NHBPEP) working group report on hypertension and chronic renal*. NIH publication1990, Bethesda, Md.: U.S. Dept. of Health and Human Services, Public Health Service, National Institutes of Health, National Heart, Lung, and Blood Institute. viii, 20 p.

36. National High Blood Pressure Education Program. Working Group on Ambulatory Blood Pressure Monitoring. and National Heart Lung and Blood Institute., *National High Blood Pressure Education Program (NHBPEP) Working Group report on ambulatory blood pressure monitoring*. NIH publication1990, Bethesda, Md.: U.S. Dept. of Health and Human Services, Public Health Service, National Institutes of Health, National Heart, Lung, and Blood Institute. vi, 21 p.
37. National High Blood Pressure Education Program. Working Group on Hypertension in Diabetes., *National High Blood Pressure Education Program Working Group report on hypertension in diabetes*, in *NIH publication no 94-3530*1994, U.S. Dept. of Health and Human Services, Public Health Service, National Institutes of Health, National Heart, Lung, and Blood Institute,: Bethesda, Md.
38. Taylor, H., et al., *Distinct component profiles and high risk among African Americans with metabolic syndrome: the Jackson Heart Study.* Diabetes care, 2008. **31**(6): p. 1248-53.
39. *Standards of medical care in diabetes.* Diabetes care, 2004. **27 Suppl 1**: p. S15-35.
40. Szabo, E., et al., *trans Octadecenoic acid and trans octadecadienoic acid are inversely related to long-chain polyunsaturates in human milk: results of a large birth cohort study.* The American journal of clinical nutrition, 2007. **85**(5): p. 1320-6.

A Linkage Analysis of Serum Lipids in African Americans: The Jackson Heart Study

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Abstract

The molecular and biochemical foundations of serum lipids are complex. The goal of our study was to identify genomic regions that influence lipids, including high density lipoprotein (HDL-C), low density lipoprotein (LDL-C), total cholesterol (TC) and triglyceride (TG) concentrations in a cohort of African Americans. We conducted heritability and linkage analysis from 264 pedigrees of 1501 African Americans in the Jackson Heart Study Family Sub-study (JHS-FSS). Heritability Analysis was done with a variance components method using two models; 1) adjusted for age, body mass index (BMI) and sex, 2) adjusted for these variables, plus dietary fiber, total saturated fat, total trans fat and polyunsaturated fat. Linkage analysis was completed using a sib-pair, Haseman-Elston Revisited Regression Analysis on 882 full and half sib-pairs in the JHS-FSS. The highest heritability estimates were h^2= 0.50 (model 1) for TC and LDL-C and h^2=0.51(model 2) for TC. All heritability estimates were modestly improved with the addition of diet, with the exception of TG, for which the heritability estimate decreased (h^2=0.40 (model 1), h^2=0.38 (model2)). The strongest LOD score associated with HDL-C was 5.1 and was located on chromosome 8. There was a LOD peak of 3.8 on chromosome 3 associated with the LDL-C. The strongest LOD score for TG was 4.9, located on chromosome 3. In total, we found six regions with LOD scores above 3.6 associated with serum lipids. Our findings suggest there is a strong genetic contribution to HDL-C, LDL-C, TG and TC in African Americans.

Keywords: Linkage, Heritability, Genetics, HDL-C-C, LDL-C, Triglyceride, African Americans

Introduction

Dyslipidemia is a major risk factor for cardiovascular disease (CVD). Lipid profiles consisting of high density lipoprotein (HDL-C), low density lipoprotein (LDL-C), triglycerides (TG) and total cholesterol (TC) concentrations are routine panels performed clinically for prevention and management of cardiovascular disease and risk. Numerous studies have been conducted on the determinants of serum lipid profiles [24, 25, 36]. Research on environmental contributors includes dietary factors like fiber, saturated fat, monounsaturated fat, specific polyunsaturated fatty acids and alcohol as key determinants [60, 91, 147, 148]. Research in the field of genetics and genomics has identified multiple genetic variants associated with various lipid phenotypes [76, 149-151]. Furthermore, nutrigenomics literature pinpoints the dynamic relationship between genes and the environment. Specifically, interactions between single nucleotide polymorphisms of lipid metabolism and reverse cholesterol transport have been shown to affect the relationship between dietary fat and serum lipids [15-17, 73, 81-83, 152]. Although the literature on the genetic determinants of lipids and their interactions is growing, the research on these genetic variants, their frequency, interactions and effect in African Americans is limited.

African Americans have high rates of cardiovascular and related diseases. National statistics put both morbidity and mortality from certain cardiovascular diseases higher in African Americans than in their white counterparts [2, 3, 89, 129, 153, 154]. Meanwhile, the National Health and Nutrition Evaluation Survey (NHANES) showed serum HDL-C higher in African Americans than in whites [24, 153]. The higher concentration of HDL-C, a cardio-protective factor, in African Americans is thought to be genetic, but has not

been proven. The lack of conclusive findings highlights the need for genetic research on dyslipidemia in this population.

The genetic components of dyslipidemia are complex. Heritability, linkage, single nucleotide polymorphism (SNP) association, genome-wide association studies (GWAS), fine mapping and admixture mapping are just a few of the tools scientists have used to understand this relationship. In this GWAS era, genetic discovery has focused on exploration. However, issues of multiple comparisons and failure to replicate SNP associations are a limitation of the GWAS approach. It is postulated that combinations of all tools are necessary for conveyance of the full picture. Thus, in addition to GWAS, which provides considerably more power in identifying SNPs in unrelated individuals, heritability, linkage, fine mapping and admixture mapping may all contribute different pieces of information.

In 2006, the Lancet published a series of papers on genetic epidemiology[86]. The first paper provided a road map to approaching genetic analysis of complex diseases. The article suggests a series of questions to inform the approach. These questions include 1) asking if there is a genetic component to disease, 2) in what region of the genome is the genetic component located 3) investigating the relationship between proposed genes and markers in those regions and their associations with phenotypes of interest [86].

The aim of this study is to address two of those questions for serum lipids in African Americans, "1) is there a genetic component to disease? and 2) where in the genome is the genetic component located?". We approach these questions by employing heritability analysis to determine the presence of a genetic component and linkage analysis to help

identify genomic regions of interest. In addition, both heritability and linkage were done using multiple models, those that included dietary covariates and models without dietary covariates.

Materials and Methods

Study Population

We evaluated data from the Jackson Heart Study (JHS), a cohort of 5,302 African Americans from Jackson, MS. JHS participants were recruited between 2000 and 2004 for the single site prospective cohort. Participant recruitment included random selection, volunteer participation, recruitment of family members, and enrollment of current participants of the Jackson, MS cohort of the Atherosclerosis Risk in Communities study (ARIC). ARIC participants comprise 31% of the total JHS cohort. Participant ages ranged from 25-85 y. Detailed descriptions of the design and recruitment for the JHS are described elsewhere [127, 155]. Institutional review board approval was provided by the University of Mississippi Medical Center. This secondary data analysis was approved under the institutional review board of the Tufts Medical Center..

JHS Family Sub-study

Pedigree data consist of 264 families and 1,501 individuals with genetic data. The pedigree structure is complex, with families of various sizes and inclusion of half siblings. Within these complex family structures, there were 689 full sibships and 193 half sibships. Data were stored in the Progeny 2000 software package and checked for single marker errors, mendelian inconsistencies, pedigree loops, incompatible age, identification errors and other detectible errors [156].

Microsatellite Data

Genotyping was done by the Mammalian Genotyping Service (MGS) (NHLBI, Bethesda, MD (*http://research.marshfieldclinic.org*)). A total of 374 short tandem repeat polymorphisms were genotyped in 1,501 participants using MGS screening set 16. Results for one participant were excluded because of poor quality. The resulting 1500 participants constitute the family study component of the JHS used in this analysis. The gender averaged mean inter-marker spacing was 9.3cM and average heterozygosity was 0.76.

Lipids

Blood samples were collected during baseline clinic interviews. Participants were requested to fast for 12 h prior to the interview. Lipid concentrations were determined at the University of Minnesota Medical Center, Fairview. Lipid assays were standardized through the Lipid Standardization Program of the Centers for Disease Control and Prevention, Atlanta, Ga. [31]. Details regarding the methods and storage of lipid assays have been previously described [36, 111].

Dietary Intake Assessment

Dietary variables were obtained from a culturally-specific, interviewer administered food frequency questionnaire (FFQ). Variables, alcohol intake, dietary cholesterol, dietary fiber, dietary saturated fat, dietary polyunsaturated fat, total dietary fat and energy were represented as grams/day. The 158-item short JHS FFQ (SFFQ) is a shortened version of a 283-item FFQ (LFFQ), originally developed for use in the US Mississippi Delta with 24h recall data from the Mississippi Delta region. This SFFQ was developed specifically

for JHS to alleviate overall participant burden during the baseline study visit. The development and validation of this regional SFFQ is described elsewhere [43, 97-99, 128].

Covariates

Data for self-reported age, sex and smoking status (used in heritability analysis) were provided by interviewer-based questionnaires administered at baseline. Body mass index (BMI) was calculated from heights and weights obtained by clinic trained nursing staff at baseline. Anthropometric measurements were obtained from trained clinic staff with participants in study administered hospital gowns without their shoes. Detailed information regarding the anthropometric measurements and other study covariates was previously reported (24- 26). Age was determined from self-reported date-of-birth. Pack years (used in linkage analysis) was computed by multiplying years smoked by the result of the number of cigarettes smoked per day divided by 20. For persons classified as having never smoked, a pack year value of zero was assigned. Both alcohol intake (g/day from SFFQ) and pack years lacked normality. The logarithm of the sum of alcohol +10 was used to transform alcohol intake. Similarly, a logarithm of the sum of pack years + 100 was used to transform the pack years variable. Transformed values of pack years and alcohol intake were used as covariates.

Statistical Analysis

T tests were used to assess differences between men and women for covariates examined. A p-value <0.05 was considered statistically significant.

Heritability Analysis

Using Solar 2.1.4 genetics software, a variance components method was used to estimate heritability. The phenotypes investigated included HDL-C, LDL-C, TG, and TC. Age, sex, BMI and waist circumference (WC) were considered the first set of covariates. Waist circumference and BMI were not used in the same model; but instead used in separate models. Additional analyses which adjusted for dietary covariates in addition to the first set of covariates were also completed. Dietary covariates included energy (kcal), dietary cholesterol, saturated fat intake, poly-unsaturated fat intake (PUFA) and dietary fiber. A Bonferonni correction 0.05/4 was applied to adjust for multiple comparisons, resulting in a p-value <0.0125 being considered statistically significant.

Linkage Analysis

S.A.G.E. statistical genetics software (Case Western Reserve University, 2005) was used to create multipoint estimates of allele sharing identical by descent (IBD) and to perform linkage analysis based on full and half-sib pairs. The statistical method used for the multipoint linkage analysis was Haseman-Elston Revisited Regression analysis (Elston, 2000). Linkage was completed for four phenotypes, HDL-C, LDL-C, TG and TC. A logarithm base 10 transformation was used to normalize the TG phenotype. Three separate models 1) no adjustment 2) adjusted for age, sex, BMI, cholesterol lowering medications, pack-years and alcohol intake (g) and 3) additionally adjusted for total energy, fiber, dietary cholesterol and dietary fat were considered. A LOD >2.2 was considered suggestive of linkage, a LOD >3.0 was considered significant linkage, a LOD >3.6 was considered highly significant.

Results

Descriptive Characteristics

Characteristics of the study population are shown in **Table 1**. Statistically significant differences between men and women were found for BMI, HDL-C and total cholesterol. All other characteristics between men and women were not significantly different.

Heritability

Results from heritability analysis are presented in **Table 2**. All of the lipid phenotypes, adjusted for BMI or WC and with adjustment including or excluding diet, were significantly heritable. Adjustment for waist circumference slightly increased heritability for HDL-C and TG when used instead of BMI. The largest difference between inclusion of BMI or WC was for HDL-C with h^2=0.44 (BMI) and h^2=0.49 (WC). HDL-C heritability estimates also increased when dietary variables were added to the model (h^2= 0.49 with BMI and diet and h^2=0.51 with WC and diet). All models adjusted for diet had slightly higher heritability estimates, with the exception of TG. The estimated genetic component for TG decreased with inclusion of diet in the model. TC and LDL-C consistently had the highest heritability, h^2= 0.50 when diet was excluded from the model, h^2=0.51 when diet was included.

Linkage

Results using previously mentioned thresholds are presented in **Table 3**. **Figures 1-3** graphically display the linkage results with LOD >2.2 for HDL-C, LDL-C, and TG. **Table 4** shows results with linkage peaks >3.0 for all three models. Chromosome 3 had the most peaks with LOD >3.0. Our strongest evidence for linkage in HDL-C was LOD score=5.1 (chromosome 8, 99.4cM); LDL-C, a LOD=3.8 (chromosome 3, 122cM); TG,

LOD score= 4.9 (chromosome 3, 147.1cM); and TC, LOD score = 3.4 (chromosome 5, 86cM). For HDL-C, the maximum LOD spanned 20 cM; LDL-C, 28cM; TG, 56cM; and TC 52cM. Table 5 contains candidate genes and quantitative trait loci (qtl) located within or near our regions of significant linkage (LOD>3.0). There were several peaks identified in chromosome 3. Table 6 lists candidate genes at or near the 3q27 region in chromosome 3.

Discussion

These results suggest that there is a significant genetic component to serum lipids in African Americans. Serum TG concentration was the only phenotype whose genetic component decreased with the addition of diet to the analysis. Previous research has suggested that TG is more affected by diet in comparison with LDL-C and HDL-C. Thus, the decrease in heritability for TG with the inclusion of diet is consistent with what is known. HDL-C had the greatest change in heritability by adjusting for diet, although this change was small. This suggests there is possible confounding by diet in the relationship between genetics and serum HDL-C.

Few studies have used linkage analysis to identify genetic regions associated with cholesterol in African Americans. The Sea Islands Genetic African American Registry (Project SUGAR) investigated the linkage of lipoprotein subclasses in Gullah Speaking African Americans. The Gullah population is an ethnically distinct population of African Americans residing in the Carolina outer banks[157]. The population is believed to have had little migration and less admixture than other African American populations. This study found heritability estimates as high as 0.56 for VLDL-C and LOD scores as high as

3.0 for small LDL-C particle concentration. The study identified a significant linkage peak located at 6p24 for small LDL-C concentration. Our results show significant linkage on chromosome 6 on the q arm of the chromosome for LDL-C, but no peak in the 6p24 region previously reported[157].

We found multiple points of linkage for TG, HDL-C and LDL-C. In total we found 18 regions of genetic linkage with serum lipid concentrations in African Americans. TC had the fewest linkage peaks, with only one peak on chromosome 5 exceeding the 3.0 threshold. We saw variability in linkage results by modeling of traits. Inclusion of diet and other covariates did not have consistent results on linkage outcomes. Given the knowledge of the different ways diet affects serum lipids and TG concentrations, the differential effects of inclusion of dietary covariates on linkage results can be expected. Furthermore, nutrients, in particular fatty acids, are thought to influence serum lipids by affecting cellular signaling, transcription and translation of proteins, cellular membrane integrity and transport of molecules throughout the body[14-16, 82, 85]. As a result of the different individual nutrients, the different biochemical and physiologic affects, the different relationships between each nutrient with specific genetic components and the synergy and interactions between each of the components, interpretation of results can be complex.

Focusing on genomic regions that showed linkage across all models, we had 12 regions of consistent linkage. There were multiple regions of linkage on chromosome 3. Previously identified qtl on chromosome 3 include blood pressure 30 (*BP30*), blood pressure 51 (*BP51*), body fluid distribution 1(*BFD1*), body weight 53(*BW53*), body

weight 24 (*BW24*), body weight (*BW23*), body weight 5 (*BW5*), and body weight 14 (BW14).

Chromosome 3 Blood Pressure Genes and QTLs

There are several genes and QTLs associated with blood pressure on chromosome 3 near to the region of linkage found in our study. These include *BP30*, *BP51* and *BFD*1. Of these three, *BFD1*, a qtl related to body fluid distribution, was specifically related to hypertension in African Americans. The body fluid distribution locus is associated with biologic impedance, measured as the ratio of extracellular fluid volume (ECF)/ total body water (TBW). Kotchen et al showed that ECF/TBW ratio was greater in hypertensive than normotensive subjects [158]. In addition they found significant correlations between ECF/TBW and both systolic and diastolic blood pressure. These findings indicate higher extracellular volume in persons with hypertension. Extracellular fluid volume is associated with solute concentration, in particular salt concentration, and movement in and out of cells. Higher extracellular fluid volume could indicate higher concentration of salt in the extracellular volume. These findings have been attributed to diuretic sensitive and possibly salt-sensitive hypertension, a condition reported in >50% of African American hypertensive patients [159]. Kotchen et al highlight that some of the variants and qtl detected have been found in obesity studies, but not in the absence of hypertension, suggesting an obesity-associated hypertension.

Chromosome 3 Obesity Related Genes

In addition to the genes and qtl related to blood pressure, there are also genes and qtl related to body weight and obesity on chromosome 3 near 3q22. The body weight (BW)

qtl are a family of genetic loci across chromosomes associated with body weight that have been found in animal and human models. These qtl have been located on both chromosome 3 and chromosome 6, both of which are near regions of significant linkage in our study. Wu et al found strong linkage to BMI on Chromosome 3 near 3q22.1 in the GENOA African American sample [160]. The *BW23* and *BW24* qtl are located in this region and may be the cause of linkage. This was the only region with significant linkage in their single genome scan. After combining IBD analyses, the study also found an additional peak 30cM away, near 3q27.

This region on chromosome 3 has been confirmed in several studies and is associated with multiple traits associated with metabolic syndrome. Vionnet et al. [161] reported significant linkage at the 3q27 region with diabetes and glucose intolerance in white Frenchman. In a genome scan with white families, Kissebah et al also found linkage associated with metabolic syndrome at this location [162]. Possible candidate genes near this region include adiponectin, GLUT2 transporter, and Apolipoprotein D (*APOD*). *APOD* variants have also been found to be associated with obesity in other studies [163]. A description of these genes is presented in **Table 5**. *APOD* has the most direct association with serum lipids. *APOD* has also been found to be associated with neurogenerative processes in Alzheimer's disease [164-166]. Genetic variation in *APOD* has been reported to be associated with Alzheimer's disease in African Americans [167]. Furthermore, several genetic variants in *APOD* are found exclusively in populations with African ancestry [168].

It is not clear if the 3q27 peak overlaps the peak at 3q22.1 or if they are separate peaks. We also found a small peak near the 3q27 location for HDL-C (LOD = 1.4, 180cM) and

TG (LOD=1.5, 210 cM). These peaks did not meet our threshold for significance. In our unadjusted linkage analysis of HDL-C, the two peaks overlapped, spanning a region from 116cM to180cM. Adjustment for BMI, sex, age, smoking status and alcohol (g) intake shortened the range of linkage to 102cM to 126cM.

Relationship of variants to dyslipidemia

The JHS has high prevalence of obesity, diabetes and hypertension [36, 40, 155]. The clustering of obesity, hypertension, dyslipidemia and insulin resistance is a well-recognized phenomenon called metabolic syndrome. Speculation of a common etiologic cause for these traits in metabolic syndrome has been the focus of much research [169, 170]. Many of the candidate genes associated with metabolic syndrome have been associated with pathways regulating obesity, free fatty acid metabolism, insulin sensitivity, lipid metabolism and inflammation [171]. Furthermore, there have been reports of interconnected pathways and genetic linkage of disease traits only in the context of other related diseases. An example of this is with obesity-related hypertension, where certain qtl associated with obesity have been only found in the studies of hypertension [158]. These interconnected pathways are especially true for CVD and related diseases [159].

Several qtl on chromosome 3 were associated with regions of significant linkage that have only been found in other African American populations (*BFD1, BW23* and *BW24*). The function of these qtl in African Americans and their association with multiple risk factors for CVD warrant further investigation. Many of these qtl have only been found

only in the context of obesity [158]. However it is not clear if these qtl are obesity related, or associated with obesity related conditions.

In our study we used the Haseman-Elston Regression method to complete the linkage analysis. Variance component analysis is another common approach to linkage analysis. Given low reproducibility of results from linkage, replication of the analysis using a variance component approach is important to confirm these results. Subsequent research using other genetic approaches, fine mapping and association analysis of genetic variants within the confirmed linkage regions is also needed is needed to further understanding. Furthermore, it is important confirm results in other populations, including other African American cohorts.

Table 1. Descriptive Statistics of JHS Family Study Participants

	Women		Men	
Phenotype	Mean	SD	Mean	SD
Age (years)	55.3	12.7	54	13
BMI*	32.7	7.7	30.0	6.1
Waist Circumference (CM)	100	17	101	15
LDL –C	126	37	129	39
HDL –C*	55.0	14.6	45.9	12.6
TG	101	65	115	91
Total Cholesterol*	201	40	197	40

BMI, Body Mass Index; LDL-C, low density lipoprotein concentration; HDL-C, high density lipoprotein concentration; TG, triglyceride concentration; SD, standard deviation
Differences between men and women were computed using the general linear models procedure in SAS 9.2
*indicates statistically significant differences between men and women $p<0.05$

Table 2. Heritability of serum lipid concentrations with and without adjustment for dietary variables in JHS family study participants.

Phenotype	h^2 (No Diet)		P-value	h^2 (Diet)		P-value
	BMI	WC		BMI	WC	
HDL-C	.44	.49	<.0001	.49	.51	<.0001
LDL-C	.50	.50	<.0001	.50	.51	<.0001
TG	.40	.42	<.0001	.38	.41	<.0001
Total Cholesterol	.50	.50	<.0001	.51	.51	<.0001

JHS, Jackson Heart Study; WC= waist circumference; BMI= body mass index; HDL-C, high density lipoprotein concentration; LDL-C, low density lipoprotein concentration; TG, triglyceride concentration
All models adjusted for age, and sex. Dietary models were adjusted for energy, dietary fiber, saturated fatty-acid, polyunsaturated fatty-acid and dietary cholesterol.
P-vlaue <0.04 was considered statistically significant.

