Interactions Between Sugar-Sweetened Beverage Consumption and Genetic Variants in the *CHREBP* Locus on Risk of Dyslipidemia in Adults

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ABSTRACT

Background: High sugar-sweetened beverage (SSB) consumption has been linked to a variety of cardiometabolic disorders, including cardiovascular disease, type 2 diabetes, metabolic syndrome, and non-alcoholic fatty liver disease. Dyslipidemia, a condition which is traditionally characterized by high triglyceride (TG), high low-density lipoprotein cholesterol (LDL-C) and/or low high-density lipoprotein cholesterol (HDL-C) concentrations, is a common risk factor shared by these conditions. The effect of SSB consumption on dyslipidemia is variable, and genetic susceptibility to dyslipidemia may be modified by SSB consumption. Data from animal models have indicated that carbohydrate-responsive element binding protein (CHREBP*)* expression is altered by sugar consumption, while in observational studies, genetic variants within or near *CHREBP (*also known as *MLXIPL*) have been associated with TG and HDL-C concentrations. This evidence suggests that *CHREBP* is a promising candidate for population-based gene-SSB interaction on TG and HDL-C concentrations.

Aims: The aims of this study were to examine the (1) association between SSB consumption and lipoprotein profiles, apolipoprotein (apo), and lipoprotein particle size concentrations; (2) longitudinal association between SSB, 100% fruit juice (FJ) and low-calorie sweetened beverage (LCSB) consumption and 4-year changes in plasma lipoprotein concentrations and incident dyslipidemia; and (3) interactions between SSB consumption and 1,606 selected single nucleotide polymorphisms (SNPs) within or near *CHREBP* on HDL-C and TG concentrations.

Methods: This study utilized data from the Framingham Heart Study (FHS) (n=6,730) and Women's Genome Health Study (WGHS) (n=21,794), as well as aggregated data from 11 cohorts who are part of the Cohorts for Heart and Aging Research in Genetic Epidemiology (CHARGE) Nutrition Working Group (n=63,599). Dietary intakes were estimated from food-frequency questionnaires, and participants were grouped by category of beverage consumption (<1 serving/month, 1-4 serving/month, 1-2 serving/week, 3-7 serving/week, >1 serving/day). In *Aim 1*, linear regression models were applied to investigate the association between SSB consumption and plasma cholesterol (LDL-C, HDL-C, non-HDL-C, remnant-like particle [RLP]-C), TG (total TG, RLP-TG), and apolipoproteins (apo B, apo A1, apo E, and apo C3) concentrations, and total cholesterol:HDL-C and apo B:apo A1 ratios among FHS and WGHS participants. Associations between SSB consumption and lipoprotein particle sizes were also explored through measures of mean lipoprotein particle size and lipoprotein particle size concentrations [triglyceride-rich lipoprotein particles (TRL-P): very large, large, medium, small, and very small; LDL-particles (LDL-P): large, medium, and small; HDL-particles (HDL-P): large, medium, and small] in linear regression models. In *Aim 2*, mixed-effect linear regression models were used to examine the association between SSB, FJ, and LCSB consumption and 4-year changes in plasma lipid concentrations (LDL-C, HDL-C, TG, and non-HDL-C) and Cox proportional hazard models were used to estimate hazard ratios (HR) for incident dyslipidemia across categories of SSB, FJ and LCSB consumption among FHS participants. In *Aim 3*, inverse-variance weighted fixed- and random-effect meta-analyses were used to quantify the following cross-sectional associations using data from 11 CHARGE consortium cohorts: 1) SSB consumption and HDL-C and TG concentrations; 2) selected SNPs (within or near *CHREBP*)

and HDL-C and TG concentrations; and 3) interactions between SSB consumption and selected SNPs, and HDL-C and TG concentrations.

Results:
Aim 1: SSB consumption was positively associated with LDL-C, apo B, TG, apo C3, RLP-TG, RLP-C and non-HDL-cholesterol concentrations, and total:HDL cholesterol and apo B: apo A1 ratios, and negatively associated with HDL-C and apoA1 concentrations (p_{trend} ranges from <0.0001 to 0.008). After further adjustment for traditional lipoprotein measures (LDL-C, HDL-C, or TG), high SSB consumers had smaller LDL-P and HDL-P size, lower concentrations of large LDL-P and medium HDL-P, and higher concentrations of small LDL-P, small HDL-P and large TRL-P (p_{trend} ranges from <0.0001 to 0.002).
Aim 2: Among FHS participants, participants in the highest category of SSB intake (>1 serving/day) had smaller mean 4-year changes in HDL-C [highest (>1 serving/day) vs. lowest consumption category (<1 serving/month) (H vs. L): β ± SE: -1.0 ± 0.3 mg/dl, p_{trend} = 0.0001] and greater mean 4-year changes in TG (H vs. L: β ± SE: 5.6 ± 2.1 mg/dl, p_{trend} = 0.0004), along with a higher incidence of low HDL-C [H vs. L HR (95% CI): 1.98 (1.20-3.28); p_{trend} = 0.01)] and high TG [HR (95% CI): 1.53 (1.01-2.31); p=0.05; p_{trend} = 0.004)] compared to those in the lowest category of SSB intake (<1 serving/month). LCSB consumption was associated with a higher incidence of high non-HDL-C [H vs. L HR (95% CI): 1.40 (1.17-1.69); p_{trend} = 0.0002)]. No other significant associations between beverage consumption and lipids were observed.
Aim 3: In meta-analyses of CHARGE consortium cohorts, SSB consumption was inversely associated with HDL-C and positively associated with TG concentrations (p_{trend} <0.0001). We replicated previously observed GWAS associations between one SNP on HDL-C and two distinct SNPs on TG concentrations (Bonferroni-corrected p<0.0001). Additionally, we identified two distinct novel SNP associations with TG concentrations (*FZD9*-rs42124 and *VPS37D*-rs10245965). One distinct SNP displayed a statistically significant difference in effect by category of SSB consumption on HDL-C (*TBL2*- rs71556729), and additional SNPs displayed a suggestive difference for both HDL-C and TG concentrations.

Conclusions: Higher consumption of SSB was adversely associated with multiple measures of plasma lipoprotein concentrations that have been linked to higher cardiometabolic risk, along with longitudinal changes in HDL-C and TG concentrations and a higher risk of incident dyslipidemia. Overall, the results from these analyses indicate that higher SSB consumption may contribute to the development of dyslipidemia. Additionally, high SSB consumption may modify the association between genetic variants within or near the *CHREBP* locus and HDL-C and TG concentrations, however, these observations warrant further investigation. These data can assist in the development of new hypotheses to investigate potential underlying mechanisms by which SSB consumption and variation in the *CHREBP* locus may influence plasma lipoprotein concentrations.

TABLE OF CONTENTS

LIST OF TABLES AND FIGURES

ACKNOWLEDGMENTS

Words cannot express the immense gratitude I feel towards all of the people and organizations that have made this research possible. The conception of this research would not have occurred if it were not for the commitment from all of the participants who are part of the cohort studies included in this research. I thank these participants for sacrificing their time and effort to enable scientific research and I thank the many funding sources that have enabled these studies to gather a wealth of data from these participants over the years.

I thank Dr. Nicola McKeown and Dr. Mark Herman for providing financial support during the majority of my doctoral training through their grant supported by the American Heart Association. I would also like to thank Dr. Alice Lichtenstein for the additional financial support provided through the National Institute of Health. And finally, thank you to the Tufts University Friedman School of Nutrition for their partial support during the completion of my master's degree, where I was able to make the connections that eventually led me to where I am now.

Thank you to Dr. Fang Fang Zhang and Dr. Nicola McKeown for providing foundational training in nutritional epidemiology research during my master's degree. This coursework and hands-on experience helped me find my niche in nutrition research and begin this journey. To Dr. Hassan Dashti, for introducing me to the world of nutrigenomics and providing early mentorship in conducting studies in large consortia. To Dr. Edward Saltzman, Dr. Nicola McKeown, Dr. Adela Hruby, and Dr. Fang Fang Zhang, for believing in me and guiding me through the most difficult times. To Dr. Daniel Chasman, for encouraging me to dig deeper and providing additional resources to enhance the scope of this research.

To my colleagues in the Nutritional Epidemiology Program at the HNRCA – Dr. Adela Hruby, Kara Livingston, Dr. Paul Jacques, and all other group members – thank you for providing a positive work environment and offering your kind advice and constructive criticism.

I could not have asked for a better group of individuals to prepare me for a research career in nutritional epidemiology, advise me throughout my doctoral candidacy, and set an example for research excellence. This work would not have been possible without the contributions from each and every member of our research team, and I am so appreciative of the time and thought they gave to this project.

To Dr. Alice Lichtenstein: thank you for lending your expertise in cardiovascular health and translational science that has helped maximize the impact of this work. Your praise has been a source of inspiration throughout this journey.

To Dr. Caren Smith: thank you for welcoming me into the CHARGE Nutrition Working Group and helping me assemble a team of research scientists to tackle this large consortia-based project. Your contributions to these projects and dedication to bringing researchers with expertise in nutrition and genetics together are so valuable.

To Dr. Gina Peloso: thank you for introducing me to statistical genetics, graciously answering my many lists of questions about statistics/statistical software, and providing assistance, expertise, and guidance throughout my analyses.

To Dr. Josee Dupuis: thank you for welcoming me to BU and providing me with a wealth of resources to conduct this research. Your expertise and quick responses were invaluable and always provided clear advice and explanations for any statistical problem.

To Dr. Mark Herman: thank you for sharing your expertise in fructose metabolism and enthusiasm to gain additional insights through genetic epidemiology. Without your role in the conception of this idea and excellent foundational research, we would not have been able to pursue this novel candidate gene approach.

To Dr. Nicola McKeown: thank you for putting your trust in me, never doubting me, and inspiring me each and every day. Your guidance throughout this process has allowed me to fulfill the very dreams I aspired to when I arrived at Tufts University, along with achievements I never thought possible. I will forever cherish this experience.

To my parents, for encouraging me to set high expectations and persevere. You have always fostered an environment of curiosity and set an example of lifelong learning that I have embraced. To my fiancé, for his unconditional love and flexibility – I will forever be thankful for the emotional and instrumental support you have provided me throughout this academic pursuit. And to all my friends and family, forgive me for my absence during this endeavor and thank you for bringing joy and happiness when I needed it most – without your unending love and support, none of this would have been achievable.

CHAPTER 1. INTRODUCTION

1.1 Background and Significance

In 2015, an estimated 17.9 million global deaths were attributed to CVD, which represents 31% of all global deaths (1). Dyslipidemia is a risk factor for CVD, characterized by high triglyceride (TG), high low-density lipoprotein cholesterol (LDL-C), and/or low high-density lipoprotein cholesterol (HDL-C) concentrations (2). An estimated 30-40% of adults in the United States can be classified as dyslipidemic (1,3), and thus are at increased risk for developing CVD. Dyslipidemia is common risk factor shared by a variety of cardiometabolic disorders, including type 2 diabetes mellitus (T2D) (4,5), metabolic syndrome (6,7), and non-alcoholic fatty liver disease (8). Lipoprotein profiles may be markers of "distinct" dyslipidemias, such as diabetic dyslipidemia (9,10) or atherogenic dyslipidemia (11,12), and dietary modification may offer a promising strategy in the primary prevention and treatment of dyslipidemia (13,14).

Dietary sugar in the form of sucrose or high-fructose corn syrup, both of which are comprised of nearly equal amounts of glucose and fructose, are used to formulate a variety of foods and beverages. Sugar-sweetened beverages (SSB) are the largest single source of dietary added sugar in the U.S. diet (15), and excess intake of SSB has been linked to dyslipidemia and increased risk for CVD and CVD-mortality (16–22). An estimated 7.4% of cardiometabolic deaths (deaths from heart disease, stroke, and T2D) were attributable to high SSB intake in 2012 (23). Currently, there is limited longitudinal evidence for how SSB and 100% fruit juice may influence lipid concentrations. The mechanisms by which they might contribute to dyslipidemia remain uncertain. Furthermore, frequent consumption of SSB may exacerbate genetic susceptibility to dyslipidemia.

Carbohydrate responsive element binding protein (ChREBP) is a transcription factor that responds to SSB consumption. Single nucleotide polymorphisms (SNPs) within the *CHREBP* (also known as *MLXIPL*) locus have been associated with TG and HDL-C concentrations in population-based studies (24–27), and animal models indicate that *CHREBP* expression is altered by dietary sugar consumption and regulates metabolic processes involved in lipid metabolism (28–32). Thus, biological and epidemiological evidence suggest that SSB consumption and SNPs in the *CHREBP* locus present a promising candidate for population-based SSB-gene interaction with TG and HDL-C concentrations.