Table 3. Chromosome and centimorgan position of (cM) linkage peaks with LOD score >2.2 for lipid phenotypes in JHS Family Study participants

Phenotype	CHR	cM	LOD	Model	Closest Marker
HDL	1	247.2	2.7	2	D1S3462
HDL	1	72.6	2.5	3	D1S3721
HDL	1	48	3.0	1	Chr1_48.0
HDL	1	72.6	4.5	1	D1S3721
LDL	1	175.6	2.5	2	D1S1677
LDL	1	175.6	2.5	3	D1S1677
LDL	1	170.8	3.1	1	D1S1679
TG	1	265.5	2.5	1	D1S1594
HDL	2	27.6	2.4	1	D2S1400
HDL	2	38.3	2.9	1	D2S1360
HDL	2	85.5	2.2	1	D2S1772
HDL	2	90.8	2.2	2	D2S1394
HDL	2	85.5	2.5	3	D2S1772
TG	2	55.5	3.3	2	D2S1788
TG	2	55.5	4.2	3	D2S1788
TG	2	67.6	3.2	3	ATA47CC04
TG	2	238	7.0	3	CHR2_238.0
TG	2	55.5	2.7	1	D2S1788
TG	2	236	5.5	1	CHR2_236.0
HDL	3	32	2.2	1	CHR3_30.0
HDL	3	164	2.8	1	CHR3_164.0
LDL	3	98.2	3.2	2	D3S3039
LDL	3	122	3.8	2	CHR3_122.0
LDL	3	98.2	3.1	3	D3S3039
LDL	3	122	3.8	3	CHR3_122.0
LDL	3	98.2	3.2	1	D3S3039
LDL	3	124	2.3	1	CH3_124.0
TG	3	52.4	3.1	2	D3S2432
TG	3	147.1	4.8	2	D3S1764
TG	3	52.4	3.0	3	D3S2432
TG	3	147.1	4.9	3	D3S1764
TG	3	146	2.3	1	CHR3_146.0
TG	4	136	2.8	2	CHR4_136
TG	4	158	3.4	2	D4S1629
TG	4	138	2.6	3	CHR4_138
TG	4	158	3.6	3	D4S1629
TG	4	138	4.7	1	CHR4_138.0
HDL	5	59.3	4.2	1	D5S1457
HDL	5	84	4.5	1	D5S1501
LDL	5	85.2	3.9	2	D5S1501
LDL	5	88	3.6	3	D5S1501
LDL	5	124.0	3.3	3	D5S1505
LDL	5	110	3.0	1	D5S1462
HDL	6	63.3	3.3	1	D6S1017
HDL	6	174	2.3	1	D6S1277
LDL	6	70	2.7	2	D6S2410
LDL	6	186	3.3	2	D6S1027
LDL	6	70	2.5	3	D6S2410
LDL	6	186	3.2	3	D6S1027
LDL	6	70	2.9	1	D6S2410
HDL	8	56	2.7	2	D8S1477
HDL	8	99.4	2.9	2	GATA8B01

HDL	8	56	2.7	3	D8S1477
HDL	8	99.4	3.9	3	GATA8B01
HDL	8	56	3.7	1	D8S1477
HDL	8	99.4	6.2	1	GATA8B01
TG	8	99.4	2.2	1	GATA8B01
TG	8	104	2.3	2	GAAT1A4
LDL	9	146.8	2.6	1	ATA63D01
TG	9	120	2.7	2	D9S930
TG	9	120	3.3	3	D9S930
TG	9	2	2.6	1	MFD455-AAT052
TG	9	128	2.5	1	D9S934
HDL	10	138.5	2.9	2	D10S1230
HDL	10	8	2.3	1	ATCC001
HDL	10	71.2	3.9	1	D10S1227
HDL	10	96.6	2.9	1	D10S2327
HDL	10	138.5	2.2	1	D10S1230
TC	10	28	3.2	1	D10S1430
HDL	11	0	3.4	3	D11S1984
TG	11	30.9	2.3	2	ATA24E08
TG	11	74	2.5	2	D11S2371
TG	11	28	2.9	3	ATA34E08
TG	11	74	3.0	3	D11S2371
TG	11	72	3.4	1	D11S2371
HDL	12	144	3.3	1	GATA21B11
HDL	12	48	3.4	2	D13S800
HDL	12	48	4.8	3	D13S800
HDL	12	46	5.0	1	D13S800
TG	12	8	2.5	1	D13S787
HDL	13	48	3.4	2	D13S800
HDL	13	48	4.8	3	D13S800
HDL	13	46	5.0	1	D13S800
TG	13	8	2.5	1	ATA5A09
HDL	14	93.1	2.5	1	D14S617
TG	14	100.7	2.5	2	D14S1434
TG	14	100.7	3.2	3	D14S1434
TG	14	93.1	4.4	1	D14S617
LDL	15	32.5	2.3	2	D15S659
LDL	15	32.5	2.3	3	D15S659
TG	15	41.3	3.6	2	D15S643
TG	15	40	3.2	3	D15S643
TG	15	41.3	4.2	1	D15S643
HDL	16	128.1	2.8	1	D16S2621
LDL	16	0	2.4	1	TTTA028
TG	16	122.4	2.5	3	D16S539
TG	16	78.8	3.0	1	AAT107
LDL	17	42	2.8	1	D17S2196
TG	17	81.4	2.4	2	D17S1290
TG	17	81.4	2.5	3	D17S1290
TG	17	0	2.3	1	D17S1308
HDL	18	4.1	2.6	1	ATA45G06
TG	18	4.1	3.6	1	ATA45G06
HDL	19	0	2.7	2	D19S591
HDL	20	35.4	3.7	1	D20S477
HDL	20	77.9	2.7	1	D20S451
LDL	20	67.8	2.5	1	D20S480

LDL	21	11.7	2.5	2	D21S2052
LDL	21	11.7	2.8	3	D21S2052
LDL	21	11.7	2.3	1	D21S2052
HDL	22	0	2.4	2	GATA198B05
HDL	22	0	2.4	3	GATA198B05

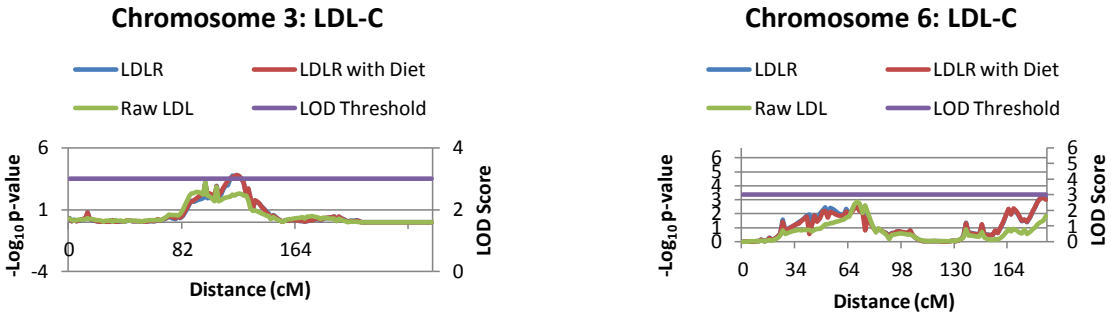
JHS, Jackson Heart Study; CHR, chromosome; cM, centimorgan; HDL, high density lipoprotein; LDL, low density lipoprotein, TG, triglycerides
Model 1: crude unadjusted model
Model 2: adjusted for age, sex, body mass index, smoking status
Model 3: adjusted for age, sex, body mass index, smoking status, energy, saturated fat, dietary fiber
In this study a LOD>2.2 indicated a region of interest, LOD >3.0 indicated significant linkage

Table 4. Linkage regions with LOD[1] >3.0 across all three models[2] and identified qtl and candidate genes in specified regions

Outcome	Chromosome	Position	Interval cM	LOD	Markers	Candidate Genes
TG	2	55.5	52-67.6	3.8*	D2S1788	*ABCG5*, *SOS1*, *CANDF1*, *LSL*, *SLEP2*, *BW53*, *OA2*, *STSL*, *CRIM1*
	3	52.4	50-73.1	3.1	D3S2432	*GPD1L*, *BP30*, *BFD1*, *GPD1L*, *AASTH43*, *SUSD5*, *TGFBR2*
	3	147.1	110-166	4.9*	D3S1764	*BW24*, *BW23*, *LOC100389498*, *RBP2*, *STAG1*, *RYK*
	4	158	122-167.5	3.5	D4S1629	*GALNTL6*, *OFC4*, *PDGFC*, *PDE4D*, *SPATA5*, *GUCY1A3*, *FSTL5*
	15	41.3	24-70	3.9*	D15S643	*FAM81A-like*, *CCPSO*, *KBTBD13*
LDL-C	3	98.2	84-110	3.2	D3S3039	*CT2*(Carnitine transporter 2), *BFD1*, *GXYLT2*
	3	122	110-138	3.8*	D3s3045	*BP51* (Blood pressure QTL 51), *MYH15*, *TRAT1*, *SLC12A8*
	6	186	160-187.2	3.3	D6S1027	*PLF*, *COPD25*, *SUMO4*, *BW5*, *BW14*, *PARK2*
HDL-C	8	99.4	92-112	5.1*	GATA8B01	*TMEM55A* (Transmembrane Protein 55A), *CNBD1*
	13	48	42-76	4.1*	D13S800	*KLF12*, *BRCA3*, *PCDH9*, *SCZD7*

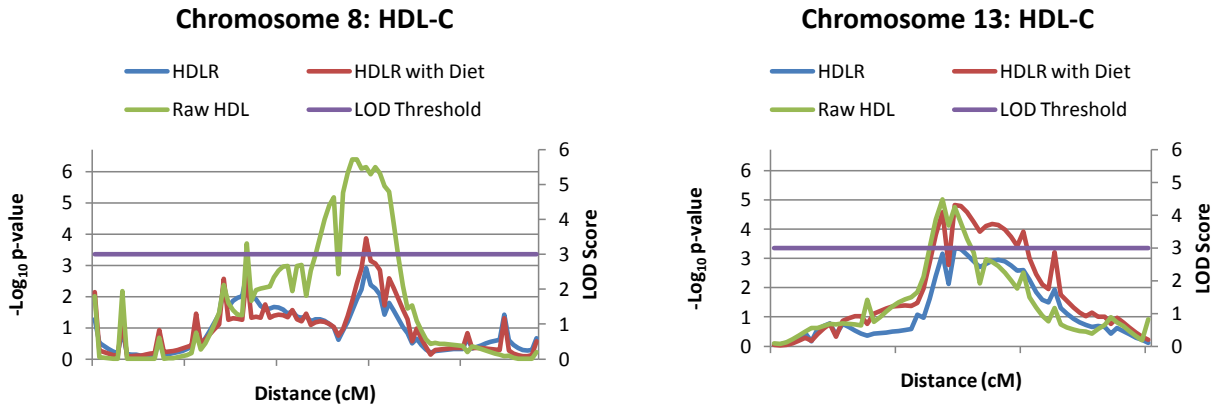
TG, triglycerides; LDL-C, low density lipoprotein concentration, HDL-C, high density lipoprotein concentration; TC total cholesterol
cM, centimorgan
[1] LOD>3.0 indicates significant linkage
[2] Models of association included: Model 1: crude unadjusted model; Model 2: adjusted for age, sex, body mass index, smoking status; Model 3: adjusted for age, sex, body mass index, smoking status, energy, saturated fat, dietary fiber
*Additionally met Kruglyak et al threshold of LOD>3.6 [172]

Figure 1. Graph of LOD scores by chromosome position in cM for three models of linkage (1. unadjusted, 2) adjusted for age, sex, bmi, , cholesterol lowering medication use, pack-years and alcohol intake , 3) adjusted for age, bmi, sex, cholesterol lowering medication use, pack-years and alcohol intake, energy, dietary fiber, dietary cholesterol and total dietary fat) of LDL-C in the Jackson Heart Study. Only results with LOD>3.0 for all three models are shown.



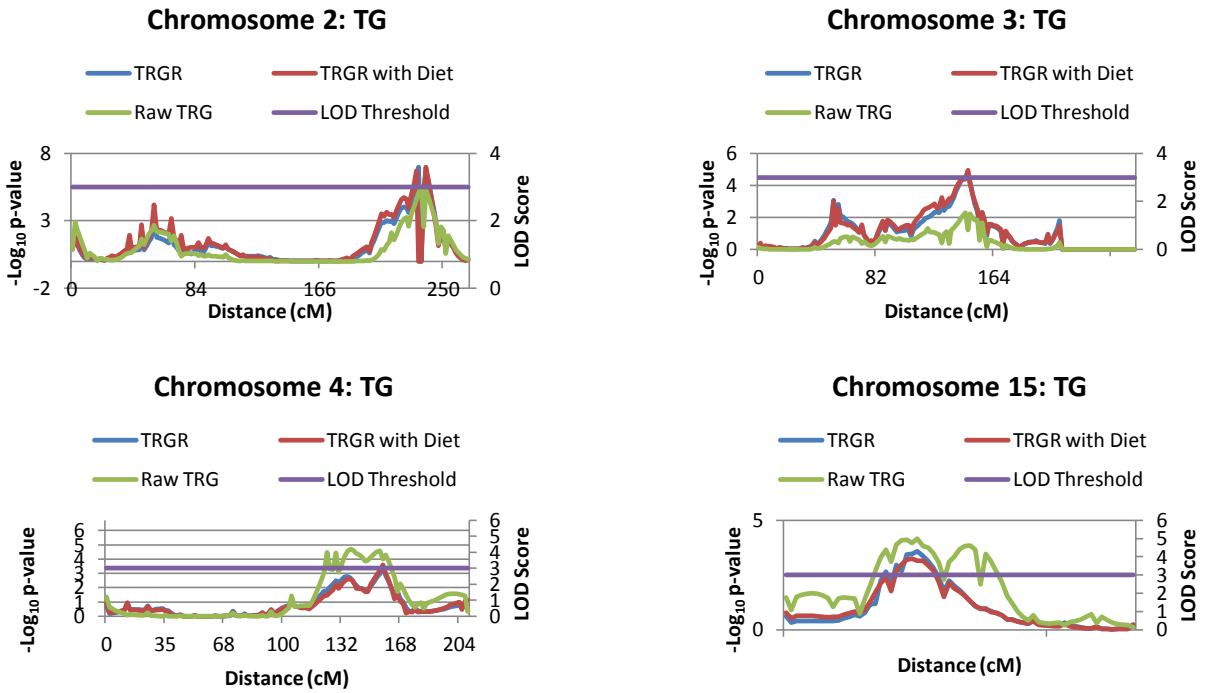
LDL-C, low density lipoprotein concentration; raw LDL, model 1; LDLR, Model2; LDLR with Diety, Model 3; LOD score, logarithm (base 10) odds; purple line indicates threshold of 3.0. ; cM, centimorgans

Figure 2. Graph of LOD scores by chromosome position in cM for three models of linkage (1. unadjusted, 2) adjusted for age, sex, bmi, cholesterol lowering medication use, pack-years and alcohol intake , 3) adjusted for age, bmi, sex, cholesterol lowering medication use, pack-years and alcohol intake, energy, dietary fiber, dietary cholesterol and total dietary fat) of HDL-C in the Jackson Heart Study. Only results with LOD>3.0 for all three models are shown.



HDL-C, high density lipoprotein concentration; raw HDL, Model 1;HDLR, Model2; HDLR with Diet, Model 3; LOD score, logarithm (base 10) odds; purple line indicates threshold of 3.0. ; cM, centimorgans

Figure 3. Graph of LOD scores by chromosome position in cM for three models of linkage (1. unadjusted, 2) adjusted for age, sex, bmi, cholesterol lowering medication use, pack-years and alcohol intake , 3) adjusted for age, bmi, sex, cholesterol lowering medication use, pack-years and alcohol intake, energy, dietary fiber, dietary cholesterol and total dietary fat) TG in the Jackson Heart Study. Only results with LOD>3.0 for all three models are shown.



TG, triglycerides; raw TRG, model 1; TRGR, Model2; TRGR with Diet, Model 3; LOD score, logarithm (base 10) odds; purple line indicates threshold of 3.0. ; cM, centimorgans

Table 5. Linkage Table of Candidate Genes and QTL Descriptions

Gene	Name	Location	Description [1]
LSL	Leptin serum levels of	2p21	Description not available
CRIM1	cysteine rich transmembrane BMP regulator 1	2p21	This gene encodes a transmembrane protein containing six cysteine-rich repeat domains and an insulin-like growth factor-binding domain. The encoded protein may play a role in tissue development though interactions with members of the transforming growth factor beta family, such as bone morphogenetic proteins. (provided by RefSeq) **Function**: May play a role in CNS development by interacting with growth factors implicated in motor neuron differentiation and survival. May play a role in capillary formation and maintenance during angiogenesis. Modulates BMP activity by affecting its processing and delivery to the cell surface
AASTH43	Allergic/atopic asthma related QTL 43		Description not available
SUSD5	sushi domain containing 5[1]		Unidentified human gene
TGFBR2	transforming growth factor, beta receptor II	3p22	This gene encodes a member of the Ser/Thr protein kinase family and the TGFB receptor subfamily. The encoded protein is a transmembrane protein that has a protein kinase domain, forms a heterodimeric complex with another receptor protein, and binds TGF-beta. This receptor/ligand complex phosphorylates proteins, which then enter the nucleus and regulate the transcription of a subset of genes related to cell proliferation. Mutations in this gene have been associated with Marfan Syndrome, Loeys-Deitz Aortic Aneurysm Syndrome, and the development of various types of tumors. Alternatively spliced transcript variants encoding different isoforms have been characterized. (provided by RefSeq) **Function**: On ligand binding, forms a receptor complex consisting of two type II and two type I transmembrane serine/threonine kinases. Type II receptors phosphorylate and activate type I receptors which autophosphorylate, then bind and activate SMAD transcriptional regulators. Receptor for TGF-beta
RBP2	retinol binding protein 2, cellular'	3q23	**RBP2** is an abundant protein present in the small intestinal epithelium. It is thought to participate in the uptake and/or intracellular metabolism of vitamin A. Vitamin A is a fat-soluble vitamin necessary for growth, reproduction, differentiation of epithelial tissues, and vision. **RBP2** may also modulate the supply of retinoic acid to the nuclei of endometrial cells during the menstrual cycle. (provided by RefSeq) **Function**: Intracellular transport of retinol
STAG1	stromal antigen 1	3q22.3	This gene is a member of the SCC3 family and is expressed in the nucleus. It encodes a component of cohesin, a multi-subunit protein complex that provides sister chromatid cohesion along the length of a chromosome from DNA replication through prophase and

			prometaphase, after which it is dissociated in preparation for segregation during anaphase. (provided by RefSeq) **Function**: Component of cohesin complex, a complex required for the cohesion of sister chromatids after DNA replication. The cohesin complex apparently forms a large proteinaceous ring within which sister chromatids can be trapped. At anaphase, the complex is cleaved and dissociates from chromatin, allowing sister chromatids to segregate. The cohesin complex may also play a role in spindle pole assembly during mitosis
RYK	**RYK** receptor-like tyrosine kinase	3q22.2	The protein encoded by this gene is an atypical member of the family of growth factor receptor protein tyrosine kinases, differing from other members at a number of conserved residues in the activation and nucleotide binding domains. This gene product belongs to a subfamily whose members do not appear to be regulated by phosphorylation in the activation segment. It has been suggested that mediation of biological activity by recruitment of a signaling-competent auxiliary protein may occur through an as yet uncharacterized mechanism. Two alternative splice variants have been identified, encoding distinct isoforms. (provided by RefSeq) **Function**: May be a coreceptor along with FZD8 of Wnt proteins, such as WNT1, WNT3, WNT3A and WNT5A. Involved in neuron differentiation, axon guidance, corpus callosum establishment and neurite outgrowth. In response to WNT3 stimulation, receptor C-terminal cleavage occurs in its transmembrane region and allows the C-terminal intracellular product to translocate from the cytoplasm to the nucleus where it plays a crucial role in neuronal development
OFC4	Orofacial cleft 4	4q21-q31	Description not available
PDGFC	platelet derived growth factor C[1]	4q32	The protein encoded by this gene is a member of the platelet-derived growth factor family. The four members of this family are mitogenic factors for cells of mesenchymal origin and are characterized by a core motif of eight cysteines. This gene product appears to form only homodimers. It differs from the platelet-derived growth factor alpha and beta polypeptides in having an unusual N-terminal domain, the CUB domain. Alternatively spliced transcript variants have been found for this gene. (provided by RefSeq) **Function**: Potent mitogen and chemoattractant for cells of mesenchymal origin. Binding of this growth factor to its affinity receptor elicits a variety of cellular responses. Appears to be involved in the three stages of wound healing: inflammation, proliferation and remodeling. Involved in fibrotic processes, in which transformation of interstitial fibroblasts into myofibroblasts plus collagen deposition occurs. Acts as a specific ligand for alpha platelet-derived growth factor receptor homodimer, and alpha and beta heterodimer. Binding to receptors induces their activation by tyrosine phosphorylation. The CUB domain has mitogenic activity in coronary artery smooth muscle cells, suggesting a role beyond the maintainance of the latency of the PDGF domain. In the nucleus, **PDGFC** seems to have additional function. Seems to be involved in palatogenesis

			(By similarity)
PDE4D	phosphodiesterase 4D, cAMP-specific[1]	5q12	This gene encodes one of four mammalian counterparts to the fruit fly 'dunce' gene. The encoded protein has 3',5'-cyclic-AMP phosphodiesterase activity and degrades cAMP, which acts as a signal transduction molecule in multiple cell types. This gene uses different promoters to generate multiple alternatively spliced transcript variants that encode functional proteins **Function**: Hydrolyzes the second messenger cAMP, which is a key regulator of many important physiological processes
Fam81A	family with sequence similarity 81, member A[1]	15q22.2	Description not available
CCPSO	Cataract, central pouch-like, with sutural opacities	15q21-q22	Description not available
KBTBD13	kelch repeat and BTB (POZ) domain containing 13	15q22.1	The gene belongs to a family of genes encoding proteins containing a BTB domain and several kelch repeats. The BTB domain functions as a protein-protein interaction module, which includes an ability to self-associate or to interact with non-BTB domain-containing proteins. The kelch motif typically occurs in groups of five to seven repeats, and has been found in proteins with diverse functions. Known functions of these family members include transcription regulation, ion channel tetramerization and gating, protein ubiquitination or degradation, and cytoskeleton regulation. The exact function of this family member has yet to be determined. (provided by RefSeq)
CT2	Carnitine Transporter-2	3	Description not available
BFD1	Body fluid distribution QTL 1	3	HTN related QTL in African Americans (kitchen, 2002)
GXYLT2	glucoside xylosyltransferase 2	3p13	**GXYLT2** is a xylosyltransferase (EC 2.4.2.-) that adds the first xylose to O-glucose-modified residues in the epidermal growth factor (EGF; MIM 131530) repeats of proteins such as NOTCH1 (MIM 190198) (Sethi et al., 2010 (PubMed 19940119)).(supplied by OMIM) **Function**: Glycosyltransferase which elongates the O-linked glucose attached to EGF-like repeats in the extracellular domain of Notch proteins by catalyzing the addition of xylose
BP51	Blood pressure QTL 51	3	GWAS of systolic and diastolic BP Quebec Study (Rice, 2000)
MYH15	Myosin, Heavy Chain	3q13.13	Myosins are a large family of motor proteins that share the common features of ATP hydrolysis, actin binding and potential for kinetic energy transduction. Originally isolated from muscle cells (hence the name),almost all eukaryotic cells are now known to contain myosins. Structurally, mysoins contain a head domain that binds to actin filaments (microfilaments) and is the site of ATP hydrolysis. The tail domain interacts with cargo molecules, and the neck acts as a linker between the head and tail and is the site of regulatory myosin light chain binding. There are 17 myosin families and the most well

			characterized is myosin II. Myosin II is found predominantly in myocytes and mediates plus-ended movement along microfilaments. It is involved in muscle contraction through cyclic interactions with actin-rich thin filaments, creating a contractile force. It is regulated by phosphorylation via myosin light chain kinase (MLCK) and by intracellular Ca2+ concentrations.
PLF	Pulmonary function	6q21-q22	Linkage and Association with pulmonary function Framingham Heart Study (Wilk, 2003).
COPD25	Chronic obstructive pulmonary disease QTL 25	6	Genetic Loci Influencing Lung Function: Framingham Heart Study (Joost, 2002).
TMEM55A	transmembrane protein 55A	8q21.3	**TMEM55A** catalyzes the degradation of phosphatidylinositol 4,5-bisphosphate (PtdIns-4,5-P2) by removing the 4-phosphate (Ungewickell et al., 2005 (PubMed 16365287)).(supplied by OMIM) **Function**: Catalyzes the hydrolysis of the 4-position phosphate of phosphatidylinositol 4,5-bisphosphate. Does not hydrolyze phosphatidylinositol 3,4,5-trisphosphate, phosphatidylinositol 3,4-bisphosphate, inositol 3,5-bisphosphate, inositol 3,4-bisphosphate, phosphatidylinositol 5-monophosphate, phosphatidylinositol 4-monophosphate and phosphatidylinositol 3-monophosphate
CNBD1	cyclic nucleotide binding domain containing 1[1]	8q21.3	Description not available
KLF12	Kruppel-like factor 12	13q22	Activator protein-2 alpha (AP-2 alpha) is a developmentally-regulated transcription factor and important regulator of gene expression during vertebrate development and carcinogenesis. The protein encoded by this gene is a member of the Kruppel-like zinc finger protein family and can repress expression of the AP-2 alpha gene by binding to a specific site in the AP-2 alpha gene promoter. Repression by the encoded protein requires binding with a corepressor, CtBP1. Two transcript variants encoding different isoforms have been found for this gene. (provided by RefSeq) **Function**: Confers strong transcriptional repression to the AP-2-alpha gene. Binds to a regulatory element (A32) in the AP-2-alpha gene promoter
BRCA3	breast cancer 3	13q21	Description not available
PCDH9	protocadherin 9	13q21.32	This gene belongs to the protocadherin gene family, a subfamily of the cadherin superfamily. The mRNA encodes a cadherin-related neuronal receptor that localizes to synaptic junctions and is putatively involved in specific neuronal connections and signal transduction. Sharing a characteristic with other protocadherin genes, this gene has a notably large exon that encodes six cadherin domains and a transmembrane region. Two alternatively spliced transcript variants encoding distinct isoforms have been found for this gene. (provided by RefSeq)