1.2 Hypothesis and Specific Aims

The overarching goal of this project was to investigate the role that SSB consumption may play in the development of dyslipidemia in adults and whether genetic variation in the *CHREBP* locus modifies risk. The *central hypothesis* was that SSB consumption and genetic variants within *CHREBP* (selected SNPs) are each associated with an unfavorable lipoprotein profile, and a genetic predisposition may interact with SSB consumption patterns to induce greater dyslipidemia, thereby predisposing susceptible individuals to greater risk of CVD. We tested our hypothesis through analysis of population-level data in the Framingham Heart Study [Offspring (FOS) and Third Generation (GEN3)] and Women's Genome Health Study (WGHS), as well as nine additional cohorts participating in the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) consortium's Nutrition Working Group: Western Australian Birth Cohort Study (Raine), Atherosclerosis Risk in Communities Study (ARIC), Netherlands Epidemiology in Obesity Study (NEO), The Fenland Study (Fenland), Young Finns Study (YFS)

(Finland), Women's Health Initiative (WHI) (USA), Multi-Ethnic Study of Atherosclerosis (MESA), Cardiovascular Health Study (CHS), and the Rotterdam Study (RS). The aims of this project were meant to contribute to the long-term goal of understanding how genes and nutrients interact to increase cardiometabolic risk and inform new preventative and therapeutic strategies for dyslipidemia. The specific aims of this thesis are as follows:

Aim 1: To examine the association between SSB consumption and lipoprotein cholesterol [LDL-cholesterol, HDL-C, TG, non-HDL-C, and total-cholesterol: HDL-C ratio], apolipoprotein (apo) (apo B, apo A1, and apo B: apo A1), and mean lipoprotein particle size [triglyceride-rich lipoprotein particles (TRL-P; very large, large, medium, small, and very small), LDL-particles (LDL-P; large, medium, and small), HDL-particles (HDL-P; large, medium, and small)] concentrations and mean lipoprotein size (TRL-P, LDL-P, HDL-P) in FOS at exam 5 (1991–95) (N=3,047) and Women's Genome Health Study (WGHS) (n=21,794).

Hypothesis: SSB consumption will be unfavorably associated with lipoprotein cholesterol, apolipoprotein, and lipoprotein particle size concentrations among adults.

Aim 2: To examine the longitudinal association of beverage consumption (SSB, LCSB, and FJ with fasting low-density lipoprotein cholesterol (LDL-C), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C) concentrations, and non-HDL-C in FOS from exam 5 (1991–95) to exam 9 (2011-14) (N=2,800) and GEN3 exam 1 (2002–05) and 2 (2008–11) (N=3,082).

Hypothesis: SSB consumption will unfavorably associate with plasma TG, LDL-C, HDL-C and non-HDL-C concentrations, while the association between LCSB or FJ and lipid traits will be attenuated compared to that of SSB.

Aim 1.3: Conduct a meta-analysis of summary data from 11 CHARGE consortium cohorts (N=63,599) to examine the following cross-sectional associations: 1) SSB consumption (a dietary exposure well-characterized across multiple cohorts) and HDL-C and TG concentrations; 2) selected SNPs and HDL-C and TG concentrations; and 3) interactions between SSB consumption and selected SNPs (within or near *CHREBP*) on HDL-C and TG concentrations.

Hypothesis: Both SSB consumption and SNPs in the CHREBP locus will be significantly associated with higher TG and lower HDL-C concentrations. Furthermore, SNPs within the CHREBP locus will interact significantly with SSB consumption to influence the association SSB on fasting TG and HDL-C concentrations.

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CHAPTER 2: LITERATURE REVIEW

2.1 Beverage Consumption Patterns

Dietary sugar in the form of sucrose or high-fructose corn syrup (HFCS), both of which are comprised of nearly equal amounts of glucose and fructose, is added to a variety of foods and beverages. Sugar-sweetened beverages (SSB), such as sodas, fruit-flavored drinks, sports drinks, and sweetened coffees and teas, are a significant source of added sugars and a major contributor to excess energy intake (1). Global averages of SSB consumption were estimated at up to one 8-ounce serving/day in 2010 (2,3), and have been estimated to contribute between 3-10% of daily energy consumption (1,4–6). In the diets of U.S adults and children, nearly 50% of added sugars in the diet are attributed to SSB (7), amounting to approximately 7% of daily calories from consuming SSB (1,4,8). Other major contributors to added sugar intake include candies, frozen desserts, and refined grain desserts, such as cakes, muffins, pastries, and cookies, contributing ~7-9% of daily energy intake in children and adolescents (9) and 4-6% in adults (7). Based on a cross-sectional analysis of data in a representative sample of the US population, SSB consumption trends between the years of 1977 and 2001 indicated a steady increase in SSB during this period (10), but more recent data from 1999 and 2014 suggest that consumption of SSB is decreasing (3,8). Despite this decline in SSB consumption over recent years, national surveys suggest that >50% of US and European youth and adults continue to consume at least one SSB daily (4–6,11–13). Similar consumption patterns exist worldwide, thus SSB consumption continues to be a major public health concern globally (14).

One hundred percent fruit juices (FJ) and low-calorie sweetened beverages (LCSB) are commonly used as "healthier" alternatives to replace dietary SSB (12,15–17). However, the evidence is mixed as to whether or not these beverages can be considered healthy alternatives to SSB (18–21). Thus, to provide useful dietary guidance, it is necessary to compare whether FJ, LCSB, and SSB consumption associate with disease or disease risk factors in a similar fashion (22–26).

Currently, LCSB provide no nutritional value and contain high intensity sweeteners such as saccharin, sucralose, aspartame, acesulfame-K, neotame, advantame, and stevia. The predominant sweeteners used in food products varies by both region and years of data collection. The lack of glucose and fructose in LCSB makes them a suitable comparison, as a negative control to SSB consumption, in observational studies. However, we do have to consider other potential mechanisms by which low-calorie sweeteners may contribute to cardiometabolic risk, such as the potential to act as chemosensory signaling compounds that may influence ingestive processes and behavior, alter glucose homeostasis, and produce changes in the gut microbiome (15,27–29). National estimates indicate that mean intakes of LCSB among adults are about one half 8-ounce serving/day, and consumption has been declining since 2006 (3). This rate of decline is much slower compared to the rate of decline in SSB consumption (3), and national data derived from 2009-2012 suggest that approximately 31% of adults consume LCSB (30).

FJ provide a similar amount of dietary sugar, by weight and volume, compared to SSB, but they contain additional nutrients such as antioxidants (e.g. vitamins C and E), polyphenols (e.g. flavonoids), and other vitamins and minerals (e.g. potassium, folate, and vitamin B6) that could also influence health (24,31). Additionally, overall dietary patterns among SSB versus FJ

consumers may differ (32) and could contribute to differences in health outcomes. Mean intakes of FJ are estimated at about 0.4 servings per day among US adults (33,34).

2.2 Current Recommendations

Dietary guidance consistently recommends limiting added sugar consumption, particularly from SSB (7,9,35). The American Heart Association (AHA) recommends that added sugars be limited to no more than 100 calories per day for women and 150 calories per day for men (6 and 9 teaspoons, respectively) (36). The 2015 Dietary Guidelines for Americans (DGA) recommend that added sugar intake be less than 10% of total energy intake in a healthy dietary pattern (34), thus suggesting that SSB should be limited. The DGA suggest that SSB are replaced with calorie-free beverages (especially water) or beverages containing beneficial nutrients, which includes limited amounts of FJ. The World Health Organization (WHO) recommends that intake of 'free sugars,' which include added sugars, sugars naturally found in honey, fruit juices, fruit concentrates, and syrups, contribute no more than 10% of total energy intake. In addition, WHO suggests that free sugar intake be further reduced to less than 5% for additional health benefits (37). This translates into 100 calories or less per day for a person on a 2000 kcal diet. Thus, the WHO recommendations suggest that both consumption of SSB and FJ should be limited.

The AHA recommends limiting consumption of LCSB where possible, and also notes that substitution of SSB for LCSB can be beneficial for those habituated to consuming sweet-tasting beverages (19). Prior reports from the Academy of Nutrition and Dietetics and the American Diabetes Association corroborate this recommendation (38,39). The American Dental Association also recommends avoiding foods containing natural sugars, added sugars, and low

pH-level acids in order to maintain optimal oral health, which suggests that SSB, LCSB, and FJ consumption should be limited (40).

2.3 Beverage Consumption and Cardiovascular Disease (CVD)

CVD is the leading cause of global death, accounting for more than 17.9 million deaths in 2015 (41). Prospective cohort studies have found an association between added sugar intake and CVD (36,42,43) and CVD-mortality (42,44), particularly in the form of SSB (42,44–47). Each additional daily serving of SSB consumption was estimated to associate with a 22% increased risk for myocardial infarction (48–50) and a 13% increased risk for stroke (48,51) in meta-analyses, each with two prospective cohort studies. A large prospective study among over 100,000 adults observed a 31% increased risk of CVD mortality among study participants consuming 2 or more SSB/day compared to those consuming <1 SSB/month (44). Consistent with these results, higher SSB consumption associates with CVD risk factors, including obesity (52–55), type 2 diabetes mellitus (T2D) (56,57), hypertension (58–60), and dyslipidemia (43,47,61–64). The potential consequences of this link are highlighted in a recent analysis using pooled data from national dietary surveys worldwide, suggesting that SSB are responsible for 45,000 deaths from CVD worldwide (14).

Fewer studies have investigated the relationship between FJ and LCSB consumption and CVD. Two prospective cohort studies observed that LCSB consumption was not significantly associated with incident heart disease after over 20 years of follow-up (47,50), and consistent with this observation, no adverse relationship has been observed between LCSB and intermediate cardiometabolic risk factors (65). A recent large prospective study observed a statically significant increase in CVD-mortality among women reporting consumption of at least

two servings of LCSB/day (44). This study also estimated that substituting one serving of SSB with LCSB was associated with a 4% lower risk of all-cause mortality, 5% lower risk of CVD mortality and 4% lower risk of cancer mortality (44). The authors note the risk of reverse causality in this study, but highlight that future studies exploring very high LCSB consumption levels and cardiometabolic risk factors may be of interest. Additional studies have observed a positive association between LCSB and CVD risk (66,67), but these studies were smaller and may suffer bias due to reverse causation. FJ consumption and has not been linked to CVD in prospective cohort studies (68,69), and one study indicates a potential protective association between citrus FJ consumption and risk of ischemic stroke (70).

2.4 Potential Mechanisms Linking SSB and Lipids

There are several potential mechanisms that may explain the associations observed between SSB consumption and CVD. One potential mechanism by which SSB consumption may increase risk for CVD is through the development of dyslipidemia, which a risk factor for CVD characterized by high triglyceride (TG) and/or low-density lipoprotein cholesterol (LDL-C), or low high-density lipoprotein cholesterol (HDL-C) concentrations (71). Several large meta-analyses associate increased SSB consumption with increased body weight, and much, though not all, of this increased weight is likely due to increased total energy consumption (52,72). Several studies suggest that increases in visceral adiposity account for much of this weight gain (73,74). Weight reduction has been correlated with improved lipoprotein concentrations (75,76), suggesting that high SSB consumption may influence lipoprotein profiles through increases in BMI, thus increasing CVD risk. However, high SSB consumption may also influence lipoprotein metabolism through other distinct mechanisms that could lead to development dyslipidemia

independent of changes in BMI.

The fructose component of sucrose and HFCS appears to be particularly harmful with respect to dyslipidemia (77,78). Fructose may contribute to hypertriglyceridemia by at least two mechanisms which include: 1) fructose increases the amount of substrate available for fatty-acid and TG synthesis, and; 2) fructose activates signaling pathways to enhance fatty-acid and TG production (78–82). Some, but not all, short-term dietary intervention studies in humans have found that overfeeding fructose, but not glucose, can increase post-prandial hypertriglyceridemia (77). Additionally, some short-term interventional studies, even those within the range of "normal" fructose consumption, show that fructose can rapidly impair intermediate physiological endpoints like circulating lipids in humans (83–85). Evidence for the potential impact of isolated fructose on cardiometabolic health is limited to intervention studies because it is difficult to distinguish the effects of glucose from fructose since they are provided together in most forms of added sugar (86). However, fructose and glucose are not consumed in isolation in the human diet. They are often consumed together in the form of sucrose or HFCS in foods and beverages. The complex interactions between sugar and other nutrients consumed in tandem may influence the relationship between sugar and health outcomes. Studying the consumption of foods and beverages versus isolated nutrients helps account for some of these complex interactions, but it also limits our ability to draw conclusions about mechanisms related to specific nutrients. Thus, although animal and human studies suggest short-term effects of fructose, but not glucose, on dyslipidemia, long-term observational studies cannot replicate this finding due to real consumption patterns among humans.

2.5 Beverage Consumption and Dyslipidemia

An estimated 40-50% of adults in the US could be classified as dyslipidemic (87,88), and thus are at increased risk for CVD. Dietary modification offers a promising strategy in the primary prevention and treatment of dyslipidemia (89,90). Some randomized-controlled trials (RCTs) suggest that added sugar and SSB intake may play a role in the modification of plasma lipid concentrations (85), while others show no effect (91). Further investigation into both the cross-sectional and longitudinal association between SSB intake and traditional lipid measurements will further elucidate this association among adults.

Evidence from Observational Studies:

The majority of cross-sectional studies have observed a significant adverse association between added sugar (92) and SSB (47,62,64,93,94) consumption and dyslipidemia among adults. Only one cross-sectional study of SSB consumption did not observe a significant association (95). Similar associations have been observed among adolescents (43,96–99) and children (96,98,100). There are fewer prospective cohort studies examining this association, and results are less consistent (6,26,61,101–103). Among adults, one prospective study found that consuming >1 soft drink per day (including both SSB and LCSB) increased odds of developing hypertriglyceridemia by 25% and low HDL concentrations by 32% over 4 years of follow-up compared to those consuming no soft drinks (61). Among adolescents and children, three prospective studies observed a significantly increased risk for hypertriglyceridemia with higher intake of SSB (6,101,102), and two prospective studies observed no significant association (26,103). In contrast, observational studies have not observed a significant association between LCSB consumption and blood lipid measures (26,65), and some evidence exists that FJ

consumption may positively influence blood lipid measures (23,104,105). Together, these studies suggest that future examination of the effect of beverage consumption on dyslipidemia is necessary.