			Function: Potential calcium-dependent cell-adhesion protein
SCZD7	schizophrenia disorder 7[1]	13q32	Description not available
RA10	Rheumatoid arthritis QTL 10	5	Genome-wide screen of RA genes (Jawaheer, 2001)
EDIL3	EGF-like repeats and discoidin I-like domains 3	5q14	The protein encoded by this gene is an integrin ligand. It plays an important role in mediating angiogenesis and may be important in vessel wall remodeling and development. It also influences endothelial cell behavior. (provided by RefSeq) **Function**: Promotes adhesion of endothelial cells through interaction with the alpha-v/beta-3 integrin receptor. Inhibits formation of vascular-like structures. May be involved in regulation of vascular morphogenesis of remodeling in embryonic development

[1] Summary from Entrez[173]; Function from UniProtKB/Swiss-Prot[174], information from both sources provided by genecards[175];

Table 6: Possible Candidate Genes in the 3q27 region

Gene	Name	Location	Description[1]
ADIPOQ	Adiponectin	3q27	This gene is expressed in **adipo**se tissue exclusively. It encodes a protein with similarity to collagens X and VIII and complement factor C1q. The encoded protein circulates in the plasma and is involved with metabolic and hormonal processes. Mutations in this gene are associated with **adipo**nectin deficiency. Multiple alternatively spliced variants, encoding the same protein, have been identified. **Function**: Important **adipo**kine involved in the control of fat metabolism and insulin sensitivity, with direct anti-diabetic, anti-atherogenic and anti-inflammatory activities. Stimulates AMPK phosphorylation and activation in the liver and the skeletal muscle, enhancing glucose utilization and fatty-acid combustion. Antagonizes TNF-alpha by negatively regulating its expression in various tissues such as liver and macrophages, and also by counteracting its effects. Inhibits endothelial NF-kappa-B signaling through a cAMP-dependent pathway. May play a role in cell growth, angiogenesis and tissue remodeling by binding and sequestering various growth factors with distinct binding affinities, depending on the type of complex, LMW, MMW or HMW
SLC2A2	Glucose transporter 2	3q26.1	Glucose transporter 2 isoform is an integral plasma membrane glycoprotein of the liver, islet beta cells, intestine, and kidney epithelium. It mediates facilitated bidirectional glucose transport. Because of its low affinity for glucose, it has been suggested as a glucose sensor. (provided by RefSeq) **Function**: Facilitative glucose transporter. This isoform likely mediates the bidirectional transfer of glucose across the plasma membrane of hepatocytes and is responsible for uptake of glucose by the beta cells; may comprise part of the glucose-sensing mechanism of the beta cell. May also participate with the Na(+)/glucose cotransporter in the transcellular transport of glucose in the small intestine and kidney
APOD	Apolipoprotein- D	3q26	This gene encodes a component of high density lipoprotein that has no marked similarity to other **apolipoprotein** sequences. It has a high degree of homology to plasma retinol-binding protein and other members of the alpha 2 microglobulin protein superfamily of carrier proteins, also known as lipocalins. This glycoprotein is closely associated with the enzyme lecithin:cholesterol acyltransferase - an enzyme involved in lipoprotein metabolism. (provided by RefSeq) **Function**: APOD occurs in the macromolecular complex with lecithin-cholesterol acyltransferase. It is probably involved in the transport and binding of bilin. Appears

	to be able to transport a variety of ligands in a number of different contexts

[1] Summary from Entrez[173]; Function from UniProtKB/Swiss-Prot[174], information from both sources provided by genecards[175]

1. Sempos, C.T., et al., *Prevalence of high blood cholesterol among US adults. An update based on guidelines from the second report of the National Cholesterol Education Program Adult Treatment Panel*. JAMA : the journal of the American Medical Association, 1993. **269**(23): p. 3009-14.
2. Talayero, B.G. and F.M. Sacks, *The role of triglycerides in atherosclerosis*. Current cardiology reports, 2011. **13**(6): p. 544-52.
3. Taylor, H.A., Jr., et al., *Dyslipidemia and the treatment of lipid disorders in African Americans*. The American journal of medicine, 2009. **122**(5): p. 454-63.
4. Artaud-Wild, S.M., et al., *Differences in coronary mortality can be explained by differences in cholesterol and saturated fat intakes in 40 countries but not in France and Finland. A paradox*. Circulation, 1993. **88**(6): p. 2771-9.
5. Mensink, R.P., et al., *Effects of dietary fatty acids and carbohydrates on the ratio of serum total to HDL cholesterol and on serum lipids and apolipoproteins: a meta-analysis of 60 controlled trials*. The American journal of clinical nutrition, 2003. **77**(5): p. 1146-55.
6. Mozaffarian, D., *Low-fat diet and cardiovascular disease*. JAMA : the journal of the American Medical Association, 2006. **296**(3): p. 279-80; author reply 280-1.
7. Vafeiadou, K., et al., *A review of the evidence for the effects of total dietary fat, saturated, monounsaturated and n-6 polyunsaturated fatty acids on vascular function, endothelial progenitor cells and microparticles*. The British journal of nutrition, 2012. **107**(3): p. 303-24.
8. Ariza, M.J., et al., *Additive effects of LPL, APOA5 and APOE variant combinations on triglyceride levels and hypertriglyceridemia: results of the ICARIA genetic sub-study*. BMC medical genetics, 2010. **11**: p. 66.
9. Arya, R., et al., *Evidence of a novel quantitative-trait locus for obesity on chromosome 4p in Mexican Americans*. American journal of human genetics, 2004. **74**(2): p. 272-82.
10. Kathiresan, S., et al., *Common variants at 30 loci contribute to polygenic dyslipidemia*. Nature genetics, 2009. **41**(1): p. 56-65.
11. Waterworth, D.M., et al., *Genetic variants influencing circulating lipid levels and risk of coronary artery disease*. Arteriosclerosis, thrombosis, and vascular biology, 2010. **30**(11): p. 2264-76.
12. Campos, H., M. D'Agostino, and J.M. Ordovas, *Gene-diet interactions and plasma lipoproteins: role of apolipoprotein E and habitual saturated fat intake*. Genetic epidemiology, 2001. **20**(1): p. 117-128.
13. Corella, D. and J.M. Ordovas, *Nutrigenomics in cardiovascular medicine*. Circulation. Cardiovascular genetics, 2009. **2**(6): p. 637-51.
14. DeBusk, R.M., et al., *Nutritional genomics in practice: where do we begin?* Journal of the American Dietetic Association, 2005. **105**(4): p. 589-98.
15. Ordovas, J.M., *Nutrigenetics, plasma lipids, and cardiovascular risk*. Journal of the American Dietetic Association, 2006. **106**(7): p. 1074-81; quiz 1083.
16. Ordovas, J.M. and D. Corella, *Nutritional genomics*. Annual review of genomics and human genetics, 2004. **5**: p. 71-118.
17. Ordovas, J.M. and V. Mooser, *Nutrigenomics and nutrigenetics*. Current opinion in lipidology, 2004. **15**(2): p. 101-8.
18. Paoloni-Giacobino, A., R. Grimble, and C. Pichard, *Genetics and nutrition*. Clinical nutrition, 2003. **22**(5): p. 429-35.
19. Paoloni-Giacobino, A., R. Grimble, and C. Pichard, *Genomic interactions with disease and nutrition*. Clinical nutrition, 2003. **22**(6): p. 507-14.

20. Gillum, R.F., M.E. Mussolino, and J.H. Madans, *Coronary heart disease risk factors and attributable risks in African-American women and men: NHANES I epidemiologic follow-up study*. American journal of public health, 1998. **88**(6): p. 913-7.
21. Gillum, R.F., M.E. Mussolino, and C.T. Sempos, *Baseline serum total cholesterol and coronary heart disease incidence in African-American women (the NHANES I epidemiologic follow-up study). National Health and Nutrition Examination Survey*. The American journal of cardiology, 1998. **81**(10): p. 1246-9.
22. Metcalf, P.A., et al., *African American-white differences in lipids, lipoproteins, and apolipoproteins, by educational attainment, among middle-aged adults: the Atherosclerosis Risk in Communities Study*. American journal of epidemiology, 1998. **148**(8): p. 750-60.
23. Minino, A.M., *Death in the United States, 2009*. NCHS data brief, 2011(64): p. 1-8.
24. Minino, A.M., et al., *Deaths: final data for 2004*. National vital statistics reports : from the Centers for Disease Control and Prevention, National Center for Health Statistics, National Vital Statistics System, 2007. **55**(19): p. 1-119.
25. Minino, A.M., et al., *Death in the United States, 2007*. NCHS data brief, 2009(26): p. 1-8.
26. Burton, P.R., M.D. Tobin, and J.L. Hopper, *Key concepts in genetic epidemiology*. Lancet, 2005. **366**(9489): p. 941-51.
27. Taylor, H.A., Jr., et al., *Toward resolution of cardiovascular health disparities in African Americans: design and methods of the Jackson Heart Study*. Ethnicity & disease, 2005. **15**(4 Suppl 6): p. S6-4-17.
28. Wyatt, S.B., et al., *Prevalence, awareness, treatment, and control of hypertension in the Jackson Heart Study*. Hypertension, 2008. **51**(3): p. 650-6.
29. Fox, E.R., et al., *Epidemiology, heritability, and genetic linkage of C-reactive protein in African Americans (from the Jackson Heart Study)*. The American journal of cardiology, 2008. **102**(7): p. 835-41.
30. *Summary of the second report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel II)*. JAMA : the journal of the American Medical Association, 1993. **269**(23): p. 3015-23.
31. Carpenter, M.A., et al., *Laboratory, reading center, and coordinating center data management methods in the Jackson Heart Study*. The American journal of the medical sciences, 2004. **328**(3): p. 131-44.
32. Carithers, T.C., et al., *Validity and calibration of food frequency questionnaires used with African-American adults in the Jackson Heart Study*. Journal of the American Dietetic Association, 2009. **109**(7): p. 1184-1193.
33. Talegawkar, S.A., et al., *Total antioxidant performance is associated with diet and serum antioxidants in participants of the diet and physical activity substudy of the Jackson Heart Study*. The Journal of nutrition, 2009. **139**(10): p. 1964-71.
34. Talegawkar, S.A., et al., *Carotenoid intakes, assessed by food-frequency questionnaires (FFQs), are associated with serum carotenoid concentrations in the Jackson Heart Study: validation of the Jackson Heart Study Delta NIRI Adult FFQs*. Public health nutrition, 2008. **11**(10): p. 989-97.
35. Tucker, K.L., et al., *A regional food-frequency questionnaire for the US Mississippi Delta*. Public health nutrition, 2005. **8**(1): p. 87-96.
36. Champagne, C.M., et al., *Dietary intake in the lower Mississippi delta region: results from the Foods of our Delta Study*. Journal of the American Dietetic Association, 2004. **104**(2): p. 199-207.

37. Divers, J., et al., *The genetic architecture of lipoprotein subclasses in Gullah-speaking African American families enriched for type 2 diabetes: the Sea Islands Genetic African American Registry (Project SuGAR)*. Journal of lipid research, 2010. **51**(3): p. 586-97.
38. Ordovas, J.M., *The quest for cardiovascular health in the genomic era: nutrigenetics and plasma lipoproteins*. The Proceedings of the Nutrition Society, 2004. **63**(1): p. 145-52.
39. Talmud, P.J. and D.M. Waterworth, *In-vivo and in-vitro nutrient-gene interactions*. Current opinion in lipidology, 2000. **11**(1): p. 31-6.
40. Kotchen, T.A., et al., *Identification of hypertension-related QTLs in African American sib pairs*. Hypertension, 2002. **40**(5): p. 634-9.
41. Kraja, A.T., et al., *Genetics of hypertension and cardiovascular disease and their interconnected pathways: lessons from large studies*. Current hypertension reports, 2011. **13**(1): p. 46-54.
42. Wu, X., et al., *A combined analysis of genomewide linkage scans for body mass index from the National Heart, Lung, and Blood Institute Family Blood Pressure Program*. American journal of human genetics, 2002. **70**(5): p. 1247-56.
43. Vionnet, N., et al., *Genomewide search for type 2 diabetes-susceptibility genes in French whites: evidence for a novel susceptibility locus for early-onset diabetes on chromosome 3q27-qter and independent replication of a type 2-diabetes locus on chromosome 1q21-q24*. American journal of human genetics, 2000. **67**(6): p. 1470-80.
44. Kissebah, A.H., et al., *Quantitative trait loci on chromosomes 3 and 17 influence phenotypes of the metabolic syndrome*. Proceedings of the National Academy of Sciences of the United States of America, 2000. **97**(26): p. 14478-83.
45. Vijayaraghavan, S., G.A. Hitman, and P.G. Kopelman, *Apolipoprotein-D polymorphism: a genetic marker for obesity and hyperinsulinemia*. The Journal of clinical endocrinology and metabolism, 1994. **79**(2): p. 568-70.
46. Boyles, J.K., L.M. Notterpek, and L.J. Anderson, *Accumulation of apolipoproteins in the regenerating and remyelinating mammalian peripheral nerve. Identification of apolipoprotein D, apolipoprotein A-IV, apolipoprotein E, and apolipoprotein A-I*. The Journal of biological chemistry, 1990. **265**(29): p. 17805-15.
47. Boyles, J.K., et al., *Identification, characterization, and tissue distribution of apolipoprotein D in the rat*. Journal of lipid research, 1990. **31**(12): p. 2243-56.
48. Terrisse, L., et al., *Increased levels of apolipoprotein D in cerebrospinal fluid and hippocampus of Alzheimer's patients*. Journal of neurochemistry, 1998. **71**(4): p. 1643-50.
49. Desai, P.P., et al., *Genetic variation in apolipoprotein D affects the risk of Alzheimer disease in African-Americans*. American journal of medical genetics. Part B, Neuropsychiatric genetics : the official publication of the International Society of Psychiatric Genetics, 2003. **116B**(1): p. 98-101.
50. Desai, P.P., et al., *Genetic variation in the apolipoprotein D gene among African blacks and its significance in lipid metabolism*. Atherosclerosis, 2002. **163**(2): p. 329-38.
51. Taylor, H., et al., *Distinct component profiles and high risk among African Americans with metabolic syndrome: the Jackson Heart Study*. Diabetes care, 2008. **31**(6): p. 1248-53.
52. Hu, G., et al., *Prevalence of the metabolic syndrome and its relation to all-cause and cardiovascular mortality in nondiabetic European men and women*. Archives of internal medicine, 2004. **164**(10): p. 1066-76.
53. Sonnenberg, G.E., G.R. Krakower, and A.H. Kissebah, *A novel pathway to the manifestations of metabolic syndrome*. Obesity research, 2004. **12**(2): p. 180-6.
54. Avery, C.L., et al., *A phenomics-based strategy identifies loci on APOC1, BRAP, and PLCG1 associated with metabolic syndrome phenotype domains*. PLoS genetics, 2011. **7**(10): p. e1002322.

55. Kruglyak, L., et al., *Parametric and nonparametric linkage analysis: a unified multipoint approach*. American journal of human genetics, 1996. **58**(6): p. 1347-63.
56. NCBI, *Entrez > Gene*.
57. Institute, E.B., *UniProt*, 2012.
58. Science, W.I.o. *The GeneCards Human Gene Database*. GeneCards 1996-2012 [cited 2012 5/12/2012]; Available from: www.genecards.org.

The Association of Single Nucleotide Polymorphisms (SNPs) in *APOE, APOA5* and *PPARGC1A* with Lipid Phenotypes in African Americans: The Jackson Heart Study

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Abstract

Multiple single nucleotide polymorphisms (SNPs) in the lipid metabolism pathway have been identified. Some of these variations have been shown to be atherogenic. In many ways, research on the genetics of lipid metabolism has set the field for genetic epidemiology of CVD. The population-based approaches contributing to the field have yielded large numbers of SNPs. However, individual SNPs must be replicated and reproduced in different populations. We aimed to show the frequency of the homozygous dominant genotype of six SNPs in *APOE, APOA5* and *PPARGC1A*. In addition, we examined the association between these polymorphisms and serum lipids in a cohort of African Americans, in the Jackson Heart Study (JHS). We found frequencies of 59.1% (*APOE*, rs7412), 80.7% 3 (*APOE*, rs429358), 78.1% (*APOA5*, rs662799), 87.6% (*APOA5*, rs3135506), 87.1% (*PPARGC1A*, rs2970869), and 84.5% (*PPARGC1A*, rs10028665) for the homozygous dominant (HOD) genotypes. HDL-C was positively associated with the HOD and heterozygous (HET) genotypes for *APOA5*, rs3135506 and the homozygous dominant genotype (HOD) in *APOE*, rs7412 ($P<0.008$). LDL-C was negatively associated with the HOD in the *APOE*, rs7412 ($p<0.0001$). LDL-C was positively associated with the HOD in *APOE*, rs429358 ($p<0.0001$). TG was negatively associated with the HOD and HET for *APOA5*, rs3135506 ($p<0.0001$). In addition, we found a significant interaction between dietary fat intake and HOD genotype for *APOA5*, rs662799 for HDL-C. These findings may prove beneficial in our understanding of lipid metabolism.

Introduction

Serum lipids are a key component to the development of atherosclerosis. Specifically elevated high-density lipoprotein cholesterol (HDL-C) and reduced low-density lipoprotein cholesterol (LDL-C) and triglyceride (TG) concentrations are considered protective factors for cardiovascular disease (CVD) [24, 25, 31]. CVD is the number one cause of death in Americans. African Americans disproportionately suffer from CVD morbidity and mortality. The causes of this health disparity are not fully known, but both genetic and environmental contributors are believed to be involved.

Lipid Metabolism Genes

Research on genetic variation of lipid metabolism genes has proven fruitful with the discovery of highly replicated genes like *APOE* and gene clusters such as *APOA1 /C3/A4/A5*. Lipid metabolism and reverse cholesterol transport genes are some of the most studied. Genes such as *APOE*, *APOA5* and *PPARGC1A* are considered highly consistent in their associations with specific phenotypes [74-76]. The *APOE* gene is associated with the HDL-C and is a key regulator of TG. SNPs in this gene have been associated with hyperlipidemia, hypercholesterolemia, type 2 diabetes (T2D), coronary artery disease and both familial and sporadic forms of Alzheimer disease [77-79]. *APOA5* is a lipoprotein associated with HDL-C and VLDL-C and related to hypertriglyceridemia, hyperlipidemia and atherosclerosis. *APOA5* is a part of a gene cluster with *APOA1, APOC3* and *APO4*. Several SNPs inside this cluster have been associated with hypertriglyceridemia and atherosclerosis [176]. *PPARGC1A*, a transcription factor, is believed to be associated with adipocyte differentiation and glucose homeostasis. Studies suggest *PPARGC1A* plays a role in obesity, diabetes, atherosclerosis and cancer [177-179].

The presence and frequency of causal or protective genetic variants differ by ancestral population. These genotypic differences could contribute to phenotypic differences across populations. In addition to the frequency of genetic variants, other factors affect the relationship between genotype and phenotype across populations. Occurrences of recombination can differ across populations. Recombination events are focused in genetic hot spots. Recent studies have shown locations of recombination hot spots can differ by ethnicity [180, 181]. A study by Hinch (2011) showed unique factors affecting recombination events in African Americans [182]. In addition to the presence and frequency of genetic variants and recombination events, gene-gene and gene-environment interactions are associated with phenotypic differences between ethnic groups [183, 184].

Although much research has been done on the genetic variants associated with lipids, there is little research on these variants in African Americans. We aim to show the genotype frequency of six SNPs in the *APOA5, APOE* and *PPARGC1A* genes and their association with dietary fat intake and serum lipids in African Americans in the Jackson Heart Study (JHS).

Materials and Methods

Study Population

We evaluated data from 3,700 African Americans from the Jackson Heart Study, who consented to have their DNA used for genetic studies. Participants were recruited between 2000 and 2004 to participate in the single site prospective cohort. Participant recruitment included random selection, volunteer participation, recruitment of family members and enrollment of current participants of the Jackson, MS cohort of the

Atherosclerosis Risk in Communities study (ARIC). ARIC participants comprise 31% of the total JHS cohort. Participant ages ranged from 35-85 years, with younger and older adults included in the family sub-study. Detailed descriptions of the design and recruitment for the JHS are described elsewhere [100, 127].

Covariates

Data for self-reported age, sex, and smoking status were provided by interviewer-based questionnaires administered at baseline. Body Mass Index (BMI) was calculated from heights and weights obtained by clinic trained nursing staff at baseline as weight (kg)/height $(m)^2$. Physical activity score was based on a validated physical activity questionnaire given at baseline. Details on the development and validation of the physical activity questionnaire, as well as the other baseline data collection were previously described [106-108].