Evidence from Randomized Controlled Trials (RCTs):

Large prospective RCTs assessing the effects of SSB consumption on fasting lipid concentrations are lacking and unlikely to occur due to prohibiting factors such as complexity, cost, compliance issues, and ethical issues. One parallel-arm, nonrandomized, double-blinded intervention study comparing the effects of consuming beverages sweetened with HFCS, glucose, and fructose at 25% of total energy intake on lipid/lipoprotein risk factors in 28 healthy adults observed a nominally significant, dose-dependent increase in fasting TG concentrations, as well as a statistically significant, dose-dependent increase in 24-h mean TG concentrations and postprandial TG concentrations with increasing SSB consumption after two weeks with no significant changes in body weight (85). Similarly, one 6-month RCT of 47 overweight individuals, with no significant changes in body weight, observed a statistically significant 32% increase in fasting TG concentrations among cola consumers, but no significant increases in fasting TG concentrations among the milk, diet cola, and water consumers (although this was not the primary outcome within this study) (74). Another 9-month RCT among 240 overweight/obese women of Mexican heritage (minor weight loss of ~1 kg) observed a significant decrease in TG concentrations among only those women who received nutritional counseling and water to substitute for their usual SSB compared to those that only received nutrition counseling (106). However, one study replacing SSB consumption with LCSB for 12-weeks, with no significant changes in body weight, did not observe a similar effect on fasting TG concentrations (107).

Evidence from RCT looking at diets high in added sugars from a variety of sources is mixed. A meta-analysis of 37 RCT evaluating the effect of dietary free sugars (includes added sugars and naturally occurring sugars) on lipids found that higher compared to lower sugar intakes significantly increased TG, total cholesterol, and LDL-C concentrations, while also decreasing HDL-C concentrations (108). Controversy exists based on differences among these RCT (109,110), but most evidence suggests that significant detrimental effects on lipoprotein concentrations seem to be observed when percent of energy from sucrose is at approximately 25% of total energy intake (111–113), but evidence is less consistent at lower percentages of energy intake from sucrose (91,110). Several short term RCT indicate no significant association between LCSB intake and lipid concentrations, (26,65) and positive and negative associations between FJ consumption and plasma lipid concentrations (114–116).

2.6 SSB Consumption and Lipoprotein Concentrations

In addition to its association with CVD, consumption of SSB has been associated with increased risk for a variety of cardiometabolic disorders, including T2D (117,118), metabolic syndrome (MetS) (119,120), and non-alcoholic fatty liver disease (NAFLD) (121). Dyslipidemia is a common risk factor shared by these conditions. Novel lipoprotein biomarkers exist that may be markers of "distinct" dyslipidemias, such as diabetic dyslipidemia or atherogenic dyslipidemia. Diabetic dyslipidemia is characterized by the high concentrations of TG and small dense LDL, and low concentrations of HDL-C, frequently found in diabetics (122,123). Although there is currently no widely accepted definition for atherogenic dyslipidemia, it has been characterized by high TG, small low-density lipoprotein particles (LDL-P) and remnant-

like particle (RLP) concentrations, and low HDL-C concentrations (124,125). Dietary modifications that may influence plasma lipid concentrations also influence additional lipoprotein measures (126–128). Thus, understanding whether SSB is associated with additional lipoprotein measures may reflect aspects of the pathogenesis and risks associated with 'distinct' dyslipidemias.

RLP result from the lipolysis of TG-rich particles (TRL-P) in circulation. Delayed removal of TRL-P has been associated with insulin resistance, T2D, and atherogenesis (129). Apolipoproteins are the main protein components of lipoprotein particles. The major apolipoproteins include apolipoprotein A1 (apo A1), apolipoprotein B (apo B), apolipoprotein E (apo E) and apolipoprotein C3 (apo C3). Apolipoprotein on lipoprotein particles mediate their metabolic fate and concentrations may also reflect aspects of the pathogenesis and risks associated with 'distinct' dyslipidemias (130,131). Plasma TG and cholesterol are carried in lipoprotein particles of varying sizes and apolipoprotein composition and include: TRL-P (very large, large, medium, small, and very small; these encompass chylomicrons, very-low-density lipoproteins, and intermediate density lipoproteins), LDL-P (large, medium, and small), and HDL-particles (HDL-P) (large, medium, and small) (132). Although the clinical utility of these lipoprotein measures is uncertain, specific lipoprotein profiles have been associated with increased cardiometabolic risk (133–139).

Evidence from Observational Studies

One large cross-sectional study in Swedish adults observed that SSB consumption was unfavorably associated with apo B and apo A1 concentrations, and apo B:apo A1 ratio (140). No previous observational studies have investigated the association of SSB consumption with RLP

concentrations or lipoprotein particle size profiles. However, there are a few studies that suggest SSB consumption may influence these measures. Apo B and apo A1 are the main apolipoproteins of LDL-C and HDL-C, respectively (139), thus previous studies linking SSB consumption to LDL-C and HDL-C concentrations would suggest that similar associations between SSB consumption and apo B and apo A1 (47,62,64,93,94). One cross-sectional study among 74 Swiss children observed that high total fructose intake from sweets, drinks, fruits, and vegetables, out of eleven categories of macronutrient intake, was the only significant predictor of LDL-P size (141). Large prospective studies have also observed that greater concentrations of large TRL-P are associated with a higher risk for T2D compared to small TRL-P (137,142) and that large TRL-P concentrations were elevated among adults with NAFLD (143). Given that SSB consumption has been associated with increased risk for T2D (117) and NAFLD (121), differences in lipoprotein concentrations could reflect mechanisms underlying this association.

Evidence from Randomized Controlled Trials:

Randomized controlled trials assessing the effects of SSB consumption on fasting lipid concentrations are sparse. One RCT with 48 participants observed that consumption of SSB at 25% of total energy intake over a 2 week period resulted in significant increases in RLP-C and RLP-TG concentrations (144). Two small (<50 participants) intervention studies comparing consumption of glucose-, fructose-, and sugar-sweetened beverages within ranges of normal intake over 2-3 week periods also observed that either consumption of fructose- or sugar-sweetened beverages led to lower LDL-P size (144,145).

To date, no other intervention studies have examined the association between SSB consumption and RLP concentrations, apolipoprotein concentrations or lipoprotein particle size profiles, but intervention studies evaluating the association between carbohydrate consumption

and lipoprotein particle size profiles are available. Two small intervention studies that compared low-carbohydrate diets vs. low-fat weight loss diets for 3-6 months observed larger decreases in large TRL-P concentrations compared to small TRL-P for the low-carbohydrate diet (146,147), suggesting a potential role of diets low in carbohydrates on TRL-P size. Two short-term (up to 4 weeks) intervention studies observed an increase in smaller LDL particles in individuals fed diets high in carbohydrates and low in fat compared to a diet high in fat and low in carbohydrate (128,148). One 9-month intervention study in overweight/obese adults observed an increase in LDL particle size in individuals following a low-carbohydrate diet, but no differences in individuals following a low-fat diet (149). Additionally, a 12-week Mediterranean-style diet (decreased carbohydrate, and increased fat and protein) intervention study among women with MetS also observed increased LDL particle size after the intervention, although this study lacked a control group (150). One potential reason for inconsistency in results across these studies could be that these studies did not account for different sources of dietary carbohydrates. Thus, more studies are necessary to further investigate the potential association between the intake of carbohydrates and lipoprotein particle sizes, especially among specific types of carbohydrates.

2.7 Gene-SSB Interactions

Genetic variation may contribute to inconsistency in previous studies looking at the association between SSB consumption and dyslipidemia (151). An understanding of how genes and diet interact in the development of dyslipidemia could lead to specific lifestyle recommendations based on an individual's genetic predisposition (152). There is a limited body of evidence describing how genes implicated in various diseases may interact with SSB

consumption to modify cardiometabolic health and chronic disease risk. We identified 9 published studies linking SSB to a variety of health outcomes with consideration for an underlying genetic predisposition (**Table 1**). Studies included cross-sectional and longitudinal analyses of population-based cohort studies, primarily from the U.S. and Europe, and meta-analytic data from population-based cohort studies. The genetic variants investigated were either limited to single loci or expanded to multiple loci that have been observed in earlier publications to associate with related health outcomes (e.g. obesity, T2D, and CVD) (**Table 2**). To date, the strongest evidence for interaction between genes and SSB consumption is for obesity. Three independent studies support a link between genetic risk for obesity and SSB consumption on the basis of longitudinal changes in adiposity (153–155). The majority of current evidence suggests that the association with SSB consumption and body weight is strongest among subgroups with a genetic predisposition for adverse metabolic outcomes, with two notable exceptions. In one study focused on body weight (155) and the other gout (156). The genetic predisposition to either condition attenuated or possibly masked the association between SSB intake and the outcome.

Cardiovascular Disease

Associations between SSB consumption and CVD risk prompted initial efforts to test whether genetic variation might interact with SSB consumption to influence CVD risk. One prospective cohort study in 26,455 adults living in Sweden investigated whether genetic risk for dyslipidemia (weighted genetic risk score for 80 known genetic variants associated with TG and HDL-C or LDL-C concentrations) interacted with SSB consumption to influence incident ischemic CVD. No significant interactions were observed (157). In contrast, another study in adults living in Costa Rica took a candidate gene approach for a locus on chromosome 9p21 and

reported a robust association with cardiovascular disease. In 3,311 adults of Hispanic origin with high SSB consumption (>2 serving/d) compared to those with low SSB consumption (<1 serving/d) ($P_{interaction}$=0.005) it was estimated that there was a 48% higher risk of myocardial infarction per each risk allele of rs4977574 (in the CDKN2B-AS1 gene within the 9p21 region) (158). No significant interactions were observed for the two other variants tested in the same region (rs2383206 and rs1333049).

Metabolic Syndrome (MetS)

One small case-control study investigated the potential interaction between SSB consumption and 3 genetic variants (rs670, rs5069, and rs5128) in the *APOA1/APOC3* locus, previously associated with dyslipidemia (159–161), on the prevalence of MetS in approximately 800 residents of Iran (162). Haplotype analysis identified an interaction between SSB consumption and the combination of two genotypes at the locus of interest (GA+AA rs670/CT+TT rs5069/CC rs5128) on MetS risk ($P_{interaction}$=0.03). Carriers of the haplotype and those who were the highest consumers of SSB had 9 times higher odds of MetS compared to those with the same genotype but who were the lowest SSB consumption. Among those with other combinations of genotypes, the observed increase in MetS risk with higher SSB consumption was blunted. However, given the large number of statistical tests, the selected 2-sided *P* value threshold of <0.05 applied in this study is not sufficiently conservative, and thus the validity of these suggestive findings is uncertain.

Dyslipidemia

The same prospective cohort study in 26,455 adults living in Sweden that investigated the interaction between SSB consumption and incident ischemic CVD also investigated whether genetic predisposition for dyslipidemia interacted with SSB consumption to influence plasma lipid concentrations (TG, HDL-C, and LDL-C) (157). No significant interaction was observed.

2.8 *CHREBP*

Carbohydrate Responsive Element Binding Protein (ChREBP) is a transcription factor that is activated by products of carbohydrate metabolism and regulates the expression of glycolytic and lipogenic gene programs in an insulin-independent manner (163). Single nucleotide polymorphisms (SNPs) in the *CHREBP* genetic locus have been previously associated with lipid concentrations and lipoprotein particle sizes in large population studies (164–168) (**Table 3**). Animal studies suggest differential activity of *CHREBP* at different levels of sugar consumption (169–171). Thus, biological and epidemiological evidence suggest that SSB consumption and SNPs in the *CHREBP* genetic locus present a promising candidate for a gene-diet interaction in the development of dyslipidemia.

There are two previous studies that have investigated gene-diet interactions involving the *CHREBP* genetic locus. One study in 7,166 adults living in Spain investigated whether the association between SNPs in *CHREBP* with plasma lipid concentrations and incident CVD events may be modified by baseline adherence to a Mediterranean diet (165). When adherence to the Mediterranean diet was high, they observed a significant association between the SNP located at rs3812316 and lower hypertriglyceridemia (OR = 0.63; 95% CI: 0.51-0.77; $p=8.6x10^{-6}$), but no significant association among those with low adherence to the Mediterranean diet (OR = 0.88; 95% CI: 0.70-1.09; p=0.219). Our group has also recently investigated how the

association between SSB consumption and fasting insulin and glucose may be modified by proteins involved in fructose metabolism and the ChREBP-FGF21 pathway. Genetic variants were selected that have been demonstrated to be critical determinants of hepatic glucose metabolism, regulation of ChREBP and plasma TG concentrations, or the metabolic hormone *FGF21* and its obligate receptor beta-klotho (*KLB*) (172). In a meta-analysis of 11 cohorts from the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium including up to 34,748 adults of European descent, we first replicated earlier observations that higher SSB consumption was positively associated with fasting insulin and glucose concentrations. We then identified a non-significant interaction between a genetic variant (rs1542423) in the *KLB* locus and fasting insulin concentrations. This interaction was not replicated in additional cohorts.