Diet

Dietary variables were obtained from a culturally specific interviewer administered food frequency questionnaire (FFQ). Alcohol intake, dietary cholesterol, dietary fiber, dietary saturated fat, dietary polyunsaturated fat, and total dietary fat were represented as grams/day. Energy was defined as kcal/day. The 158-item short JHS FFQ (SFFQ) is a shortened version of a 283-item FFQ (LFFQ), originally developed for use in the southern US, based on 24h recall data from the Mississippi Delta region. This SFFQ was developed specifically for JHS to alleviate overall participant burden during the baseline study visit. The development and validation of this regional SFFQ has been reported [43, 97-99, 128].

Lipids

Blood samples were collected during baseline clinic interviews. Participants were requested to fast for 12 h prior to the clinic interview. Lipid concentrations were determined at the University of Minnesota Medical Center, Fairview. Lipid assays were standardized through the Lipid Standardization Program of the Centers for Disease Control and Prevention, Atlanta, Ga. [31]. Details regarding the methods and storage of lipid assays were previously reported [36, 111].

Selection of Single Nucleotide Polymorphisms

Genes and SNPs were selected as a result of previous research on gene-nutrient interactions involving the lipid metabolism pathway [14, 73, 81, 83-85, 184]. Pairs of SNPs in three different genes believed to be involved in interactions between lipid metabolism and dietary fat were selected. In addition, previous bioinformatic analysis in other populations helped to inform SNP selection.

Genotyping

SNPs were examined using TaqMan SNP genotyping assays. TaqMan assay uses a hybridized probe where fluorogenic and quencher tags are cleaved by the 5'nuclease activity of TaqDNA polymerase during polymerase chain reaction (PCR) amplification. This cleavage produces fluorescence. Two probes were used, each specific to an allele of the SNP and labeled with different tags, thus, allowing both alleles to be detected in a single tube. Allele specific TaqMan probes were used with assays purchased from Applied Biosystems (Applied Biosystems, Foster City, CA). For each SNP genotyped, 10 ng of DNA was diluted and aliquoted into 96 well plates. A RapidPlate robotic system

was used in aliquoting of samples. A TECAN and RapidPlate robotic system was used to mix DNA with water for PCR. Prism 7900 HT Sequence Detection System (Applied Biosystems, Foster City, CA) was used for allelic discrimination. Microsoft Excel macros were used to create the plate records with allele base codes for cleaning. Output was captured directly to databases and then transferred via File Transfer Protocol (FTP) to the data manager. Genotyping was completed in 3,771 JHS participants.

Statistical Analysis

Data were analyzed using SAS 9.2 statistical software. Genotype frequency was used to compute allele frequency (Table 3). In addition, comparisons of the minor allele frequency (MAF) with reference populations from the low-coverage panel of HAPMAP Yoruba (HAPMAP cohort: Yoruba, Nigerian population), HAPMAP CEU (HAPMAP cohort: UTAH residents of European ancestry), CEPH (Minnesota residents, French and Venezuelan) and CABG (North American Cohort with coronary artery disease, predominantly Caucasian) cohorts were performed by chi-square analysis. Hardy-Weinberg equilibrium (HWE), linkage disequilibrium (LD) and LD graphs were computed using Haploview genetics software, Copyright ©2003-2006 Broad Institute of MIT and Harvard [185, 186].

The general linear models (GLM) procedure was used to evaluate differences in covariates between the sexes. Serum lipid concentrations were evaluated for normality and measures of central tendency. Serum TG was transformed using logarithm based 10. All other lipid values were normally distributed. Outliers were examined using the residual method. Covariates included age, sex, BMI, smoking status (yes, no) and alcohol

intake (g). The logarithm of the sum of alcohol +10 was used to transform alcohol intake. Serum lipid concentrations and dietary predictors were analyzed as continuous variables.

In our primary analysis, associations between genotype and serum cholesterol were assessed using analysis of covariance (ANCOVA) modeling in GLM. Genotype exposure was defined as homozygous dominant (HOD) and heterozygous (HET) compared to the homozygous recessive (HOR). Covariates included age, sex, BMI, smoking status, and alcohol intake. A secondary analysis using a recessive mode of inheritance with HOD coded as 1 and a combined variable with HOR and HET coded as 0 was also used to examine the association between genotype and serum lipids. The secondary analysis was completed using three different models for the outcomes; Model 1 adjusted for age and sex ; Model 2 adjusted for age, sex, diabetes and hypertension status; Model 3 adjusted for age, sex, BMI, smoking status and alcohol intake. Stratified analysis of the associations between SNPs and serum lipids concentrations by dichotomous percent fat intake (35.1 ≥ vs. < 35.1%) was completed using GLM. SNP exposure in the stratified analysis was defined as HOD, HET compared to HOR. Regression analysis of the interaction between percent fat intake (35.1 ≥ vs. < 35.1%) and SNP genotype (binary HOD) was also examined. Bonferroni correction was used to adjust for multiple comparisons. A p-value <0.008 was considered statistically significant.

Results

Descriptive Characteristics of JHS Participants

On average, men were older than women, had higher alcohol intake, physical activity, LDL-C and TG. Women had higher BMI, HDL-C and TC. Both groups had mean BMI ≥

30. On average, women had HDL-C concentration approximately 10 mg/dL higher than men. Differences in BMI, physical activity score, HDL-C and total cholesterol were statistically significant (P<0.05) **(Table1).**

Disease Frequency

Frequency of type 2 diabetes was higher in women (~12%) compared to men (9%). Similarly, HbA1c ≥ 6.5 % was higher in women (12%) than men (11%). Approximately 30% of both men and women were classified as having hypercholesterolemia, while 12-13% reported use of lipid lowering medications. Prevalence of hypertension was around 60% for both men and women. More women (75%) reported having never smoked than men (53%) **(Table2).**

Genotype Frequency

The frequency of the heterozygous genotype was highest for the rs7412 *APOE* SNP (34.8%), corresponding with the highest MAF (24.7%). Frequencies of the HET for both the rs429358 SNP in *APOE* and the rs662799 in *APOA5* were close to 20%, corresponding with a MAF of ~14%. SNPs rs3135506 (*APOA5*), rs2970869 (*PPARGC1A*) and rs10028665 (*PPARGC1A*) had lower frequencies of the heterozygous genotype (12-14%), corresponding to 9-12% of the MAF. All MAF frequencies were statistically different than the YRI, CEU, CEPH and CABG comparison populations. **(Table3).**

Characteristics of Selected Single Nucleotide Polymorphisms

All SNPs were in HWE **(Table 4).** The pairwise LD D' and R^2 indicate that there is linkage disequilibrium within the *APOE* SNP pair in JHS participants **(Table 5).** No other pairs showed significant LD with each other. Visualization of the LD between the selected SNPs in *APOE*, *APOA5* and *PPARGC1A* polymorphisms is shown in **Figures 1 and 2**.

Association between Mean Serum Lipid Concentration and Genotype for Selected Single Nucleotide Polymorphisms

The mean serum lipid concentrations by SNP genotype are presented in **Table 6**. In some cases differences in concentrations appear to be additive. In others, the simple presence or absence of the minor allele reflects differences between serum lipids by genotype. In ANCOVA analyses **(Table 7),** the HOD and HET genotype in *APOA5*, rs3135505 were negatively associated with TG concentration. The HOD genotype in *APOA5*, rs3135506 was also positively associated with HDL-C. Similarly the HOD genotype in the *APOE*, rs7412 was also positively associated with HDL-C. The HOD genotype in the *APOE*, rs7412 was negatively associated with LDL-C and total cholesterol. In contrast, the HOD genotype in *APOE*, rs429358 was positively associated with LDL-C and total cholesterol. No associations between *APOA5*, rs662799, *PPARGC1A*, rs2970869 or rs10028665 were observed with lipid measures.

In a subsequent analysis, with the HOD genotype (coded as 1,0) **(Table 8),** the HOD genotype for *APOE*, rs7412 showed a marginal positive association with HDL-C ($P<0.05$) and negative association with LDL-C ($p<0.0001$) and TG ($p<0.008$). Inclusion of smoking status, BMI and alcohol led to attenuation of the association between TG and

APOE, rs7412. The HOD genotype for *APOE*, rs429358 showed marginal negative association with HDL-C ($p<0.05$) and positive association with LDL-C ($p<0.0001$). TG concentration was negatively associated with the HOD genotype for the rs3135506 SNP ($p<0.0001$) in *APOA5*. The HOD genotype for *PPARGC1A*, rs2970869 was positively associated with LDL-C. There was no association between serum lipids and the rs10028665 variant in *PPARGC1A*.

Association between Gene Variants, Fat Intake and Lipid Concentrations

In stratified analysis by fat intake (≥ vs. < 35.1 % energy from fat) **(Table 9)** and regression analysis of the interaction term **(Table 10),** we found evidence of significant interaction between percent fat intake and *APOA5*, rs662799 in serum HDL-C ($p<0.008$) and *PPARGC1A*, rs10028665 in LDL-C ($p<0.05$) (Table 10).

Discussion

The MAF frequency of selected SNPs in JHS participants was significantly different than that in YRI or CEU reference populations. This could result from genotyping error. To examine the possibility of genotyping error, we plan to examine these relationships using genome wide scans previously completed in the JHS. In addition, a genome wide association analysis (GWAS) would allow us to examine additional markers near our SNPs of interest. The possibility of genotyping error should be taken into consideration when interpreting these results. If results from GWAS confirm our analysis, then genotype frequencies of these selected SNPs are different in the JHS cohort in comparison to the HAPMAP CEU, YRI and the CEPH and CABG cohorts. Differences in genotype frequency for non-Hispanic whites in the dbSNPs database were found

between the CEPH and CABG and the HAPMAP CEU cohort for APOE rs7412, rs429358 and PPARGC1A rs2970869, rs10028665 (significance testing not shown).

Assuming there was no genotyping error, we found that after inclusion of BMI, the association between *APOA5*, rs3135506 and HDL-C was no longer significant. This suggests that BMI is an important component in this relationship. BMI has been shown to be both independently associated, a confounding factor and an effect modifier in relationships between *APOA5* and cardiovascular risk factors [187-192]. The mechanism underlying these associations is not well understood. Modeling of the association between genotype and serum lipid concentration produced some differences. Comparing the combined variable of HOD and HET to HOR showed a significant association between the HOD genotype in the *APOA5*, rs3135506 and HDL-C ($p<0.008$), while the binary HOD, defined as HOD genotype compared to the combined variable of HOR and HET genotype, showed only marginal significance ($p<0.05$). Similarly, the binary HOD genotype showed a significant association between the *APOE*, rs7412 and TG ($P<0.0001$), while the combined HOD and HET variable compared to HOR showed a marginally significant association with TG ($P<0.05$).

Comparison with Other Studies

Using case-control modeling of ancestry, Deo et al previously found a strong positive association between the *APOA5*, rs3135506 minor allele and TG in JHS participants [193]. This association was seen in those classified as having either European or African ancestry at the locus. In addition, *APOA5*, rs662799, approached a significant association with TG ($p=0.07$), but with a positive interaction by ancestry. JHS participants classified

with European ancestry showed an effect of 21 ± 5.5 between the minor allele and TG, while participants classified with African ancestry showed an effect size of 1.1±1.0 [193]. Other studies have shown a strong association between this variant and TG in Europeans [194]. JHS is the first population to show a significant interaction with ancestry at this locus. In a study of *APOA5*, Ken-Dror et al found associations between the rs662799 variant and TG in Ashkenazi and Yemeni Jews, but not in Sephardic (North African, Asian) Jews [195]. This further suggests an interaction with ancestry for this genetic variant. We found negative associations with the HOD and HET genotypes for *APOA5* and TG, and we did not see an association between *APOA5*, rs662799 and TG in our models. This may be explained by the ancestral interaction found between *APOA5*, rs662799 and TG noted in previous studies in the JHS.

Using data from the National Health and Examination Survey (NHANES), Chang et al found a negative association ($p<0.0001$) between LDL-C and the rs7412 variant in *APOE* in African Americans [74]. These effects were strengthened after adjustment for age, sex, education, BMI, smoking status, alcohol intake, physical activity and dietary fat intake, and further strengthened in haplotype analysis. The direction of effect was consistent across all ethnicities: non-Hispanic whites, non-Hispanic blacks and Mexican Americans [74]. Our findings are consistent with those reported by NHANES. However, in addition, we found a positive association between *APOE*, rs7412 with HDL-C and TG.

In association analysis of the *APOE*, rs429358 variant, Chang et al found a positive association with LDL-C in both non-Hispanic whites and Mexican Americans, but not in African Americans. In contrast, we found a positive association between LDL-C and *APOE*, rs42958 ($p<0.0001$) in the JHS African Americans. In addition, we found a

marginally significant negative association between *APOE*, rs429358 with HDL-C ($p<0.05$) in JHS. The differences between NHANES (n=683 African Americans) and our analysis in the JHS (n=3,771 African Americans) may be a result of limited power due to a smaller sample size in the NHANES cohort.

PPARGC1A has been associated with obesity, cholesterol, diabetes, blood pressure and inflammation. Brito et al found a significant association with the rs2970869 variant of *PPARGC1A* and systolic blood pressure [178]. Kothari et al found higher A-allele frequency in Caucasians compared to African Americans[196]. This frequency was consistent with lower rates of diastolic dysfunction. *PPARGC1A* variants have been associated with obesity phenotypes [197]. Sarzynski et al found associations between *PPARGC1A*, rs2970869 and maximal weight loss and weight regain [198]. We found associations between *PPARGC1A*, rs2970869 and LDL-C ($P<0.008$). LDL-C, obesity and systolic and diastolic dysfunction are CVD risk factors and components of metabolic syndrome. It is postulated that these traits share a common etiology. *PPARGC1A* may play a role in CVD pathogenesis through effects on each of these traits. No association was found with the *PPARC1A*, rs10028665 variant and serum lipids.

We found a significant interaction between *APOA5* rs662799 and percent fat intake on HDL-C ($p<0.008$) in the JHS cohort. In addition to ancestral interactions, interactions with diet may help to explain differences in associations between this variant and lipids in this population. We found a marginally significant interaction between *PPARGC1A*, rs10028665 and percent fat intake with LDL-C ($p<0.05$). These interactions with diet may help explain the lack of association of rs10028665 and serum lipids in previous studies.

If genotyping is confirmed, our findings show that multiple genetic variants are associated with serum lipids in African Americans. In addition to genetic differences, interactions with diet may, in part, explain differences in observed association between these variants and serum lipid concentrations. To our knowledge, this study is the first to examine gene-diet interactions in a large all African American population. Additional research on gene-diet interactions may further understandings of health disparities in this population. It is important to confirm these findings using several different approaches 1) within the JHS cohort by comparing to JHS genome-wide scan data, 2) using JHS genome-wide scan data to examine other SNPs in these genes, 3) replicating these analyses in other populations, including other African American populations.

Table 1. Mean Descriptive characteristics of JHS participants

	Men	**Women**
	Mean ± SD	Mean ± SD
Age (years)	53.8± 13.4	54.6± 13.6
BMI *	29.7± 6.27	32.7± (7.7
Alcohol (gm)	2.7± 0.55	2.4± (0.2
Physical Activity Score*	8.7± 2.67	8.3± (2.6
HDL-C mg/dL*	46.1± 13.1	54.9± (14.4
LDL-C mg/dL	126± 37	126 ± (36
Triglycerides mg/dL	120± 110	98.8± (56.3
Total Cholesterol mg/dL*	196± 40	200± (39

JHS, Jackson Heart Study; BMI, Body Mass Index; LDL-C, low density lipoprotein; HDL-C, high Density lipoprotein. SD, standard deviation
Differences between men and women were computed using the general linear models procedure in SAS 9.2
*Indicates statistically significant differences between men and women
$p<0.05$

Table 2. Frequency of certain CVD risk factors in JHS participants

	Men % (n)	**Women** % (n)
Hypercholesterolemia	31.6(942)	30.0(1678)
Hypertension	59.4(1020)	61.8(1770)
Type 2 Diabetes	9.4 (1007)	11.8(1760)
HbA1c Status	10.9(1099)	12.0(1911)
Cholesterol Lowering Medication	13.2(991)	11.6(1754)
Never Smoked *	53.4(545)	74.7(1318)

JHS, Jackson Heart Study; n= number of JHS participants in group
Hypercholesterolemia was determined by participant's report of lipid lowering medications use in the previous 2 weeks, a fasting LDL-C ≥ 160mg/dL or a TC concentration ≥ 240 mg/dL [48]. The hypercholesterolemia classification is based on the third report of the Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults, Adult Treatment Panel III [1, 30].
Hypertension was determined by use of antihypertensive medication, or systolic blood pressure ≥ 140mm Hg or diastolic blood pressure ≥ 90mm Hg. Criteria for hypertension status are based on the 7th Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC7) [30, 49-52]. Type 2 diabetes was defined by fasting plasma glucose ≥126 mg/dL or current use of insulin or oral hypoglycemic medications [30, 53]. These criteria for type 2 diabetes are in accordance with the American Diabetic Association 2004 Guidelines [54]. HbA1c≥ 6.5%.This criteria for glycosylated hemoglobin in accordance with the International Expert Committee (2009) International Expert Committee report of the role of the A1 assay in the diagnosis and treatment with diabetes (2009).
*indicates statistically significant difference between men and women ($p<0.05$)

Table 3. Genotype Frequency of six select SNPs in *APOE*, *APOA5* and *PPARGC1A* in JHS participants.

	Percent Genotype Frequency		**MAF**	**MAF Reference population[175, 199]**		
	Dominant	**Heterozygote**	**JHS**	**YRI**	**CEU**	**CEPH[1]/CABG[2]**
APOE **rs7412**	59.1	34.8	24.7	9.3***	6.7***	28.1[1] ***
APOE **rs429358**	80.7	17.5	13.4	14.4***	9.2***	15.5[2]***
APOA5 **rs662799**	78.1	20.0	13.8	8.5***	7.5***	---
APOA5 **rs3135506**	87.6	11.6	8.9	2.5***	4.2***	---
PPARGC1A **rs2970869**	87.1	12.0	9.4	3.4***	20.8***	28.3[1] ***
PPARGC1A **rs10028665**	84.5	14.2	11.6	6.8***	31.7***	23.9[1] ***

n=3771;SNPs, single nucleotide polymorphisms, JHS, Jackson Heart Study; *APOE*, Apolipoprotein E; *APOA5*, Apolipoprotein A5; *PPARGC1A*, Peroxisome Proliferating-Activated Receptor Gamma, Coactivator 1 alpha; Dominant, Homozygous Dominant Genotype; Heterozygote, Heterozygous genotype; MAF, Minor Allele Frequency;
YRI- HAPMAP Yoruba as the reference populations (n=110)
CEU- HAPMAP Utah residents with ancestry from northern and western Europe (n=120)
[1]CEPH- Utah (93%), French (4%), Venezuelan (3%) (n=92)
[2]CABG- North American population (n=1113)
--- Genotype frequency not available
*** indicates significant differences between groups using alpha 0.001

Table 4. Descriptive Information for SNPs in *APOE*, *APOA5* and *PPARGC1A*

Gene	SNP Name[1]	rs number	CHR	Location	Type	ABI Assay[2]	HWE p-value[3]
APOE	R176C	rs7412	19	Q13.1-3	Ref, mis	C____904973_10	1.0
APOE	C130R	rs429358	19	Q13.1-3	exe, mis, ref	C___3084793_20	0.5
APOA5	m1223	rs662799	11	Q23	us2k	C___2310403_10	0.2
APOA5	S16W	rs3135506	11	Q23	Ref, mis	C__25638153_10	0.4
PPARGC1A	m1668	rs2970869	4	P15.1	Us2k	C__26497367_10	0.5
PPARGC1A	i15867	rs10028665	4	P15.1	int	C__30441792_10	1.0

SNPs, single nucleotide polymorphisms; HWE, Hardy-Weinberg equilibrium; LD, linkage disequilibrium
APOE, Apolipoprotein E; *APOA5*, Apolipoprotein A5; *PPARGC1A*, Peroxisome Proliferating-Activated Receptor Gamma, Coactivator-1 alpha;
[1]Variant name for amino acid change, or intronic(i), promoter (m), or in/near3'UTR(3U)position from mRNA start.
Mis, missense; ref, reference allele observed in reference contig sequence; ese, exonic splicing enhancer; us2k - upstream-variant-2KB sequence variant within 2KB 5' of gene; int - intron-variant-- variation in intron, but not in first 2 or last 2 bases of intron
[2]ABI assays from Applied Biosystems (Foster City, CA)
[3]HWE p-value indicates values calculated within this Analysis in JHS participants
Information SNPs provided by dbSNPs (URL: http://www.ncbi.nlm.nih.gov/projects/SNP/)

Table5. Pairwise Linkage Disequilibria D' and R^2 between selected SNPs in *APOE*, *APOA5* and *PPARGC1A* in JHS participants

	APOA5 *rs3135506*	***APOE*** *rs7412*	***APOE*** *rs429358*	***PPARGC1A*** *rs2970869*	***PPARGC1A*** *rs10028665*
Cohort	D'/r^2	D'/r^2	D'/r^2	D'/r^2	D'/r^2
APOA5 *rs662799*	0.3/0	0.10/0.002	0.2/0	0.6/0.002	0.01/0
APOA5 *rs3135506*		0.06/0.001	0.003/0	0.02/0	0.04/0.001
APOE *rs7412*			1.0/0.03*	0.08/0	0.02/0
APOE *rs429358*				0.02/0	0.3/0.001
PPARGC1A *rs2970869*					0.9/0.005

SNPs, single nucleotide polymorphisms; LD, linkage disequilibrium
APOE, Apolipoprotein E; *APOA5*, Apolipoprotein A5; *PPARGC1A*, Peroxisome Proliferating-Activated Receptor Gamma, Coactivator 1 alpha;
*Indicates significant LD in Haploview [185]

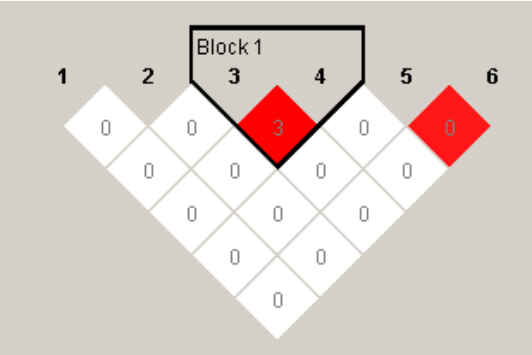

Figure 1. R-squared Between SNPS in *APOE, APOA5* and *PPARGC1A*

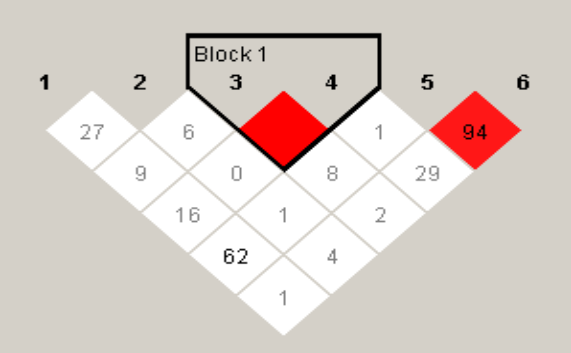

Figure 2. D' Between SNPS in *APOE*, *APOA5* and *PPARGC1A*

1=*APOA5* rs662799
2=*APOA5* rs3135506
3=*APOE* rs7412
4=*APOE* rs429358
5=*PPARGC1A* rs2970869
6=*PPARGC1A* rs10028665