2.9 Summary

In summary, high SSB consumption has been linked to a variety of cardiometabolic disorders, including CVD, T2D, MetS, and NAFLD. Dyslipidemia, a condition which is traditionally characterized by TG, high LDL-C and/or low HDL-C concentrations, is a common risk factor shared by these conditions. The effect of SSB consumption on dyslipidemia is variable, and genetic susceptibility to dyslipidemia may be modified by SSB consumption. Data from animal models have indicated that CHREBP expression is altered by sugar consumption, while in observational studies, genetic variants within or near *CHREBP* have been associated with TG and HDL-C concentrations. This evidence suggests that *CHREBP* is a promising candidate for population-based gene-SSB interaction on TG and HDL-C concentrations.

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Table 2.1. Population-based studies of the interaction between genetics and sugar-sweetened beverage (SSB) consumption on various health outcomes.

Author (reference)	Year	n	Region	Age, y	Female, %	BMI, kg/m^2	Total Energy Intake, kcal/d	SSB Consumption, serving/d*	Outcomes	Key Observations
Qi (153)	2012	33,097	United States	53.2 ± 7.17	86.6	25.6 ± 4.64	1742 ± 518	0.28 ± 0.60	4-year change in BMI, incident obesity	Significant positive interaction between SSB consumption and BMI genetic risk score on 4-year change in BMI and incident obesity (p<0.001). The increases in BMI per increment of 10 risk alleles were 1.00 for a consumption of less than one serving per month, 1.12 for one to four servings per month, 1.38 for two to six servings per week, and 1.78 for one or more servings per day (P<0.001 for interaction) The relative risks of incident obesity per increment of 10 risk alleles were 1.19 (95% confidence interval [CI], 0.90 to 1.59), 1.67 (95% CI, 1.28 to 2.16), 1.58 (95% CI, 1.01 to 2.47), and 5.06 (95% CI, 1.66 to 15.5) (P = 0.02 for interaction).

Batt (156)	2014	1,634 (n=925 cases)	New Zealand (Polynesian and Caucasian)	50.2 (17-94)	34.5	32.0 (18.1-77.0)	NA	NA	Gout, and Serum Urate Concentrations	Significant positive interaction between SSB consumption and SLC2A9 genotype on gout risk (p =0.010), whereas each extra daily SSB serving, carriers of the gout-protective allele of SLC2A9 associated with a 15% increase in risk (p=0.078), compared with a 12% increase in non-carriers (p=0.002). The interaction term was significant in pooled ($p_{Interaction}$=0.01), but not meta-analyzed ($p_{Interaction}$=0.99) data. In the US cohort, with each extra daily serving, a greater increase in serum uric acid protective allele carriers (0.005 (p=8.7×10−5) compared with 0.002 (p=0.016) mmol/L).
		7,075 (n=148 cases)	United States (Caucasian)	53.8 (44-65)	52.6	26.4 (14.4-54.6)				
Nobili (173)	2014	200	Italy	11 (10, 13)	56.0	25.1 (22, 27.4)	NA	NA	Steatosis Severity (%)	Significant positive interaction between consumption of SSB and PNPLA3 I148M genotype on severity of steatosis (p=0.033).
Sonestedt (157)	2015	26,455	Sweden	57.9 ± NA	62.5	25.7 ± NA	2280 ± NA	0.23 ± NA	incident CVD, TG, HDL-C, and LDL-C concentrations	No significant interactions observed between SSB consumption and outcomes (p>0.05).
Brunkwall (154)	2016	26,726	Sweden	56.3 ± 7.87	62.1	25.7 ± 3.8	2173 ± 606	0.31 ± 0.57	BMI	Significant positive interaction between SSB consumption and BMI genetic risk score on BMI (p<0.05).

Olsen (155)	2016	4,765	Denmark	47.6 ± NA	50.3	NA	2143 ± NA	0.05 ± NA	Change in Body Weight, Waist Circumference, and WC regressed on BMI	Significant negative interaction between soft drink consumption and waist circumference genetic risk score on change in body weight ($p<0.01$). Significant positive interaction between soft drink consumption and both BMI and adiposity genetic risk scores on waist circumference change ($p=0.001$).
Zheng (158)	2016	3,311 (*n*=1,560 cases)	Costa Rica	57.7 ± 11.7	24.5	26.2 ± 4.11	2598 ± 862	1.79 ± 1.44	Myocardial Infarction (based on WHO criteria)	Significant positive interaction between SSB consumption and per-risk allele of rs4977574 increased risk of myocardial infarction ($p<0.05$). A genetic risk score derived from 3 SNPs in the same locus also showed a significant interaction with SSB consumption on MI risk.
Hosseini-Esfahani (162)	2017	828 (*n* =414 cases)	Iran	42.3 ± 12.5	44.0	24.5 ± 4.0	2338 ± 1025	NA	Metabolic Syndrome (based on modified National Cholesterol Education Program/Adult Treatment panel III (ATP III) definition)	Significant positive interaction between SSB consumption and specific haplotypes at *APOA1/APOC3* loci (GA+AA rs670/CT+TT rs5069/CC rs5128 genotypes) on risk of MetS (*P* =0.03). Note: when accounting for multiple comparisons, interaction no longer significant.

McKeown & Dashti (172)	2017	37,748	United States, Netherlands, Finland, Denmark, Sweden Australia	55.7 ± 7.1	56.4	26.9 ± 4.44	1994 ± 644	0.31 ± 0.67	Fasting Glucose and Fasting Insulin	Suggestive interaction was observed between genetic variant in *KLB* (rs1542423) and SSB consumption on FI. No other significant interactions found between selected genetic variants in CHREBP-FGF21 Pathway and FG/FI.

* SSB consumption was ascertained by a semi-quantitative food frequency questionnaire alone (Qi et al., Hosseini et al., Zheng et al., Nobili et al., Batt et al., and Sonestedt et al.) or a combination of semi-quantitative food frequency questionnaire or 7-day food diary (McKeown & Dashti et al., Olsen et al., and Brunkwall et al.). SSB consumption includes fruit juices in the following studies: Qi et al., McKeown & Dashti et al., Zheng et al., and Batt et al.).

Abbreviations: BMI, body mass index; CVD, cardiovascular disease; FG, fasting glucose; FI, fasting insulin; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; MetS, metabolic syndrome; MI, myocardial infarction; SSB, sugar-sweetened beverages; TG, triglyceride; WHO, World Health Organization.