Table 6. Mean serum lipid concentration by single nucleotide variants in *APOE*, *APOA5* and *PPARGC1A* in Jackson Heart Study participants

	Genotype	**HDL-C** mg/dL	**LDL-C** mg/dL	**Triglycerides** mg/dL	**Total Cholesterol** mg/dL
APO A5 M1123	AA	52	126	106	198
(rs662799)	AG	52	128	108	201
	GG	50	125	128	199
APO A5 s16w	GG	52	126	105	199
(rs3135506)	GC	51	125	120	199
	CC	40	132.	260	210
APOE C130R	TT	53	122	105	195
(rs7412)	CT	51	131	110	204
	CC	49	137	125	209
APOE R176C	CC	52	130	107	202
(rs429358)	CT	53	111	105	185
	TT	54	100	128	181
PPARGC1A m1668	CC	52	127	108	200
(rs2970869)	CT	53	119	100	192
	CC	52	114	103	187
PPARGC1A	CC	52	126	107	198
I15867	CT	52	128	108	201
(rs10028665)	CC	49	117	108	188

HDL-C, high density lipoprotein; LDL-C, low density lipoprotein
APOE, Apolipoprotein E; *APOA5*, Apolipoprotein A5; *PPARGC1A*, Peroxisome Proliferating-Activated Receptor Gamma, Coactivator 1 alpha
Values presented are means

Table 7. Association of serum lipid concentration and selected SNPs[1] in *APOA5*, *APOE* and *PPARGC1A* (HOD and HET compared to HOR) in JHS participants

	HDL-C	**LDL-C**	**Triglycerides**[2]
SNPs	β (SE)	β (SE)	β (SE)
APOA5 rs662799			
0	2.6 (2.1)	0.6(5.8)	-21.4(12.8)
1	2.1 (2.2)	2.9(6.0)	-19.6(13.2)
APOA5 rs3135506			
0	12.3 (4.1)**	-6.0(12.2)	-155(24.3)***
1	10.8(4.2)*	-7.2(12.4)	-140(24.7)***
APOE rs7412			
0	3.6(1.3)**	-15.1(3.6)***	-20.5(7.9)*
1	2.5(1.4)	-5.8(3.7)	-15.1(8.1)
APOE rs429358			
0	-2.5(2.5)	29.3(6.6)***	-21.6(14.6)
1	-0.8(2.5)	10.2(6.7)	-23.7(15.1)
PPARGC1A rs2970869			
0	-0.3(4.5)	12.9(12.1)	4.9(27.1)
1	0.8 (4.6)	5.4(12.3)	-3.1(27.4)
PPARGC1A rs10028665			
0	3.3(3.3)	8.6(8.7)	-1.0(19.8)
1	3.4(3.4)	10.4(9.0)	0.2(20.1)

[1]SNP, single nucleotide polymorphism; β, Beta; JHS, Jackson Heart Study; HDL-C, high density lipoprotein concentration; LDL-C , low density lipoprotein concentration; *APOE*, Apolipoprotein E; *APOA5*, Apolipoprotein A5; *PPARGC1A*, Peroxisome Proliferating-Activated Receptor Gamma 1-alpha HOD, homozygous; In SNPs, 0=Homozygous Dominant Genotype; 1= Heterozygous Genotype. The comparison group is the Homozygous Recessive Genotype.[2]Triglyceride concentrations have been log transformed
Models adjusted for age, sex, BMI, smoking status and alcohol intake
Bonferroni correction (p-value/6 = 0.008); P-value <0.008 indicates significant linkage adjusted for multiple comparisons
*P<0.05; **P<0.008; ***P<0.001

Table 8. Association of serum lipid concentration and the HOD (binary)[1] genotype for select SNPs in Jackson Heart Study Participants

	HDL-C	LDL-C	Triglycerides[2]
	β (SE)	β (SE)	β (SE)
***APOE* rs7412**			
Model 1	1.27(0.5)*	-9.50 (1.4)***	-8.41(3.1)**
Model 2	1.29(0.5)*	-9.22(1.4)***	-8.51(3.1)**
Model 3	1.44(0.5)*	-9.68(1.5)***	-7.35(3.3)*
***APOE* rs429358**			
Model 1	-1.36(0.7)*	19.4(1.7)***	-0.61(3.8)
Model 2	-1.35(0.7)*	19.5(1.7)***	-0.46(3.8)
Model 3	-1.78(0.7)*	19.4(1.8)***	-0.04(4.1)
***APOA5* rs662799**			
Model 1	0.23(0.6)	-1.90(1.7)	-2.26(3.6)
Model 2	0.43(0.6)	-1.52(1.7)	-3.21(3.6)
Model 3	0.58(0.6)	-2.16(1.7)	3.43(3.9)
***APOA5* rs3135506**			
Model 1	1.38(0.8)*	0.18(2.1)	-18.0(4.6)***
Model 2	1.34(0.8)	0.08(2.2)	-19.6(4.6)***
Model 3	1.56(0.8)	0.84(2.2)	-20.0(4.9)***
***PPARGC1A* rs2970869**			
Model 1	-1.27(0.8)	6.83(2.1)**	7.55(4.5)
Model 2	-1.29(0.8)	6.54(2.1)**	8.28(4.5)
Model 3	-1.13(0.8)	7.19(2.1)**	8.28(4.8)
***PPARGC1A* rs10028665**			
Model 1	-0.31(0.7)	-0.84(1.9)	-2.98(4.1)
Model 2	-0.32(0.7)	-0.79(1.9)	-2.58(4.1)
Model 3	0.06(0.7)	-1.40(2.0)	-1.60(4.4)

JHS, Jackson Heart Study; SNPs, single nucleotide polymorphisms; SE, standard error; *APOE*, apolipoprotein-E; *APOA5*, apolipoprotein-A5; *PPARGC1A*, peroxisome proliferator-activated receptor gamma, coactivator 1 alpha; HDL-C, high density lipoprotein concentration; LDL-C, low density lipoprotein concentration; SE. standard error

[1] HOD compared to the combined variable of HOR and HET

[2] Triglyceride concentrations have been log transformed

Model1,adjusted for age and sex ;

Model 2, adjusted for age, sex, diabetes status and HTN status; Model 3, adjusted for age, sex, body mass index, smoking status and alcohol intake;

Bonferroni correction ;P-value <0.008 indicates significance after adjusting for multiple comparisons

*P<0.05; **P<0.008; ***P<0.0001

Table 9. Association between selected SNPs[1] and serum lipids, stratified by fat intake in Jackson Heart Study Participants

Gene	SNP	Phenotype	High Fat Intake		Low Fat Intake	
			β(SE) 0	β(SE) 1	β(SE) 0	β(SE) 1
APOE	rs7412	HDL-C	2.85(1.8)	1.59(1.8)	4.5(2.0)	3.46(2.0)
		LDL-C	-18.4(4.8)***	-8.19(4.9)	-12.2(5.4)*	-4.1(5.5)
		TG	-13.2(8.9)	-9.68(9.1)	-27.9(13.3)*	-20.7(13.6)
	rs429358	HDL-C	0.48(3.7)	2.17(3.8)	-4.63(3.3)	-3.0(3.4)
		LDL-C	40.0(10.2)***	22.0(10.4)*	-21.4(8.6)*	1.16(8.9)
		TG	-35.2(18.4)	-36.8(18.8)	-11.2(22.3)	-14.3(23.0)
APOA5	rs662799	HDL-C	-0.01(3.1)	1.3(3.1)	4.6 0(3.0)	2.07(3.1)
		LDL-C	-1.28(8.5)	1.17(8.7)	2.38(8.0)	4.5(8.3)
		TG	-24.7(15.3)	-23.3(15.7)	-18.0(20.2)	-15.9(0.4)
	rs3135506	HDL-C	15.6(4.7)	14.2(4.8)	4.39(8.1)	2.94(8.1)
		LDL-C	-2.59(14.7)	-4.92(14.9)	-12.6(21.4)	-12.6(21.6)
		TG	-186(22.8)***	-183(23.4)***	-72.7(5.9)	-44.2(54.4)
PPARGC1A	rs2970869	HDL-C	-1.91(4.7)	-0.94(4.8)	13.9(14.0)	15.0(14.0)
		LDL-C	5.2(12.7)	-4.52(13.0)	79.8(36.9)*	74.5(37.0)*
		TG	-1.50(23.5)	-2.74(24.0)	44.5(93.6)	30.6(93.9)
	rs10028665	HDL-C	0.17(4.7)	0.23(4.8)	5.96(4.7)	6.17(4.8)
		LDL-C	18.5(12.7)	16.1(12.9)	-0.27(12.4)	5.57(12.6)
		TG	-3.81(23.4)	-1.8(23.9)	3.61(31.4)	4.45(32.0)

SNPs, single nucleotide polymorphisms; *APOE*, apolipoprotein-E; *APOA5*, apolipoprotein-A5*; PPARGC1A*, peroxisome proliferator-activated receptor gamma, coactivator 1 alpha; HDL-C, high density lipoprotein concentration; LDL-C, low density lipoprotein concentration; TG, triglyceride concentration; high fat intake ≥35.1 percent energy from fat; low fat intake 35.1percent energy from fat.

[1] SNP exposure was defined as HOD, HET compared to HOR

Bonferroni adjusted P-value <0.008 indicates significance after adjusting for multiple comparisons

Models adjusted for age, sex, body mass index, smoking status, energy and alcohol intake

* p<0.05; ** P<0.008; *** P<0.0001

Table 10. Interactions between dietary fat intake and SNP genotypes[1] with serum lipid outcomes in Jackson Heart Study participants

		SNP	**High Fat intake**	**Interaction**
		β (SE)	β (SE)	β (SE)
APOE				
HDL-C	rs7412			
	β(SE)	-1.43(0.8)	-0.74(0.7)	0.008(1.1)
	rs429358			
	β(SE)	1.46(1.0)	-0.86(0.6)	1.37(1.4)
LDL-C	rs7412			
	β(SE)	11.0(2.9)***	1.00(1.9)	-2.59(2.9)
	rs429358			
	β(SE)	-18.9(2.6)***	0.15(1.6)	-1.06(3.6)
Triglycerides	rs7412			
	β(SE)	4.59(4.6)	018 (4.2)	6.1(6.5)
	rs429358			
	β(SE)	1.72(5.8)	4.11(3.6)	-3.40(8.2)
APOA5				
HDL-C	rs662799			
	β (SE)	1.47(0.9)	0.18(0.6)	-4.15(1.3)**
	rs3135506			
	β (SE)	-1.82(1.1)	-0.83(0.6)	0.49(1.7)
LDL-C	rs662799			
	β (SE)	2.39(2.5)	0.06(1.7)	-0.48(3.5)
	rs3135506			
	β (SE)	-2.25(3.1)	-0.45(1.6)	3.04(4.5)
Triglycerides	rs662799			
	β (SE)	2.77(5.4)	0.06 (1.7)	-0.48(3.5)
	rs3135506			
	β (SE)	12.6(6.8)	-1.97 (3.5)	16.5 (9.9)
PPARGC1A				
HDL-C	rs2970869			
	β (SE)	1.24(1.1)	-0.69(0.6)	-0.67(1.5)
	rs10028665			
	β (SE)	0.30(1.1)	-0.63(0.6)	-0.67(1.5)
LDL-C	rs2970869			
	β (SE)	-8.66(3.0)**	-0.52 (1.6)	2.90(4.3)
	rs10028665			
	β (SE)	-3.49(2.8)	-1.58(1.6)	9.39(3.9)*
Triglycerides	rs2970869			
	β (SE)	-2.25(6.7)	5.06(3.5)	-12.7(9.5)
	rs10028665			
	β (SE)	0.63 (6.3)	3.20 (3.6)	1.63(8.7)

JHS, Jackson Heart Study; SNP, single nucleotide polymorphism; SE, standard error; HDL-C, high density lipoprotein concentration; LDL-C, low-density lipoprotein concentration; high fat intake, ≥35.1 percent fat intake; Interaction, statistical interaction between dietary fat (≥35.1, <35.1 percent intake) and SNP; *APOE*, Apolipoprotein-E*; APOA5*, Apolipoprotein -A5; *PPARGC1A*, Peroxisome Proliferator-Activated Receptor Gamma, cofactor 1 Alpha

adjusted for age, sex, body mass index, smoking status, alcohol, >35.1 percent fat intake, energy, selected SNP and the interaction between >35.1 percent fat and selected SNP;[1] SNP genotype defined as HOD compared to the combined variable of HOR and HET
Bonferroni adjusted P-value <0.008 indicates significance after adjusting for multiple comparisons).
* p<0.05; ** P<0.008; *** P<0.0001

1. *Summary of the second report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel II)*. JAMA : the journal of the American Medical Association, 1993. **269**(23): p. 3015-23.
2. Sempos, C.T., et al., *Prevalence of high blood cholesterol among US adults. An update based on guidelines from the second report of the National Cholesterol Education Program Adult Treatment Panel*. JAMA : the journal of the American Medical Association, 1993. **269**(23): p. 3009-14.
3. Talayero, B.G. and F.M. Sacks, *The role of triglycerides in atherosclerosis*. Current cardiology reports, 2011. **13**(6): p. 544-52.
4. Chang, M.H., et al., *Genetic variants associated with fasting blood lipids in the U.S. population: Third National Health and Nutrition Examination Survey*. BMC medical genetics, 2010. **11**: p. 62.
5. Dong, C., et al., *Genetic loci for blood lipid levels identified by linkage and association analyses in Caribbean Hispanics*. Journal of lipid research, 2011. **52**(7): p. 1411-9.
6. Waterworth, D.M., et al., *Genetic variants influencing circulating lipid levels and risk of coronary artery disease*. Arteriosclerosis, thrombosis, and vascular biology, 2010. **30**(11): p. 2264-76.
7. Erkkila, A.T., et al., *APOE polymorphism and the hypertriglyceridemic effect of dietary sucrose*. The American journal of clinical nutrition, 2001. **73**(4): p. 746-52.
8. Moreno, J.A., et al., *Apolipoprotein E gene promoter -219G->T polymorphism increases LDL-cholesterol concentrations and susceptibility to oxidation in response to a diet rich in saturated fat*. The American journal of clinical nutrition, 2004. **80**(5): p. 1404-9.
9. Rubin, J. and L. Berglund, *Apolipoprotein E and diets: a case of gene-nutrient interaction?* Current opinion in lipidology, 2002. **13**(1): p. 25-32.
10. Lai, C.Q., L.D. Parnell, and J.M. Ordovas, *The APOA1/C3/A4/A5 gene cluster, lipid metabolism and cardiovascular disease risk*. Current opinion in lipidology, 2005. **16**(2): p. 153-66.
11. Benetti, E., N.S. Patel, and M. Collino, *The role of PPARbeta/delta in the management of metabolic syndrome and its associated cardiovascular complications*. Endocrine, metabolic & immune disorders drug targets, 2011. **11**(4): p. 273-84.
12. Brito, E.C., et al., *PPARGC1A sequence variation and cardiovascular risk-factor levels: a study of the main genetic effects and gene x environment interactions in children from the European Youth Heart Study*. Diabetologia, 2009. **52**(4): p. 609-13.
13. Jurkowski, W., et al., *PPARgamma population shift produces disease-related changes in molecular networks associated with metabolic syndrome*. Cell death & disease, 2011. **2**: p. e192.
14. Auton, A. and G. McVean, *Estimating recombination rates from genetic variation in humans*. Methods in molecular biology, 2012. **856**: p. 217-37.
15. Stumpf, M.P. and G.A. McVean, *Estimating recombination rates from population-genetic data*. Nature reviews. Genetics, 2003. **4**(12): p. 959-68.
16. Hinch, A.G., et al., *The landscape of recombination in African Americans*. Nature, 2011. **476**(7359): p. 170-5.
17. Cheng, K.F. and J.Y. Lee, *Assessing the joint effect of population stratification and sample selection in studies of gene-gene (environment) interactions*. BMC genetics, 2012. **13**: p. 5.
18. Corella, D., et al., *APOA2, dietary fat, and body mass index: replication of a gene-diet interaction in 3 independent populations*. Archives of internal medicine, 2009. **169**(20): p. 1897-906.

19. Fuqua, S.R., et al., *Recruiting African-American research participation in the Jackson Heart Study: methods, response rates, and sample description*. Ethnicity & disease, 2005. **15**(4 Suppl 6): p. S6-18-29.
20. Taylor, H.A., Jr., et al., *Toward resolution of cardiovascular health disparities in African Americans: design and methods of the Jackson Heart Study*. Ethnicity & disease, 2005. **15**(4 Suppl 6): p. S6-4-17.
21. Dubbert, P.M., et al., *Physical activity assessment methods in the Jackson Heart Study*. Ethnicity & disease, 2005. **15**(4 Suppl 6): p. S6-56-61.
22. Smitherman, T.A., et al., *Validation of the Jackson Heart Study Physical Activity Survey in African Americans*. Journal of physical activity & health, 2009. **6 Suppl 1**: p. S124-32.
23. Hertogh, E.M., et al., *Validity of the modified Baecke questionnaire: comparison with energy expenditure according to the doubly labeled water method*. The international journal of behavioral nutrition and physical activity, 2008. **5**: p. 30.
24. Carithers, T.C., et al., *Validity and calibration of food frequency questionnaires used with African-American adults in the Jackson Heart Study*. Journal of the American Dietetic Association, 2009. **109**(7): p. 1184-1193.
25. Talegawkar, S.A., et al., *Total antioxidant performance is associated with diet and serum antioxidants in participants of the diet and physical activity substudy of the Jackson Heart Study*. The Journal of nutrition, 2009. **139**(10): p. 1964-71.
26. Talegawkar, S.A., et al., *Carotenoid intakes, assessed by food-frequency questionnaires (FFQs), are associated with serum carotenoid concentrations in the Jackson Heart Study: validation of the Jackson Heart Study Delta NIRI Adult FFQs*. Public health nutrition, 2008. **11**(10): p. 989-97.
27. Tucker, K.L., et al., *A regional food-frequency questionnaire for the US Mississippi Delta*. Public health nutrition, 2005. **8**(1): p. 87-96.
28. Champagne, C.M., et al., *Dietary intake in the lower Mississippi delta region: results from the Foods of our Delta Study*. Journal of the American Dietetic Association, 2004. **104**(2): p. 199-207.
29. Carpenter, M.A., et al., *Laboratory, reading center, and coordinating center data management methods in the Jackson Heart Study*. The American journal of the medical sciences, 2004. **328**(3): p. 131-44.
30. Taylor, H.A., Jr., et al., *Dyslipidemia and the treatment of lipid disorders in African Americans*. The American journal of medicine, 2009. **122**(5): p. 454-63.
31. Santos, J.L., et al., *Genotype-by-nutrient interactions assessed in European obese women. A case-only study*. European journal of nutrition, 2006. **45**(8): p. 454-62.
32. Campos, H., M. D'Agostino, and J.M. Ordovas, *Gene-diet interactions and plasma lipoproteins: role of apolipoprotein E and habitual saturated fat intake*. Genetic epidemiology, 2001. **20**(1): p. 117-128.
33. Corella, D. and J.M. Ordovas, *Nutrigenomics in cardiovascular medicine*. Circulation. Cardiovascular genetics, 2009. **2**(6): p. 637-51.
34. Ordovas, J.M., *The quest for cardiovascular health in the genomic era: nutrigenetics and plasma lipoproteins*. The Proceedings of the Nutrition Society, 2004. **63**(1): p. 145-52.
35. Paoloni-Giacobino, A., R. Grimble, and C. Pichard, *Genomic interactions with disease and nutrition*. Clinical nutrition, 2003. **22**(6): p. 507-14.
36. Talmud, P.J. and D.M. Waterworth, *In-vivo and in-vitro nutrient-gene interactions*. Current opinion in lipidology, 2000. **11**(1): p. 31-6.
37. Shen, J., et al., *Association of vitamin B-6 status with inflammation, oxidative stress, and chronic inflammatory conditions: the Boston Puerto Rican Health Study*. The American journal of clinical nutrition, 2010. **91**(2): p. 337-42.

38. Mattei, J., et al., *Apolipoprotein A5 polymorphisms interact with total dietary fat intake in association with markers of metabolic syndrome in Puerto Rican older adults*. The Journal of nutrition, 2009. **139**(12): p. 2301-8.
39. Mattei, J., et al., *Disparities in allele frequencies and population differentiation for 101 disease-associated single nucleotide polymorphisms between Puerto Ricans and non-Hispanic whites*. BMC genetics, 2009. **10**: p. 45.
40. Lai, C.Q., et al., *Population admixture associated with disease prevalence in the Boston Puerto Rican health study*. Human genetics, 2009. **125**(2): p. 199-209.
41. Barrett, J.C., et al., *Haploview: analysis and visualization of LD and haplotype maps*. Bioinformatics, 2005. **21**(2): p. 263-5.
42. Wigginton, J.E., D.J. Cutler, and G.R. Abecasis, *A note on exact tests of Hardy-Weinberg equilibrium*. American journal of human genetics, 2005. **76**(5): p. 887-93.
43. Elosua, R., et al., *Variants at the APOA5 locus, association with carotid atherosclerosis, and modification by obesity: the Framingham Study*. Journal of lipid research, 2006. **47**(5): p. 990-6.
44. Kawamoto, R., et al., *An association between body mass index and estimated glomerular filtration rate*. Hypertension research : official journal of the Japanese Society of Hypertension, 2008. **31**(8): p. 1559-64.
45. Lo, J.C., et al., *GFR, body mass index, and low high-density lipoprotein concentration in adults with and without CKD*. American journal of kidney diseases : the official journal of the National Kidney Foundation, 2007. **50**(4): p. 552-8.
46. Smith, C.E., et al., *Apolipoprotein A5 and lipoprotein lipase interact to modulate anthropometric measures in Hispanics of Caribbean origin*. Obesity, 2010. **18**(2): p. 327-32.
47. Vattikuti, S., J. Guo, and C.C. Chow, *Heritability and genetic correlations explained by common SNPs for metabolic syndrome traits*. PLoS genetics, 2012. **8**(3): p. e1002637.
48. Manning, A.K., et al., *A genome-wide approach accounting for body mass index identifies genetic variants influencing fasting glycemic traits and insulin resistance*. Nature genetics, 2012.
49. Deo, R.C., et al., *Genetic differences between the determinants of lipid profile phenotypes in African and European Americans: the Jackson Heart Study*. PLoS genetics, 2009. **5**(1): p. e1000342.
50. Willer, C.J., et al., *Newly identified loci that influence lipid concentrations and risk of coronary artery disease*. Nature genetics, 2008. **40**(2): p. 161-9.
51. Ken-Dror, G., U. Goldbourt, and R. Dankner, *Different effects of apolipoprotein A5 SNPs and haplotypes on triglyceride concentration in three ethnic origins*. Journal of human genetics, 2010. **55**(5): p. 300-7.
52. Kothari, P.H.G., C.C; Davila-Roman, V.G., *Evaluation of PGC1alpha Gene variants in Modulating Hypertensive Heart Disease Phenotypes*: St. Louis, MO.
53. Rankinen, T., et al., *The human obesity gene map: the 2005 update*. Obesity, 2006. **14**(4): p. 529-644.
54. Sarzynski, M.A., et al., *Associations of markers in 11 obesity candidate genes with maximal weight loss and weight regain in the SOS bariatric surgery cases*. International journal of obesity, 2011. **35**(5): p. 676-83.
55. *dbSNP* NCBI.
56. Science, W.I.o. *The GeneCards Human Gene Database*. GeneCards 1996-2012 [cited 2012 5/12/2012]; Available from: www.genecards.org.

SUMMARY AND DISCUSSION

Nutritional Determinants of Disease

ω-3 Fat and Serum Lipid Concentration

In our investigation, dietary ω-3 fatty acids showed an inverse relationship with total serum cholesterol. We did not find a significant association with individual dietary ω-3 fatty acids and low density lipoprotein cholesterol (LDL-C) or triglycerides (TG). Other studies have shown negative associations between LDL-C and dietary ω-3 and strong positive associations with high density lipoprotein (HDL-C) and dietary ω-3 [64].

American Heart Association recommendations for marine sources of ω-3fatty acids highlight fatty fish like salmon or trout as good sources of ω-3[53]. Catfish has been considered a poor source of ω-3 [200, 201]. A report from the Agency for Healthcare Research and Quality on ω-3 fatty acids also characterizes the docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) content of catfish as low [45]. It is not clear if quantity of consumption can compensate for omega-3 quality/quantity of certain fish. Fried fish (unknown white fish) or fried catfish were the top contributors to omega-3 intake among JHS participants.

Issues in capturing nutrient content

The Nutrition Data System for Research (NDSR) showed catfish and trout as having high content of the marine based ω-3 DPA. Many of the entries we examined for ω-3 content of fish in databases and published articles didn't report DPA. Further investigation of ω-3 content of catfish showed variable estimates. The USDA nutrient database provides nutrient values for multiple species and cooking preparations of catfish. The database

results showed that catfish prepared by deep-frying had lower ω-3 content than catfish prepared by other cooking methods, including battered and pan fried. The Food and Drug Administration (FDA) documents 37 families of catfish and multiple strains or types within those subgroups.[202]. There is some evidence that ω-3 content of wild catfish differs by type and season caught [203]. Wild catfish can be found in Mississippi rivers, lakes and public and private ponds where they are often stocked [204]. Thus, it is possible that many JHS study participants consumed wild catfish. However, this is not likely to contribute greatly to total intake. This level of detail as to wild vs. farm-raised catfish was not included in the JHS nutrient dataset. Therefore, we used a default of channel catfish, the most common farm-raised catfish [202]. This provides a possible source or error in our study.

Furthermore, the major preparation of catfish is fried. The frying process results in loss of water and increase in fat content of foods, thus changing the nutrient content of foods [205]. Thus, frying can result in both losses and gains of nutrients during cooking [206]. The research on the effect of cooking preparations on ω-3 content of fish is limited. However, some studies suggest that the effect of frying on ω-3 content is dictated by the type of fish and oil used to fry[207]. Olive-oil, canola oil and sunflower oil when used for frying may have less of the negative effects on nutrition and health associated with frying [205-208]. Investigation on the type of oils used for frying and the effect on the nutrient content and subsequent effect on health are needed in JHS.

Trans Fat Intake and Status

A recent NHANES study reports the intake of *trans* fat (TFA) in the U.S. is decreasing [209]. This is attributed to policy changes mandating labeling of foods, restaurants bans on TFA in some cities and the media attention to the negative effects of TFA on health [57, 62, 63, 209, 210]. TFA is prevalent in the U.S. food supply. Major contributors to TFA intake include fast food, baked goods, salty snacks and partially hydrogenated vegetable oils[145]. Studies show a deleterious effect of TFA on health [64, 211]. TFA are associated with increase in atherosclerosis, LDL-C, systemic inflammation, endothelial dysfunction, thrombogenesis and glucose intolerance [58, 65, 209, 212]. Given these negative effects and the non-essential nature of TFA many have called for an elimination of all TFA from the human diet[61].

Given the volume of literature and media attention dedicated to the negative effects of TFA, it is surprising to find a potential benefit to the intake of TFA. Natural forms of TFA, produced by bacteria, can be found in meats, butter and dairy products [68, 69]. We found a positive association between 16:1t and HDL-C. 16:1t was also correlated with fast food, margarine and meat. It was the only TFA correlated with meat. Mozaffarian et al, also reported beneficial association with 16:1t and insulin resistance, despite negative associations between total TFA and insulin resistance [66]. However, a review by Brouwer et all suggests that "all fatty acids with a double bond in the *trans* configuration raise the ratio of plasma LDL-C to HDL-C" [140]. Failure to see a positive association between TFA and LDL-C or TG may due to the limited sample size. Post-hoc calibration of the nutrient database for the short FFQ form in the Jackson Heart Study may improve the correlation between the SFFQ and plasma phospholipid TFA. Analysis in the entire cohort is important to confirm these findings.

Genetic Determinants of Disease

There are no magic bullets in health

Single gene diseases known as Mendelian diseases are diseases that result from a genetic change in a single gene. Complex diseases are those whose susceptibility involves the contribution of many genes and their relationships with molecular, biochemical, physiologic and physical environments [213]. Understanding the genetic foundation of complex diseases has been difficult. Many polymorphisms and diseases have failed to be replicated in other studies. Genotypic frequency, other modes of inheritance (mitochondrial, imprinting, anticipation), incomplete penetrance, phenocopy, genetic heterogeneity, polygenic inheritance, population substructure, differences of recombination, high frequency of the disease causing allele, different environmental exposures, interactions with other genes, interactions with environment, and differences in study design have all been named possible reasons for this inconsistency [214, 215].

There are some genes whose importance to complex disease, like apolipoprotein-E (*APOE)*, has been highly consistent. Associations between *APOE* and CVD have not only been shown in multiple populations, but the effects have been shown across multiple phenotypes [77-80]. We cannot expect for all genetic variants to have as large and as consistent an effect as we see with *APOE*. The aforementioned issues related to understanding the genetics of complex diseases provide a significant obstacle for reproducing results. Therefore absence of reproducibility of results in every population does not make results invalid in a single population.

Exploration without vision just gets you lost

One of the seven criteria of Hill's causal criteria is biologic plausibility. In genetic epidemiology, many single nucleotide polymorphisms (SNP) discovery studies allow researchers to identify SNPs associated with disease without considering biologic plausibility. Geneticists have used gene-mapping to identify variants associated with phenotypic traits for a long time [216]. Exploratory research is common in many fields of study. For example, in nutritional epidemiology, nutritionists use cluster analysis and principal component analysis to derive previously unknown dietary patterns. However, after the exploration, it is important to consider biochemical and molecular mechanisms underlying the associations. Bioinformatic methods, like pathway and network analyses, some of which use linear programming to provide visual frameworks of all variables in a system, are a start. However, functional studies in cell and animal models are needed to truly implicate a gene on a biological level.

All eggs in the GWAS basket

With the completion of the human genome project came the unprecedented possibility to identify the underlying genetic causes of disease. However, the methods and computational power are still being developed and updated. Statistical methods that had been employed for Mendelian diseases were the starting point. These methods included heritability and linkage analysis. The development of genome-wide analysis (GWAS) was developed following the human genome project and has increasingly dominated the genetic epidemiologic literature. Concurrently, linkage studies have become increasingly rare [216-218] making it challenging to secure funding for linkage studies and publication of linkage results. Popularity of GWAS over linkage has been blamed on the

inconsistency of results of linkage due to problems of unknown inheritance patterns and cost associated with family based studies.

Should Linkage be dead?

Are there any benefits to linkage? In an article published about the relative merit of linkage analysis, Burton et al points out the presence of 1) Mendelian forms of complex disease traits do exist and 2) genes of modest effect are amenable to the linkage approach of complex traits [86]. Examples of Mendelian subforms of complex disease are Alzheimer's disease (beta-amyloid precursor protein and presenilin-1 and 2) and breast cancer (*BRCA-1* and *BRCA-2*) [86].

Burton goes on to explain that knowledge of these rare subforms could provide insight on more common genetic variants as in the case of the primary open-angle glaucoma genes. In addition, Burton provides *APOE*, which was identified by linkage, not association mapping, as an example of a common disease allele of modest effect. They suggest the best approach incorporates an integration of the two, ex: a linkage test using genome wide scans followed by association testing in significant regions.

In a publication on the Genetic Analysis Workshop (GAW) group 15, Knight et al showed linkage studies have increased ability to capture epistatic effects when ascertainment of extreme quantitative values is an issue [219]. Furthermore, they found that linkage models had the power to detect other modes of inheritance (maternal effect, imprinting etc.).

This suggests that there remains an intrinsic value of linkage analysis. Synergism from linkage and associations approaches may provide the best method for investigation of the genetic determinants of disease.

Gene-Nutrient Interactions: An example of it depends!

A statistical interaction occurs when the relationship between two variables is dependent on a third variable. Thus, when an interaction is present, the relationship between two variables can be qualified by "**it depends**". Gene-nutrient interactions have been well studied in dyslipidemia. However, research including the presence of gene-nutrient interactions in outcomes affecting African Americans is limited.

We found significant interactions with percent fat intake and rs662799 (apolipoprotein-A5 (*APOA5*)) in HDL-C and rs2970869 (peroxisome proliferator-activated receptor gamma -1 alpha, (*PPARGC1A*)) in TC. These findings suggest that inclusion of dietary fat in models of the relationship between rs662799 (*APOA5*) or rs2970869 (*PPARGC1A*) and serum cholesterol may be important. In our linkage analysis, we failed to show consistent results in modeling of serum lipid outcomes. Future studies may want to include adjustments for diet in phenotypes previously shown to have interactions in modeling of genetic studies.

The importance of Research on Health Disparities

On January 14, 2011, the U.S. Centers for Disease Control and Prevention (CDC) released a health disparities and inequality report which stated, "…black men and women are much more likely to die of heart disease and stroke than their white counterparts. Coronary heart disease and stroke are not only leading causes of death in the United

States but also account for the largest proportion of inequality in life expectancy between whites and blacks, despite the existence of low-cost, highly effective preventive treatment" [220]. A key concept in health disparities research is that differences in disease burdens are thought to be preventable. Through research to better understand the cause(s) of these differences in health between populations we can administer treatments, conduct programs and enact policy to prevent them. The presence of health disparities in the U.S. can be defined as a health inequality. If we believe that health and healthcare are rights then these inequalities are classified as health inequities [87, 221-225].

Health disparities, inequalities and inequities are a part of a larger debate on healthcare and policy change on a national level. Regardless of the value judgment of health and healthcare as a right, there is another argument for health disparity research. Differences in health can minimally be attributed to differences in exposure to a variety of different factors; healthcare, diet, environment, income, education, genetics, physical activity and other diseases. Quantifying all of the potential contributors to disease in a way that is meaningful to population based research is difficult. Research on the relationship between an exposure and disease that focuses on one segment of the population limits the understanding of the relationship to one portion of the exposure and disease relationship. In a single study, this makes sense as it can reduce the noise associated with statistical modeling. However, if the entire field of research is predominantly based on a single segment of the population, the knowledge of understanding is being unnecessarily limited. In order for science to advance in any number of exposure-disease relationships, research on the full range of exposures, systems in which they exist, contributing factors and range of outcomes is needed.

Some of our findings differed from those previously reported. We also had differences in our genetic and dietary exposures, as well as differences in covariates and outcomes. The interpretations of our findings are speculative, as the research is limited. The gaps in knowledge uncovered provide an opportunity for future research and advancement of knowledge.

Future Research

Our findings provide many questions to be answered by additional studies. Research including food analysis of the ω-3 content of catfish, with additional survey questions about the sources of catfish (wild or farm-raised) consumed by JHS participants would be helpful in defining the ω-3 exposure in this population. Furthermore, studies on the effect of different cooking processes, using the commonly used oils and methods, and subsequent effects on nutrient content of fish and other foods would also be useful.

Post-hoc calibration of the SFFQ with data from dietary recalls and the LFFQ may allow for validation of the short FFQ with plasma phospholipid TFA [226]. This step is important for future research on TFA in JHS.

Additional genetic analysis using variance component linkage, GWAS and combined methods is essential to confirmation of linkage and association results. Possible modeling of interactions within linkage and association tests should also be considered. In addition, studies looking at the effect of ancestry on these relationships are needed.

Study of the differential effects of genes, environmental factors, like nutrients and the synergistic and antagonistic effects between them is complex. New methods, like pathway analysis and network analysis have been developed to try and provide visual

frameworks of the statistical relationships between multiple variables associated with a disease. Such methods should be employed to the understanding of the genetic and nutritional determinants of dyslipidemia in African Americans.

In conclusion, our findings are cross-sectional and provide only a start to understanding the relationship between dietary fat, genes and serum lipids in JHS participants. Longitudinal analysis including dietary intake, serum lipid outcomes and covariates are needed to further this understanding. In addition, the Jackson Heart Study represents one geographical segment of the African American population. Additional analyses in other African American populations, as well as other ethnic populations are needed to establish the consistency of these relationships given the full range of exposures, outcomes and physiological and physical environments. These analyses can help us further the science of dyslipidemia and CVD. However, R.W. Smithells in a paper on folate reminds us that "knowledge of all contributory factors is not essential for prevention"[227]. Thus, identification of some contributing factors, like dietary fat intake and dyslipidemia, may be sufficient to support implementation of preventative programs for CVD in this population, and health disparities for CVD in Americans.

1. Booker, C.S. and J.I. Mann, *Trans fatty acids and cardiovascular health: translation of the evidence base*. Nutrition, metabolism, and cardiovascular diseases : NMCD, 2008. **18**(6): p. 448-56.
2. Lichtenstein, A.H., et al., *Diet and lifestyle recommendations revision 2006: a scientific statement from the American Heart Association Nutrition Committee*. Circulation, 2006. **114**(1): p. 82-96.
3. Bosire, C.R., Jill; Krebs-Smith, Susan M.; , *Sources of Energy and Selected Nutrient Intakes Among the US Population, 2005-2006: A Report Prepared for the 2010 Dietary Guidelines Advisory Committee*, 2009, Risk Factor Monitoring and Methods Branch: National Cancer Institute.
4. Gebauer, S.K., et al., *n-3 fatty acid dietary recommendations and food sources to achieve essentiality and cardiovascular benefits*. The American journal of clinical nutrition, 2006. **83**(6 Suppl): p. 1526S-1535S.
5. Balk, E., et al., *Effects of omega-3 fatty acids on cardiovascular risk factors and intermediate markers of cardiovascular disease*. Evidence report/technology assessment, 2004(93): p. 1-6.
6. Safety, E.C.f.C.R.a.F., *Catfish Risk Profile*, 2010-2011.
7. Nettleton, J.A. and J. Exler, *Nutrients in Wild and Farmed Fish and Shellfish*. Journal of Food Science, 1992. **57**(2): p. 257-260.
8. Mississippi Wildlife, F.a.P.S. *Fishing and Boating*. 2012 [cited 2011 11/01/2011]; Available from: http://www.mdwfp.com/media-relations/press-releases/fishing-boating/mdwfp-and-mdeq-release-fish-to-repopulate-lower-pearl-river.aspx.
9. Pokorn, J., J. Panek, and L. Trojakova, *Effect of food component changes during frying on the nutrition value of fried food*. Forum of nutrition, 2003. **56**: p. 348-50.
10. Fillion, L. and C.J. Henry, *Nutrient losses and gains during frying: a review*. International journal of food sciences and nutrition, 1998. **49**(2): p. 157-68.
11. Ansorena, D., et al., *Effect of fish and oil nature on frying process and nutritional product quality*. Journal of Food Science, 2010. **75**(2): p. H62-7.
12. Guallar-Castillon, P., et al., *Consumption of fried foods and risk of coronary heart disease: Spanish cohort of the European Prospective Investigation into Cancer and Nutrition study*. BMJ, 2012. **344**: p. e363.
13. Vesper, H.W., et al., *Levels of plasma trans-fatty acids in non-Hispanic white adults in the United States in 2000 and 2009*. JAMA : the journal of the American Medical Association, 2012. **307**(6): p. 562-3.
14. Lopez-Garcia, E., et al., *Consumption of trans fatty acids is related to plasma biomarkers of inflammation and endothelial dysfunction*. The Journal of nutrition, 2005. **135**(3): p. 562-6.
15. Mello, M.M., *New York City's war on fat*. The New England journal of medicine, 2009. **360**(19): p. 2015-20.
16. Mozaffarian, D. and M.J. Stampfer, *Removing industrial trans fat from foods*. BMJ, 2010. **340**: p. c1826.
17. Van Camp, D., N.H. Hooker, and C.T. Lin, *Changes in fat contents of US snack foods in response to mandatory trans fat labelling*. Public health nutrition, 2012: p. 1-8.
18. Lemar, L. *Fatty Acid Data in the USDA National Nutrient Databank: Data handling and currency issues*. in *32nd Nutrient Databank Conference*. 2008. USDA.
19. Mozaffarian, D. and W.C. Willett, *Trans fatty acids and cardiovascular risk: a unique cardiometabolic imprint?* Current atherosclerosis reports, 2007. **9**(6): p. 486-93.
20. Lemaitre, R.N., et al., *Trans-fatty acids and sudden cardiac death*. Atherosclerosis. Supplements, 2006. **7**(2): p. 13-5.

21. Micha, R. and D. Mozaffarian, *Trans fatty acids: effects on metabolic syndrome, heart disease and diabetes*. Nature reviews. Endocrinology, 2009. **5**(6): p. 335-44.
22. Mozaffarian, D., *Trans fatty acids - effects on systemic inflammation and endothelial function*. Atherosclerosis. Supplements, 2006. **7**(2): p. 29-32.
23. Coombes, R., *Trans fats: chasing a global ban*. BMJ, 2011. **343**: p. d5567.
24. Jakobsen, M.U., et al., *Intake of ruminant trans fatty acids in the Danish population aged 1-80 years*. European journal of clinical nutrition, 2006. **60**(3): p. 312-8.
25. Jakobsen, M.U., et al., *Intake of ruminant trans fatty acids and risk of coronary heart disease*. International journal of epidemiology, 2008. **37**(1): p. 173-82.
26. Mozaffarian, D., et al., *Trans-palmitoleic acid, metabolic risk factors, and new-onset diabetes in U.S. adults: a cohort study*. Annals of internal medicine, 2010. **153**(12): p. 790-9.
27. Brouwer, I.A., A.J. Wanders, and M.B. Katan, *Effect of animal and industrial trans fatty acids on HDL and LDL cholesterol levels in humans--a quantitative review*. PloS one, 2010. **5**(3): p. e9434.
28. Craig, J., *Complex Diseases: Research and Applications*. Nature Education, 2008. **1**(1).
29. Baron, M., *The search for complex disease genes: fault by linkage or fault by association?* Molecular psychiatry, 2001. **6**(2): p. 143-9.
30. Menzel, S., *Genetic and molecular analyses of complex metabolic disorders: genetic linkage*. Annals of the New York Academy of Sciences, 2002. **967**: p. 249-57.
31. Erkkila, A.T., et al., *APOE polymorphism and the hypertriglyceridemic effect of dietary sucrose*. The American journal of clinical nutrition, 2001. **73**(4): p. 746-52.
32. Moreno, J.A., et al., *Apolipoprotein E gene promoter -219G->T polymorphism increases LDL-cholesterol concentrations and susceptibility to oxidation in response to a diet rich in saturated fat*. The American journal of clinical nutrition, 2004. **80**(5): p. 1404-9.
33. Rubin, J. and L. Berglund, *Apolipoprotein E and diets: a case of gene-nutrient interaction?* Current opinion in lipidology, 2002. **13**(1): p. 25-32.
34. Seripa, D., et al., *The genetics of the human APOE polymorphism*. Rejuvenation research, 2011. **14**(5): p. 491-500.
35. Lander, E.S. and N.J. Schork, *Genetic dissection of complex traits*. Science, 1994. **265**(5181): p. 2037-48.
36. Bush, W.S. and J. Haines, *Overview of linkage analysis in complex traits*. Current protocols in human genetics / editorial board, Jonathan L. Haines ... [et al.], 2010. **Chapter 1**: p. Unit 1 9 1-18.
37. Kovac, I.P., et al., *Linkage and association analyses of type 2 diabetes/impaired glucose metabolism and adiponectin serum levels in Japanese Americans from Hawaii*. Diabetes, 2007. **56**(2): p. 537-40.
38. Burton, P.R., M.D. Tobin, and J.L. Hopper, *Key concepts in genetic epidemiology*. Lancet, 2005. **366**(9489): p. 941-51.
39. Knight, S., H.W. Uh, and M. Martinez, *Summary of contributions to GAW Group 15: family-based samples are useful in identifying common polymorphisms associated with complex traits*. Genetic epidemiology, 2009. **33 Suppl 1**: p. S99-104.
40. Frieden, T.R., *Forward: CDC Health Disparities and Inequalities Report - United States, 2011*. MMWR. Surveillance summaries : Morbidity and mortality weekly report. Surveillance summaries / CDC, 2011. **60 Suppl**: p. 1-2.
41. Braveman, P., *Health disparities and health equity: concepts and measurement*. Annual review of public health, 2006. **27**: p. 167-94.
42. Braveman, P. and C. Cubbin, *Optimal SES indicators cannot be prescribed across all outcomes*. American journal of public health, 2003. **93**(1): p. 12-3; author reply 13.
43. Braveman, P. and S. Gruskin, *Poverty, equity, human rights and health*. Bulletin of the World Health Organization, 2003. **81**(7): p. 539-45.

44. Braveman, P. and S. Gruskin, *Defining equity in health*. Journal of epidemiology and community health, 2003. **57**(4): p. 254-8.
45. Braveman, P.A., *Monitoring equity in health and healthcare: a conceptual framework*. Journal of health, population, and nutrition, 2003. **21**(3): p. 181-92.
46. Carter-Pokras, O. and C. Baquet, *What is a "health disparity"?* Public health reports, 2002. **117**(5): p. 426-34.
47. Spiegelman, D., A. McDermott, and B. Rosner, *Regression calibration method for correcting measurement-error bias in nutritional epidemiology*. The American journal of clinical nutrition, 1997. **65**(4 Suppl): p. 1179S-1186S.
48. Smithells, R.W., S. Sheppard, and C.J. Schorah, *Vitamin dificiencies and neural tube defects*. Archives of disease in childhood, 1976. **51**(12): p. 944-50.

Bibliography

1. Lloyd-Jones, D., et al., *Heart disease and stroke statistics--2009 update: a report from the American Heart Association Statistics Committee and Stroke Statistics Subcommittee.* Circulation, 2009. **119**(3): p. e21-181.
2. Minino, A.M., *Death in the United States, 2009.* NCHS data brief, 2011(64): p. 1-8.
3. Minino, A.M., et al., *Deaths: final data for 2004.* National vital statistics reports : from the Centers for Disease Control and Prevention, National Center for Health Statistics, National Vital Statistics System, 2007. **55**(19): p. 1-119.
4. Lloyd-Jones, D., et al., *Heart disease and stroke statistics--2009 update: a report from the American Heart Association Statistics Committee and Stroke Statistics Subcommittee.* Circulation, 2009. **119**(3): p. 480-6.
5. Lloyd-Jones, D., et al., *Heart disease and stroke statistics--2010 update: a report from the American Heart Association.* Circulation, 2010. **121**(7): p. e46-e215.
6. Mokdad, A.H., D.F. Stroup, and W.H. Giles, *Public health surveillance for behavioral risk factors in a changing environment. Recommendations from the Behavioral Risk Factor Surveillance Team.* MMWR. Recommendations and reports : Morbidity and mortality weekly report. Recommendations and reports / Centers for Disease Control, 2003. **52**(RR-9): p. 1-12.
7. Akil, L. and H.A. Ahmad, *Relationships between obesity and cardiovascular diseases in four southern states and Colorado.* Journal of health care for the poor and underserved, 2011. **22**(4 Suppl): p. 61-72.
8. Ahluwalia, I.B., et al., *State-specific prevalence of selected chronic disease-related characteristics--Behavioral Risk Factor Surveillance System, 2001.* MMWR. Surveillance summaries : Morbidity and mortality weekly report. Surveillance summaries / CDC, 2003. **52**(8): p. 1-80.
9. Budoff, M.J., et al., *Ethnic differences of the presence and severity of coronary atherosclerosis.* Atherosclerosis, 2006. **187**(2): p. 343-50.
10. Roger, V.L., et al., *Heart disease and stroke statistics--2011 update: a report from the American Heart Association.* Circulation, 2011. **123**(4): p. e18-e209.
11. Roger, V.L., et al., *Heart disease and stroke statistics--2012 update: a report from the American Heart Association.* Circulation, 2012. **125**(1): p. e2-e220.
12. Harris-Hooker, S. and G.L. Sanford, *Lipids, lipoproteins and coronary heart disease in minority populations.* Atherosclerosis, 1994. **108 Suppl**: p. S83-104.
13. Hemminki, K., J. Lorenzo Bermejo, and A. Forsti, *The balance between heritable and environmental aetiology of human disease.* Nat Rev Genet, 2006. **7**(12): p. 958-965.
14. Ordovas, J.M., *The quest for cardiovascular health in the genomic era: nutrigenetics and plasma lipoproteins.* The Proceedings of the Nutrition Society, 2004. **63**(1): p. 145-52.
15. Ordovas, J.M. and D. Corella, *Nutritional genomics.* Annual review of genomics and human genetics, 2004. **5**: p. 71-118.
16. Ordovas, J.M. and V. Mooser, *Nutrigenomics and nutrigenetics.* Current opinion in lipidology, 2004. **15**(2): p. 101-8.
17. Paoloni-Giacobino, A., R. Grimble, and C. Pichard, *Genetics and nutrition.* Clinical nutrition, 2003. **22**(5): p. 429-35.
18. Roger, V.L., et al., *Executive summary: heart disease and stroke statistics--2012 update: a report from the American Heart Association.* Circulation, 2012. **125**(1): p. 188-97.

19. Egan, B.M., Y. Zhao, and R.N. Axon, *US trends in prevalence, awareness, treatment, and control of hypertension, 1988-2008.* JAMA : the journal of the American Medical Association, 2010. **303**(20): p. 2043-50.
20. Health, N.H.L.a.B.I.P.S., *2012 NHLBI Morbidity and Mortality Chart Book.* 2012.
21. MMWR, *Prevalence of Coronary Heart Disease---United States, 2006-2010.* Morbidity and Mortality Weekly Report, 2011. **60**(40): p. 1377-1381.
22. *Diet, nutrition and the prevention of chronic diseases.* World Health Organization technical report series, 2003. **916**: p. i-viii, 1-149, backcover.
23. Lichtenstein, A.H., et al., *Summary of American Heart Association Diet and Lifestyle Recommendations revision 2006.* Arteriosclerosis, thrombosis, and vascular biology, 2006. **26**(10): p. 2186-91.
24. Sempos, C.T., et al., *Prevalence of high blood cholesterol among US adults. An update based on guidelines from the second report of the National Cholesterol Education Program Adult Treatment Panel.* JAMA : the journal of the American Medical Association, 1993. **269**(23): p. 3009-14.
25. Talayero, B.G. and F.M. Sacks, *The role of triglycerides in atherosclerosis.* Current cardiology reports, 2011. **13**(6): p. 544-52.
26. Yang, B., *A Change of Heart- How the people of Framingham, Massachusetts, Helped Unravel the Mysteries of Cardiovascular Disease.* Discovery Medicine, 2009. **5**(25): p. 104-1111.
27. Health, U.S.D.o.E.a.N.I.o. *Human Genome Project Information*. 2011 July 25, 2011 [cited 2012 5/30/2012]; Available from: www.ornl.gov/hgmis.
28. Collins, F.S., M. Morgan, and A. Patrinos, *The Human Genome Project: lessons from large-scale biology.* Science, 2003. **300**(5617): p. 286-90.
29. Collins, F.S., et al., *New goals for the U.S. Human Genome Project: 1998-2003.* Science, 1998. **282**(5389): p. 682-9.
30. Kaput, J., et al., *The case for strategic international alliances to harness nutritional genomics for public and personal health.* The British journal of nutrition, 2005. **94**(5): p. 623-32.
31. *Summary of the second report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel II).* JAMA : the journal of the American Medical Association, 1993. **269**(23): p. 3015-23.
32. Lewington, S., et al., *Blood cholesterol and vascular mortality by age, sex, and blood pressure: a meta-analysis of individual data from 61 prospective studies with 55,000 vascular deaths.* Lancet, 2007. **370**(9602): p. 1829-39.
33. Yang, Q., et al., *Trends in cardiovascular health metrics and associations with all-cause and CVD mortality among US adults.* JAMA : the journal of the American Medical Association, 2012. **307**(12): p. 1273-83.
34. *Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report.* Circulation, 2002. **106**(25): p. 3143-421.
35. Lepor, N.E. and R.E. Vogel, *Summary of the third report of the National Cholesterol Education Program Adult Treatment Panel III.* Reviews in cardiovascular medicine, 2001. **2**(3): p. 160-5.
36. Taylor, H.A., Jr., et al., *Dyslipidemia and the treatment of lipid disorders in African Americans.* The American journal of medicine, 2009. **122**(5): p. 454-63.

37. Morrison, J.A., et al., *Black-white differences in plasma lipids and lipoproteins in adults: The Cincinnati lipid research clinic population study.* Preventive medicine, 1979. **8**(1): p. 34-39.
38. Tyroler, H.A., et al., *Black-white differences in serum lipids and lipoproteins in Evans County.* Preventive medicine, 1975. **4**(4): p. 541-549.
39. Rodriguez, C., et al., *Comparison of modifiable determinants of lipids and lipoprotein levels among African-Americans, Hispanics, and Non-Hispanic Caucasians > or =65 years of age living in New York City.* The American journal of cardiology, 2002. **89**(2): p. 178-83.
40. Taylor, H., et al., *Distinct component profiles and high risk among African Americans with metabolic syndrome: the Jackson Heart Study.* Diabetes care, 2008. **31**(6): p. 1248-53.
41. Thomson, J.L., et al., *Food and beverage choices contributing to dietary guidelines adherence in the Lower Mississippi Delta.* Public health nutrition, 2011: p. 1-11.
42. McCabe-Sellers, B.J., et al., *Assessment of the diet quality of US adults in the Lower Mississippi Delta.* The American journal of clinical nutrition, 2007. **86**(3): p. 697-706.
43. Tucker, K.L., et al., *A regional food-frequency questionnaire for the US Mississippi Delta.* Public health nutrition, 2005. **8**(1): p. 87-96.
44. Talegawkar, S.A., et al., *Serum carotenoid and tocopherol concentrations vary by dietary pattern among African Americans.* Journal of the American Dietetic Association, 2008. **108**(12): p. 2013-20.
45. Balk, E., et al., *Effects of omega-3 fatty acids on cardiovascular risk factors and intermediate markers of cardiovascular disease.* Evidence report/technology assessment, 2004(93): p. 1-6.
46. Bernstein, A.M., et al., *A meta-analysis shows that docosahexaenoic acid from algal oil reduces serum triglycerides and increases HDL-cholesterol and LDL-cholesterol in persons without coronary heart disease.* The Journal of nutrition, 2012. **142**(1): p. 99-104.
47. Esposito, K., et al., *Effect of a mediterranean-style diet on endothelial dysfunction and markers of vascular inflammation in the metabolic syndrome: a randomized trial.* JAMA : the journal of the American Medical Association, 2004. **292**(12): p. 1440-6.
48. Harris, W.S., *n-3 Fatty acid fortification: opportunities and obstacles.* The British journal of nutrition, 2007. **97**(4): p. 593-5.
49. Hu, F.B. and J.E. Manson, *Omega-3 Fatty Acids and Secondary Prevention of Cardiovascular Disease--Is It Just a Fish Tale?: Comment on "Efficacy of Omega-3 Fatty Acid Supplements (Eicosapentaenoic Acid and Docosahexaenoic Acid) in the Secondary Prevention of Cardiovascular Disease".* Archives of internal medicine, 2012.
50. Kwak, S.M., et al., *Efficacy of Omega-3 Fatty Acid Supplements (Eicosapentaenoic Acid and Docosahexaenoic Acid) in the Secondary Prevention of Cardiovascular Disease: A Meta-analysis of Randomized, Double-blind, Placebo-Controlled Trials.* Archives of internal medicine, 2012.
51. Institute of Medicine (U.S.). Panel on Macronutrients. and Institute of Medicine (U.S.). Standing Committee on the Scientific Evaluation of Dietary Reference Intakes., *Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein, and amino acids*2005, Washington, DC: National Academies Press. xxv, 1331 p.
52. Thompson, T.G. and National Institutes of Health (U.S.), *HHS Secretary Thompson and USDA Secretary Veneman announce Dietary guidelines for Americans, 2005 edition*, 2005, National Institutes of Health,: Bethesda, Md.

53. Lichtenstein, A.H., et al., *Diet and lifestyle recommendations revision 2006: a scientific statement from the American Heart Association Nutrition Committee.* Circulation, 2006. **114**(1): p. 82-96.
54. Finnegan, Y.E., et al., *Plant and marine derived (n-3) polyunsaturated fatty acids do not affect blood coagulation and fibrinolytic factors in moderately hyperlipidemic humans.* The Journal of nutrition, 2003. **133**(7): p. 2210-3.
55. Hu, F.B., et al., *Fish and omega-3 fatty acid intake and risk of coronary heart disease in women.* JAMA : the journal of the American Medical Association, 2002. **287**(14): p. 1815-21.
56. Clifton, P.M., J.B. Keogh, and M. Noakes, *Trans fatty acids in adipose tissue and the food supply are associated with myocardial infarction.* The Journal of nutrition, 2004. **134**(4): p. 874-9.
57. Lopez-Garcia, E., et al., *Consumption of trans fatty acids is related to plasma biomarkers of inflammation and endothelial dysfunction.* The Journal of nutrition, 2005. **135**(3): p. 562-6.
58. Mozaffarian, D., *Trans fatty acids - effects on systemic inflammation and endothelial function.* Atherosclerosis. Supplements, 2006. **7**(2): p. 29-32.
59. Mozaffarian, D., et al., *Trans fatty acids and cardiovascular disease.* The New England journal of medicine, 2006. **354**(15): p. 1601-13.
60. Mensink, R.P., et al., *Effects of dietary fatty acids and carbohydrates on the ratio of serum total to HDL cholesterol and on serum lipids and apolipoproteins: a meta-analysis of 60 controlled trials.* The American journal of clinical nutrition, 2003. **77**(5): p. 1146-55.
61. Coombes, R., *Trans fats: chasing a global ban.* BMJ, 2011. **343**: p. d5567.
62. Mello, M.M., *New York City's war on fat.* The New England journal of medicine, 2009. **360**(19): p. 2015-20.
63. Van Camp, D., N.H. Hooker, and C.T. Lin, *Changes in fat contents of US snack foods in response to mandatory trans fat labelling.* Public health nutrition, 2012: p. 1-8.
64. Booker, C.S. and J.I. Mann, *Trans fatty acids and cardiovascular health: translation of the evidence base.* Nutrition, metabolism, and cardiovascular diseases : NMCD, 2008. **18**(6): p. 448-56.
65. Micha, R. and D. Mozaffarian, *Trans fatty acids: effects on metabolic syndrome, heart disease and diabetes.* Nature reviews. Endocrinology, 2009. **5**(6): p. 335-44.
66. Mozaffarian, D., et al., *Trans-palmitoleic acid, metabolic risk factors, and new-onset diabetes in U.S. adults: a cohort study.* Annals of internal medicine, 2010. **153**(12): p. 790-9.
67. Jakobsen, M.U., et al., *Intake of ruminant trans fatty acids and risk of coronary heart disease-an overview.* Atherosclerosis. Supplements, 2006. **7**(2): p. 9-11.
68. Jakobsen, M.U., et al., *Intake of ruminant trans fatty acids in the Danish population aged 1-80 years.* European journal of clinical nutrition, 2006. **60**(3): p. 312-8.
69. Jakobsen, M.U., et al., *Intake of ruminant trans fatty acids and risk of coronary heart disease.* International journal of epidemiology, 2008. **37**(1): p. 173-82.
70. Arnett, D.K., et al., *Relevance of genetics and genomics for prevention and treatment of cardiovascular disease: a scientific statement from the American Heart Association Council on Epidemiology and Prevention, the Stroke Council, and the Functional Genomics and Translational Biology Interdisciplinary Working Group.* Circulation, 2007. **115**(22): p. 2878-901.

71. Bentley, P., et al., *Causal relationship of susceptibility genes to ischemic stroke: comparison to ischemic heart disease and biochemical determinants.* PloS one, 2010. **5**(2): p. e9136.
72. Fan, A.Z., et al., *Gene polymorphisms in association with emerging cardiovascular risk markers in adult women.* BMC medical genetics, 2010. **11**: p. 6.
73. Campos, H., M. D'Agostino, and J.M. Ordovas, *Gene-diet interactions and plasma lipoproteins: role of apolipoprotein E and habitual saturated fat intake.* Genetic epidemiology, 2001. **20**(1): p. 117-128.
74. Chang, M.H., et al., *Genetic variants associated with fasting blood lipids in the U.S. population: Third National Health and Nutrition Examination Survey.* BMC medical genetics, 2010. **11**: p. 62.
75. Dong, C., et al., *Genetic loci for blood lipid levels identified by linkage and association analyses in Caribbean Hispanics.* Journal of lipid research, 2011. **52**(7): p. 1411-9.
76. Waterworth, D.M., et al., *Genetic variants influencing circulating lipid levels and risk of coronary artery disease.* Arteriosclerosis, thrombosis, and vascular biology, 2010. **30**(11): p. 2264-76.
77. Erkkila, A.T., et al., *APOE polymorphism and the hypertriglyceridemic effect of dietary sucrose.* The American journal of clinical nutrition, 2001. **73**(4): p. 746-52.
78. Moreno, J.A., et al., *Apolipoprotein E gene promoter -219G->T polymorphism increases LDL-cholesterol concentrations and susceptibility to oxidation in response to a diet rich in saturated fat.* The American journal of clinical nutrition, 2004. **80**(5): p. 1404-9.
79. Rubin, J. and L. Berglund, *Apolipoprotein E and diets: a case of gene-nutrient interaction?* Current opinion in lipidology, 2002. **13**(1): p. 25-32.
80. Seripa, D., et al., *The genetics of the human APOE polymorphism.* Rejuvenation research, 2011. **14**(5): p. 491-500.
81. Corella, D. and J.M. Ordovas, *Nutrigenomics in cardiovascular medicine.* Circulation. Cardiovascular genetics, 2009. **2**(6): p. 637-51.
82. Ordovas, J.M., *Nutrigenetics, plasma lipids, and cardiovascular risk.* Journal of the American Dietetic Association, 2006. **106**(7): p. 1074-81; quiz 1083.
83. Paoloni-Giacobino, A., R. Grimble, and C. Pichard, *Genomic interactions with disease and nutrition.* Clinical nutrition, 2003. **22**(6): p. 507-14.
84. Santos, J.L., et al., *Genotype-by-nutrient interactions assessed in European obese women. A case-only study.* European journal of nutrition, 2006. **45**(8): p. 454-62.
85. Talmud, P.J. and D.M. Waterworth, *In-vivo and in-vitro nutrient-gene interactions.* Current opinion in lipidology, 2000. **11**(1): p. 31-6.
86. Burton, P.R., M.D. Tobin, and J.L. Hopper, *Key concepts in genetic epidemiology.* Lancet, 2005. **366**(9489): p. 941-51.
87. Braveman, P., *Health disparities and health equity: concepts and measurement.* Annual review of public health, 2006. **27**: p. 167-94.
88. Taylor, H.A., Jr., *The Jackson Heart Study: an overview.* Ethnicity & disease, 2005. **15**(4 Suppl 6): p. S6-1-3.
89. Metcalf, P.A., et al., *African American-white differences in lipids, lipoproteins, and apolipoproteins, by educational attainment, among middle-aged adults: the Atherosclerosis Risk in Communities Study.* American journal of epidemiology, 1998. **148**(8): p. 750-60.
90. Newby, P.K., et al., *Race and region are associated with nutrient intakes among black and white men in the United States.* The Journal of nutrition, 2011. **141**(2): p. 296-303.

91. Vafeiadou, K., et al., *A review of the evidence for the effects of total dietary fat, saturated, monounsaturated and n-6 polyunsaturated fatty acids on vascular function, endothelial progenitor cells and microparticles.* The British journal of nutrition, 2012. **107**(3): p. 303-24.
92. Willett, W., *Nutritional epidemiology*. Monographs in epidemiology and biostatistics1990, New York: Oxford University Press. vii, 396 p.
93. Willett, W., *Nutritional epidemiology*. 2nd ed. Monographs in epidemiology and biostatistics1998, New York: Oxford University Press. xiv, 514 p.
94. Carithers, T., et al., *Dietary assessment in African Americans: methods used in the Jackson Heart Study.* Ethnicity & disease, 2005. **15**(4 Suppl 6): p. S6-49-55.
95. Boucher, B., et al., *Validity and reliability of the Block98 food-frequency questionnaire in a sample of Canadian women.* Public health nutrition, 2006. **9**(01): p. 84-93.
96. Willett, W.C., et al., *Reproducibility and validity of a semiquantitative food frequency questionnaire.* American journal of epidemiology, 1985. **122**(1): p. 51-65.
97. Talegawkar, S.A., et al., *Total antioxidant performance is associated with diet and serum antioxidants in participants of the diet and physical activity substudy of the Jackson Heart Study.* The Journal of nutrition, 2009. **139**(10): p. 1964-71.
98. Talegawkar, S.A., et al., *Carotenoid intakes, assessed by food-frequency questionnaires (FFQs), are associated with serum carotenoid concentrations in the Jackson Heart Study: validation of the Jackson Heart Study Delta NIRI Adult FFQs.* Public health nutrition, 2008. **11**(10): p. 989-97.
99. Carithers, T.C., et al., *Validity and calibration of food frequency questionnaires used with African-American adults in the Jackson Heart Study.* Journal of the American Dietetic Association, 2009. **109**(7): p. 1184-1193.
100. Fuqua, S.R., et al., *Recruiting African-American research participation in the Jackson Heart Study: methods, response rates, and sample description.* Ethnicity & disease, 2005. **15**(4 Suppl 6): p. S6-18-29.
101. Exler, J.L., Linda; Smith, Julie, *Fat and Fatty Acid Content of Selected Foods Containing Trans-Fatty Acids*, A.R.S.N.D. Laboratory, Editor 1989, 1993: Beltsville, MD 20705. p. 38.
102. Matthan, N.R., et al., *Long-term fatty acid stability in human serum cholesteryl ester, triglyceride, and phospholipid fractions.* Journal of lipid research, 2010. **51**(9): p. 2826-32.
103. Folch, J., M. Lees, and G.H. Sloane Stanley, *A simple method for the isolation and purification of total lipides from animal tissues.* The Journal of biological chemistry, 1957. **226**(1): p. 497-509.
104. Agren, J.J., A. Julkunen, and I. Penttila, *Rapid separation of serum lipids for fatty acid analysis by a single aminopropyl column.* Journal of lipid research, 1992. **33**(12): p. 1871-6.
105. Morrison, W.R. and L.M. Smith, *Preparation of Fatty Acid Methyl Esters and Dimethylacetals from Lipids with Boron Fluoride--Methanol.* Journal of lipid research, 1964. **5**: p. 600-8.
106. Hertogh, E.M., et al., *Validity of the modified Baecke questionnaire: comparison with energy expenditure according to the doubly labeled water method.* The international journal of behavioral nutrition and physical activity, 2008. **5**: p. 30.
107. Dubbert, P.M., et al., *Physical activity assessment methods in the Jackson Heart Study.* Ethnicity & disease, 2005. **15**(4 Suppl 6): p. S6-56-61.
108. Smitherman, T.A., et al., *Validation of the Jackson Heart Study Physical Activity Survey in African Americans.* Journal of physical activity & health, 2009. **6 Suppl 1**: p. S124-32.

109. Ainsworth, B.E., et al., *Evaluation of the kaiser physical activity survey in women.* Medicine and science in sports and exercise, 2000. **32**(7): p. 1327-38.
110. Richardson, M.T., et al., *Ability of the Atherosclerosis Risk in Communities (ARIC)/Baecke Questionnaire to assess leisure-time physical activity.* International journal of epidemiology, 1995. **24**(4): p. 685-93.
111. Carpenter, M.A., et al., *Laboratory, reading center, and coordinating center data management methods in the Jackson Heart Study.* The American journal of the medical sciences, 2004. **328**(3): p. 131-44.
112. Sun, Q., et al., *Comparison between plasma and erythrocyte fatty acid content as biomarkers of fatty acid intake in US women.* The American journal of clinical nutrition, 2007. **86**(1): p. 74-81.
113. (ARS), A.R.S., *USDA National Nutrient Database for Standard Reference*, 2011, USDA: Nutrient Data Lab.
114. Hu, F.B. and W.C. Willett, *Optimal diets for prevention of coronary heart disease.* JAMA : the journal of the American Medical Association, 2002. **288**(20): p. 2569-78.
115. Yokoyama, M., et al., *Effects of eicosapentaenoic acid on major coronary events in hypercholesterolaemic patients (JELIS): a randomised open-label, blinded endpoint analysis.* Lancet, 2007. **369**(9567): p. 1090-8.
116. Daviglus, M.L., et al., *Fish consumption and the 30-year risk of fatal myocardial infarction.* The New England journal of medicine, 1997. **336**(15): p. 1046-53.
117. Kaushik, M., et al., *Long-chain omega-3 fatty acids, fish intake, and the risk of type 2 diabetes mellitus.* The American journal of clinical nutrition, 2009. **90**(3): p. 613-20.
118. Carpentier, Y.A., L. Portois, and W.J. Malaisse, *n-3 fatty acids and the metabolic syndrome.* The American journal of clinical nutrition, 2006. **83**(6 Suppl): p. 1499S-1504S.
119. MacLean, C.H., et al., *Effects of omega-3 fatty acids on lipids and glycemic control in type II diabetes and the metabolic syndrome and on inflammatory bowel disease, rheumatoid arthritis, renal disease, systemic lupus erythematosus, and osteoporosis.* Evidence report/technology assessment, 2004(89): p. 1-4.
120. Wang, C., et al., *Effects of omega-3 fatty acids on cardiovascular disease.* Evidence report/technology assessment, 2004(94): p. 1-8.
121. Sirtori, C.R., et al., *One-year treatment with ethyl esters of n-3 fatty acids in patients with hypertriglyceridemia and glucose intolerance: reduced triglyceridemia, total cholesterol and increased HDL-C without glycemic alterations.* Atherosclerosis, 1998. **137**(2): p. 419-27.
122. Woodman, R.J., et al., *Effects of purified eicosapentaenoic and docosahexaenoic acids on glycemic control, blood pressure, and serum lipids in type 2 diabetic patients with treated hypertension.* The American journal of clinical nutrition, 2002. **76**(5): p. 1007-15.
123. Dunstan, D.W., et al., *The independent and combined effects of aerobic exercise and dietary fish intake on serum lipids and glycemic control in NIDDM. A randomized controlled study.* Diabetes care, 1997. **20**(6): p. 913-21.
124. Roger, V.G., AS; Lloyd-Jones, DM; Benjamin, EJ; Berry, JD; Borden, WB; Bravata, DM; Dai, S; Ford, ES; Fox, CS; Fullerton, HJ; Gillespie, C; Halipern, SM; Heit, JA; Howard, VJ; Kissela, BM; Kittner, SJ; Lackland, DT; Lichtman, JH, Lisabeth, LD; Makuc, DM; Marcus, GM; Marelli, A; Matchar, DB; Moy, CS; Mozaffarian, D; Mussolino, ME; Nichol, G; Paynter, NP; Soliman, EZ; Sorlie, PD; Sotoodehnia, N; Turan, TN, Virani, SS; Wong, ND; Woo, D; Turner, MB; on behalf of the American Heart Association Statistics Committee and STroke Statistics Subcommitee. , *Heart disease and stroke statistics--2012 update: a report from the American Heart Association.* Circulation 2012.

125. Benton, J.L., et al., *Predictors of low-density lipoprotein particle size in a high-risk African-American population.* The American journal of cardiology, 2005. **95**(11): p. 1320-3.
126. Liu, J., et al., *Impact of abdominal visceral and subcutaneous adipose tissue on cardiometabolic risk factors: the Jackson Heart Study.* The Journal of clinical endocrinology and metabolism, 2010. **95**(12): p. 5419-26.
127. Taylor, H.A., Jr., et al., *Toward resolution of cardiovascular health disparities in African Americans: design and methods of the Jackson Heart Study.* Ethnicity & disease, 2005. **15**(4 Suppl 6): p. S6-4-17.
128. Champagne, C.M., et al., *Dietary intake in the lower Mississippi delta region: results from the Foods of our Delta Study.* Journal of the American Dietetic Association, 2004. **104**(2): p. 199-207.
129. Gillum, R.F., M.E. Mussolino, and J.H. Madans, *Coronary heart disease risk factors and attributable risks in African-American women and men: NHANES I epidemiologic follow-up study.* American journal of public health, 1998. **88**(6): p. 913-7.
130. National High Blood Pressure Education Program. and National Heart Lung and Blood Institute., *Primary prevention of hypertension : clinical and public health advisory from the National High Blood Pressure Education Program*. NIH publication2002, Bethesda, Md.: National Institutes of Health, National Heart, Lung, and Blood Institute. vi, 17 p.
131. National High Blood Pressure Education Program. and National High Blood Pressure Education Program. Working Group on Hypertension and Chronic Renal Failure., *National High Blood Pressure Education Program (NHBPEP) working group report on hypertension and chronic renal*. NIH publication1990, Bethesda, Md.: U.S. Dept. of Health and Human Services, Public Health Service, National Institutes of Health, National Heart, Lung, and Blood Institute. viii, 20 p.
132. National High Blood Pressure Education Program. Working Group on Ambulatory Blood Pressure Monitoring. and National Heart Lung and Blood Institute., *National High Blood Pressure Education Program (NHBPEP) Working Group report on ambulatory blood pressure monitoring*. NIH publication1990, Bethesda, Md.: U.S. Dept. of Health and Human Services, Public Health Service, National Institutes of Health, National Heart, Lung, and Blood Institute. vi, 21 p.
133. National High Blood Pressure Education Program. Working Group on Hypertension in Diabetes., *National High Blood Pressure Education Program Working Group report on hypertension in diabetes*, in *NIH publication no 94-3530*1994, U.S. Dept. of Health and Human Services, Public Health Service, National Institutes of Health, National Heart, Lung, and Blood Institute,: Bethesda, Md.
134. *Standards of medical care in diabetes.* Diabetes care, 2004. **27 Suppl 1**: p. S15-35.
135. Jacobson, T.A., et al., *Effects of eicosapentaenoic acid and docosahexaenoic acid on low-density lipoprotein cholesterol and other lipids: a review.* Journal of clinical lipidology, 2012. **6**(1): p. 5-18.
136. Wei, M.Y. and T.A. Jacobson, *Effects of eicosapentaenoic acid versus docosahexaenoic acid on serum lipids: a systematic review and meta-analysis.* Current atherosclerosis reports, 2011. **13**(6): p. 474-83.
137. Tardy, A.L., et al., *Dairy and industrial sources of trans fat do not impair peripheral insulin sensitivity in overweight women.* The American journal of clinical nutrition, 2009. **90**(1): p. 88-94.
138. Chardigny, J.M., et al., *Do trans fatty acids from industrially produced sources and from natural sources have the same effect on cardiovascular disease risk factors in healthy*

subjects? Results of the trans Fatty Acids Collaboration (TRANSFACT) study. The American journal of clinical nutrition, 2008. **87**(3): p. 558-66.
139. Motard-Belanger, A., et al., *Study of the effect of trans fatty acids from ruminants on blood lipids and other risk factors for cardiovascular disease.* The American journal of clinical nutrition, 2008. **87**(3): p. 593-9.
140. Brouwer, I.A., A.J. Wanders, and M.B. Katan, *Effect of animal and industrial trans fatty acids on HDL and LDL cholesterol levels in humans--a quantitative review.* PloS one, 2010. **5**(3): p. e9434.
141. Lemaitre, R.N., et al., *Plasma phospholipid trans fatty acids, fatal ischemic heart disease, and sudden cardiac death in older adults: the cardiovascular health study.* Circulation, 2006. **114**(3): p. 209-15.
142. Kuhnt, K., et al., *Trans fatty acid isomers and the trans-9/trans-11 index in fat containing foods.* European journal of lipid science and technology : EJLST, 2011. **113**(10): p. 1281-1292.
143. Micha, R., et al., *Food sources of individual plasma phospholipid trans fatty acid isomers: the Cardiovascular Health Study.* The American journal of clinical nutrition, 2010. **91**(4): p. 883-93.
144. Mozaffarian, D., M.F. Jacobson, and J.S. Greenstein, *Food reformulations to reduce trans fatty acids.* The New England journal of medicine, 2010. **362**(21): p. 2037-9.
145. Lemar, L. *Fatty Acid Data in the USDA National Nutrient Databank: Data handling and currency issues*. in *32nd Nutrient Databank Conference*. 2008. USDA.
146. Szabo, E., et al., *trans Octadecenoic acid and trans octadecadienoic acid are inversely related to long-chain polyunsaturates in human milk: results of a large birth cohort study.* The American journal of clinical nutrition, 2007. **85**(5): p. 1320-6.
147. Artaud-Wild, S.M., et al., *Differences in coronary mortality can be explained by differences in cholesterol and saturated fat intakes in 40 countries but not in France and Finland. A paradox.* Circulation, 1993. **88**(6): p. 2771-9.
148. Mozaffarian, D., *Low-fat diet and cardiovascular disease.* JAMA : the journal of the American Medical Association, 2006. **296**(3): p. 279-80; author reply 280-1.
149. Ariza, M.J., et al., *Additive effects of LPL, APOA5 and APOE variant combinations on triglyceride levels and hypertriglyceridemia: results of the ICARIA genetic sub-study.* BMC medical genetics, 2010. **11**: p. 66.
150. Arya, R., et al., *Evidence of a novel quantitative-trait locus for obesity on chromosome 4p in Mexican Americans.* American journal of human genetics, 2004. **74**(2): p. 272-82.
151. Kathiresan, S., et al., *Common variants at 30 loci contribute to polygenic dyslipidemia.* Nature genetics, 2009. **41**(1): p. 56-65.
152. DeBusk, R.M., et al., *Nutritional genomics in practice: where do we begin?* Journal of the American Dietetic Association, 2005. **105**(4): p. 589-98.
153. Gillum, R.F., M.E. Mussolino, and C.T. Sempos, *Baseline serum total cholesterol and coronary heart disease incidence in African-American women (the NHANES I epidemiologic follow-up study). National Health and Nutrition Examination Survey.* The American journal of cardiology, 1998. **81**(10): p. 1246-9.
154. Minino, A.M., et al., *Death in the United States, 2007.* NCHS data brief, 2009(26): p. 1-8.
155. Wyatt, S.B., et al., *Prevalence, awareness, treatment, and control of hypertension in the Jackson Heart Study.* Hypertension, 2008. **51**(3): p. 650-6.
156. Fox, E.R., et al., *Epidemiology, heritability, and genetic linkage of C-reactive protein in African Americans (from the Jackson Heart Study).* The American journal of cardiology, 2008. **102**(7): p. 835-41.

157. Divers, J., et al., *The genetic architecture of lipoprotein subclasses in Gullah-speaking African American families enriched for type 2 diabetes: the Sea Islands Genetic African American Registry (Project SuGAR).* Journal of lipid research, 2010. **51**(3): p. 586-97.
158. Kotchen, T.A., et al., *Identification of hypertension-related QTLs in African American sib pairs.* Hypertension, 2002. **40**(5): p. 634-9.
159. Kraja, A.T., et al., *Genetics of hypertension and cardiovascular disease and their interconnected pathways: lessons from large studies.* Current hypertension reports, 2011. **13**(1): p. 46-54.
160. Wu, X., et al., *A combined analysis of genomewide linkage scans for body mass index from the National Heart, Lung, and Blood Institute Family Blood Pressure Program.* American journal of human genetics, 2002. **70**(5): p. 1247-56.
161. Vionnet, N., et al., *Genomewide search for type 2 diabetes-susceptibility genes in French whites: evidence for a novel susceptibility locus for early-onset diabetes on chromosome 3q27-qter and independent replication of a type 2-diabetes locus on chromosome 1q21-q24.* American journal of human genetics, 2000. **67**(6): p. 1470-80.
162. Kissebah, A.H., et al., *Quantitative trait loci on chromosomes 3 and 17 influence phenotypes of the metabolic syndrome.* Proceedings of the National Academy of Sciences of the United States of America, 2000. **97**(26): p. 14478-83.
163. Vijayaraghavan, S., G.A. Hitman, and P.G. Kopelman, *Apolipoprotein-D polymorphism: a genetic marker for obesity and hyperinsulinemia.* The Journal of clinical endocrinology and metabolism, 1994. **79**(2): p. 568-70.
164. Boyles, J.K., L.M. Notterpek, and L.J. Anderson, *Accumulation of apolipoproteins in the regenerating and remyelinating mammalian peripheral nerve. Identification of apolipoprotein D, apolipoprotein A-IV, apolipoprotein E, and apolipoprotein A-I.* The Journal of biological chemistry, 1990. **265**(29): p. 17805-15.
165. Boyles, J.K., et al., *Identification, characterization, and tissue distribution of apolipoprotein D in the rat.* Journal of lipid research, 1990. **31**(12): p. 2243-56.
166. Terrisse, L., et al., *Increased levels of apolipoprotein D in cerebrospinal fluid and hippocampus of Alzheimer's patients.* Journal of neurochemistry, 1998. **71**(4): p. 1643-50.
167. Desai, P.P., et al., *Genetic variation in apolipoprotein D affects the risk of Alzheimer disease in African-Americans.* American journal of medical genetics. Part B, Neuropsychiatric genetics : the official publication of the International Society of Psychiatric Genetics, 2003. **116B**(1): p. 98-101.
168. Desai, P.P., et al., *Genetic variation in the apolipoprotein D gene among African blacks and its significance in lipid metabolism.* Atherosclerosis, 2002. **163**(2): p. 329-38.
169. Hu, G., et al., *Prevalence of the metabolic syndrome and its relation to all-cause and cardiovascular mortality in nondiabetic European men and women.* Archives of internal medicine, 2004. **164**(10): p. 1066-76.
170. Sonnenberg, G.E., G.R. Krakower, and A.H. Kissebah, *A novel pathway to the manifestations of metabolic syndrome.* Obesity research, 2004. **12**(2): p. 180-6.
171. Avery, C.L., et al., *A phenomics-based strategy identifies loci on APOC1, BRAP, and PLCG1 associated with metabolic syndrome phenotype domains.* PLoS genetics, 2011. **7**(10): p. e1002322.
172. Kruglyak, L., et al., *Parametric and nonparametric linkage analysis: a unified multipoint approach.* American journal of human genetics, 1996. **58**(6): p. 1347-63.
173. NCBI, *Entrez > Gene*.
174. Institute, E.B., *UniProt*, 2012.

175. Science, W.I.o. *The GeneCards Human Gene Database*. GeneCards 1996-2012 [cited 2012 5/12/2012]; Available from: www.genecards.org.
176. Lai, C.Q., L.D. Parnell, and J.M. Ordovas, *The APOA1/C3/A4/A5 gene cluster, lipid metabolism and cardiovascular disease risk.* Current opinion in lipidology, 2005. **16**(2): p. 153-66.
177. Benetti, E., N.S. Patel, and M. Collino, *The role of PPARbeta/delta in the management of metabolic syndrome and its associated cardiovascular complications.* Endocrine, metabolic & immune disorders drug targets, 2011. **11**(4): p. 273-84.
178. Brito, E.C., et al., *PPARGC1A sequence variation and cardiovascular risk-factor levels: a study of the main genetic effects and gene x environment interactions in children from the European Youth Heart Study.* Diabetologia, 2009. **52**(4): p. 609-13.
179. Jurkowski, W., et al., *PPARgamma population shift produces disease-related changes in molecular networks associated with metabolic syndrome.* Cell death & disease, 2011. **2**: p. e192.
180. Auton, A. and G. McVean, *Estimating recombination rates from genetic variation in humans.* Methods in molecular biology, 2012. **856**: p. 217-37.
181. Stumpf, M.P. and G.A. McVean, *Estimating recombination rates from population-genetic data.* Nature reviews. Genetics, 2003. **4**(12): p. 959-68.
182. Hinch, A.G., et al., *The landscape of recombination in African Americans.* Nature, 2011. **476**(7359): p. 170-5.
183. Cheng, K.F. and J.Y. Lee, *Assessing the joint effect of population stratification and sample selection in studies of gene-gene (environment) interactions.* BMC genetics, 2012. **13**: p. 5.
184. Corella, D., et al., *APOA2, dietary fat, and body mass index: replication of a gene-diet interaction in 3 independent populations.* Archives of internal medicine, 2009. **169**(20): p. 1897-906.
185. Barrett, J.C., et al., *Haploview: analysis and visualization of LD and haplotype maps.* Bioinformatics, 2005. **21**(2): p. 263-5.
186. Wigginton, J.E., D.J. Cutler, and G.R. Abecasis, *A note on exact tests of Hardy-Weinberg equilibrium.* American journal of human genetics, 2005. **76**(5): p. 887-93.
187. Elosua, R., et al., *Variants at the APOA5 locus, association with carotid atherosclerosis, and modification by obesity: the Framingham Study.* Journal of lipid research, 2006. **47**(5): p. 990-6.
188. Kawamoto, R., et al., *An association between body mass index and estimated glomerular filtration rate.* Hypertension research : official journal of the Japanese Society of Hypertension, 2008. **31**(8): p. 1559-64.
189. Lo, J.C., et al., *GFR, body mass index, and low high-density lipoprotein concentration in adults with and without CKD.* American journal of kidney diseases : the official journal of the National Kidney Foundation, 2007. **50**(4): p. 552-8.
190. Smith, C.E., et al., *Apolipoprotein A5 and lipoprotein lipase interact to modulate anthropometric measures in Hispanics of Caribbean origin.* Obesity, 2010. **18**(2): p. 327-32.
191. Vattikuti, S., J. Guo, and C.C. Chow, *Heritability and genetic correlations explained by common SNPs for metabolic syndrome traits.* PLoS genetics, 2012. **8**(3): p. e1002637.
192. Manning, A.K., et al., *A genome-wide approach accounting for body mass index identifies genetic variants influencing fasting glycemic traits and insulin resistance.* Nature genetics, 2012.

193. Deo, R.C., et al., *Genetic differences between the determinants of lipid profile phenotypes in African and European Americans: the Jackson Heart Study.* PLoS genetics, 2009. **5**(1): p. e1000342.
194. Willer, C.J., et al., *Newly identified loci that influence lipid concentrations and risk of coronary artery disease.* Nature genetics, 2008. **40**(2): p. 161-9.
195. Ken-Dror, G., U. Goldbourt, and R. Dankner, *Different effects of apolipoprotein A5 SNPs and haplotypes on triglyceride concentration in three ethnic origins.* Journal of human genetics, 2010. **55**(5): p. 300-7.
196. Kothari, P.H.G., C.C; Davila-Roman, V.G., *Evaluation of PGC1alpha Gene variants in Modulating Hypertensive Heart Disease Phenotypes*: St. Louis, MO.
197. Rankinen, T., et al., *The human obesity gene map: the 2005 update.* Obesity, 2006. **14**(4): p. 529-644.
198. Sarzynski, M.A., et al., *Associations of markers in 11 obesity candidate genes with maximal weight loss and weight regain in the SOS bariatric surgery cases.* International journal of obesity, 2011. **35**(5): p. 676-83.
199. *dbSNP* NCBI.
200. Bosire, C.R., Jill; Krebs-Smith, Susan M.; , *Sources of Energy and Selected Nutrient Intakes Among the US Population, 2005-2006: A Report Prepared for the 2010 Dietary Guidelines Advisory Committee*, 2009, Risk Factor Monitoring and Methods Branch: National Cancer Institute.
201. Gebauer, S.K., et al., *n-3 fatty acid dietary recommendations and food sources to achieve essentiality and cardiovascular benefits.* The American journal of clinical nutrition, 2006. **83**(6 Suppl): p. 1526S-1535S.
202. Safety, E.C.f.C.R.a.F., *Catfish Risk Profile*, 2010-2011.
203. Nettleton, J.A. and J. Exler, *Nutrients in Wild and Farmed Fish and Shellfish.* Journal of Food Science, 1992. **57**(2): p. 257-260.
204. Mississippi Wildlife, F.a.P.S. *Fishing and Boating*. 2012 [cited 2011 11/01/2011]; Available from: http://www.mdwfp.com/media-relations/press-releases/fishing-boating/mdwfp-and-mdeq-release-fish-to-repopulate-lower-pearl-river.aspx.
205. Pokorn, J., J. Panek, and L. Trojakova, *Effect of food component changes during frying on the nutrition value of fried food.* Forum of nutrition, 2003. **56**: p. 348-50.
206. Fillion, L. and C.J. Henry, *Nutrient losses and gains during frying: a review.* International journal of food sciences and nutrition, 1998. **49**(2): p. 157-68.
207. Ansorena, D., et al., *Effect of fish and oil nature on frying process and nutritional product quality.* Journal of Food Science, 2010. **75**(2): p. H62-7.
208. Guallar-Castillon, P., et al., *Consumption of fried foods and risk of coronary heart disease: Spanish cohort of the European Prospective Investigation into Cancer and Nutrition study.* BMJ, 2012. **344**: p. e363.
209. Vesper, H.W., et al., *Levels of plasma trans-fatty acids in non-Hispanic white adults in the United States in 2000 and 2009.* JAMA : the journal of the American Medical Association, 2012. **307**(6): p. 562-3.
210. Mozaffarian, D. and M.J. Stampfer, *Removing industrial trans fat from foods.* BMJ, 2010. **340**: p. c1826.
211. Mozaffarian, D. and W.C. Willett, *Trans fatty acids and cardiovascular risk: a unique cardiometabolic imprint?* Current atherosclerosis reports, 2007. **9**(6): p. 486-93.
212. Lemaitre, R.N., et al., *Trans-fatty acids and sudden cardiac death.* Atherosclerosis. Supplements, 2006. **7**(2): p. 13-5.
213. Craig, J., *Complex Diseases: Research and Applications.* Nature Education, 2008. **1**(1).

214. Baron, M., *The search for complex disease genes: fault by linkage or fault by association?* Molecular psychiatry, 2001. **6**(2): p. 143-9.
215. Menzel, S., *Genetic and molecular analyses of complex metabolic disorders: genetic linkage.* Annals of the New York Academy of Sciences, 2002. **967**: p. 249-57.
216. Lander, E.S. and N.J. Schork, *Genetic dissection of complex traits.* Science, 1994. **265**(5181): p. 2037-48.
217. Bush, W.S. and J. Haines, *Overview of linkage analysis in complex traits.* Current protocols in human genetics / editorial board, Jonathan L. Haines ... [et al.], 2010. **Chapter 1**: p. Unit 1 9 1-18.
218. Kovac, I.P., et al., *Linkage and association analyses of type 2 diabetes/impaired glucose metabolism and adiponectin serum levels in Japanese Americans from Hawaii.* Diabetes, 2007. **56**(2): p. 537-40.
219. Knight, S., H.W. Uh, and M. Martinez, *Summary of contributions to GAW Group 15: family-based samples are useful in identifying common polymorphisms associated with complex traits.* Genetic epidemiology, 2009. **33 Suppl 1**: p. S99-104.
220. Frieden, T.R., *Forward: CDC Health Disparities and Inequalities Report - United States, 2011.* MMWR. Surveillance summaries : Morbidity and mortality weekly report. Surveillance summaries / CDC, 2011. **60 Suppl**: p. 1-2.
221. Braveman, P. and C. Cubbin, *Optimal SES indicators cannot be prescribed across all outcomes.* American journal of public health, 2003. **93**(1): p. 12-3; author reply 13.
222. Braveman, P. and S. Gruskin, *Poverty, equity, human rights and health.* Bulletin of the World Health Organization, 2003. **81**(7): p. 539-45.
223. Braveman, P. and S. Gruskin, *Defining equity in health.* Journal of epidemiology and community health, 2003. **57**(4): p. 254-8.
224. Braveman, P.A., *Monitoring equity in health and healthcare: a conceptual framework.* Journal of health, population, and nutrition, 2003. **21**(3): p. 181-92.
225. Carter-Pokras, O. and C. Baquet, *What is a "health disparity"?* Public health reports, 2002. **117**(5): p. 426-34.
226. Spiegelman, D., A. McDermott, and B. Rosner, *Regression calibration method for correcting measurement-error bias in nutritional epidemiology.* The American journal of clinical nutrition, 1997. **65**(4 Suppl): p. 1179S-1186S.
227. Smithells, R.W., S. Sheppard, and C.J. Schorah, *Vitamin dificiencies and neural tube defects.* Archives of disease in childhood, 1976. **51**(12): p. 944-50.