Table 2.2. Health outcomes that have been associated with increased sugar-sweetened beverage consumption (SSB) and have been studied in regard to SSB by gene interaction

Health Outcomes	Mapped Gene	rsID	Minor Allele Frequency*	References
Obesity	32 BMI-Associated Genetic Variants	rs543874, rs1514175, rs1555543, rs2815752, rs2890652, rs887912, rs713586, rs2867125, rs13078807, rs9816226, rs13107325, rs10938397, rs4836133, rs2112347, rs987237, rs206936, rs10968576, rs3817334, rs4929949, rs10767664, rs7138803, rs4771122, rs11847697, rs10150332, rs2241423, rs7359397, rs1558902, rs12444979, rs571312, rs29941, rs3810291, rs2287019	0.03-0.49	Qi et al. 2012 (153)
Body Mass Index	30-33 BMI-Associated Genetic Variants	rs10938397, rs2815752, rs9816226, rs987237, rs7138803, rs713586, rs12444979, rs2241423, rs2287019, rs13107325, rs2112347, rs10968576, rs3810291, rs887912, rs13078807, rs11847697, rs2867125, rs571312, rs7359397, rs3817334, rs29941, rs543874, rs1514175, rs206936, rs1555543, rs1558902, rs4929949, rs10767664, rs10150332, rs4771122	0.04-0.48	Qi et al. 2012 (153) Brunkwall et al. 2016 (154) Olsen et al. 2016 (155)
Fasting Glucose Fasting Insulin	ChREBP/FGF21-Related Loci	rs10819937, rs10819931, rs174546, rs838133, rs4607517, rs1260326, rs2119026, rs1542423,rs799166, rs799168, rs799160, rs11974409, rs11920090, rs11924032, rs5438, rs3820034, rs5840, rs2954029	0.05-0.45	McKeown & Dashti et al. 2017 (172)
CVD	Lipid-Associated Genetic Variants	(all TG, HDL-C, and LDL-C associated genetic variants below)	0.02-0.48	Sonestedt et al. 2015 (157)
TG	TG-Associated Genetic Variants	rs1042034, rs4846914, rs1260326, rs2972146, rs645040, rs442177, rs9686661, rs6882076, rs2247056, rs17145738, rs11776767, rs1495741, rs12678919, rs2954029, rs2068888, rs174546, rs964184, rs11613352, rs4765127, rs2412710, rs2929282, rs1532085, rs11649653, rs3764261, rs10401969, rs439401, and rs5756931	0.02-0.47	Sonestedt et al. 2015 (157)

HDL-C	HDL-C-Associated Genetic Variants	rs4660293, rs1689800, rs4846914, rs1042034, rs12328675, rs2972146, rs13107325, rs6450176, rs2814944, rs605066, rs17145738, rs9987289, rs12678919, rs2293889, rs2954029, rs581080, rs1883025, rs2923084, rs3136441, rs174546, rs964184, rs7941030, rs7134375, rs11613352, rs7134594, rs4759375, rs4765127, rs1532085, rs2652834, rs3764261, rs16942887, rs2925979, rs11869286, rs4129767, rs7241918, rs12967135, rs7255436, rs737337, rs4420638, rs1800961, and rs181362	0.04-0.47	Sonestedt et al. 2015 (157)
LDL-C	LDL-C Associated Genetic Variants	rs12027135, rs2479409, rs629301, rs514230, rs1367117, rs4299376, rs12916, rs6882076, rs3757354, rs1800562, rs3177928, rs9488822, rs1564348, rs12670798, rs9987289, rs2081687, rs2954029, rs9411489, rs2255141, rs174546, rs964184, rs11220462, rs11065987, rs1169288, rs8017377, rs3764261, rs2000999, rs6511720, rs10401969, rs4420638, rs2902940, and rs6029526	0.05-0.48	Sonestedt et al. 2015 (157)
Myocardial Infarction	Chromosome 9p21 Loci	rs4977574, rs2383206, and rs1333049	0.41 to 0.50	Zheng et al. 2016 (158)
Metabolic Syndrome	*APOA1* *APOC3*	rs670, rs5069 rs5128	0.19-0.29	Hosseini-Esfahani et al. 2017 (162)
Waist Circumference	6 Waist-circumference-Associated Loci	rs10146997, rs1121980, rs7138803, rs12970134, rs545854, rs987237	0.08-0.48	Olsen et al. 2016 (155)
	14 Waist: Hip Ratio-Associated Loci	rs1011731, rs10195252, rs1055144, rs1294421, rs1443512, rs2605100, rs4823006, rs6784615, rs6795735, rs6861681, rs6905288, rs718314, rs9491696, rs984222	0.02-0.48	
	33 Adiposity-Associated Loci	rs10508503, rs10838738, rs10938397, rs10968576, rs11847697, rs12444979, rs13107325, rs1424233, rs1514175, rs1555543, rs17782313, rs1805081, rs206936, rs2112347, rs2241423, rs2287019, rs2568958, rs2890652, rs29941, rs3810291, rs4712652, rs4771122, rs4929949,	0.04-0.48	

		rs543874, rs6013029, rs6232, rs6602024, rs713586, rs7647305, rs9939609, rs10146997, rs1121980, rs7138803		
Gout	*SLC2A9*	rs11942223 (NZ population)	0.09	Batt et al. 2014 (156)
Uric Acid Concentration		rs6449173 (US population; surrogate marker)	0.22	
Hepatic Steatosis	*PNPLA3*	rs738409 (I148M PNPLA3)	0.48*	Nobili et al. 2014 (173)

* Minor allele frequency is based on study population

Abbreviations: BMI, body mass index; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; NZ, New Zealand; SSB, sugar-sweetened beverages; TG, triglyceride.

Table 2.3. Previous SNPs within the *CHREBP* genetic locus associated with TG or HDL concentrations

Unique Loci	Top SNP	Other SNPs		Nearest gene(s)	MAF	Effect size
Triglycerides						
1	rs17145738 (164,174–179)	rs3812316 (167,178–182) rs1714052 (176,183) rs1051921 (183) rs1178979 (184) rs2074755 (183) rs2240466 (185)	rs11974409 (183) rs12056034 (167) rs17145713 (183) rs17145732 (167) rs17145750 (183) rs13247874 (186)	*BAZ1B, BCL7B, TBL2, CHREBP, VPS37D, & FZD9*	~0.1-0.2	~10%
2	rs2286276 (183,187)	rs7800944 (183) rs12539316 (183)		*CHREBP & TBL2*	~0.1-0.3	~5%
3	rs799160 (167)	-		*CHREBP,VPS37D, & WBSCR18*	~0.42	3.3%
HDL						
1	rs7811265 (164,176)	-		*CHREBP*	0.19	0.57 mg/dl

CHAPTER 3: METHOD DEVELOPMENT

This chapter provides an overview of selected methods and analyses that informed the design of this dissertation research but is not described in detail within further chapters. It is included in this thesis to provide a resource for other students interested in exploring gene-diet interactions.

3.1 Data Management

A wealth of free software is available online that incorporate features to aid in the efficient conduct of complex genetic data analysis [e.g. PLINK (https://www.cog-genomics.org/plink2/) (1), GCTA (http://www.complextraitgenomics.com/software/gcta/) (2), SHAPEIT (https://mathgen.stats.ox.ac.uk/genetics_software/shapeit/shapeit.html) (3), METAL (www.sph.umich.edu/csg/abecasis/metal) (4), to name just a few]. Although some these applications run on Windows and OS operating systems, many of them were created for, or are only executable, in the Linux operating system. In this fast-moving research area, development of new software and updates to existing software are ongoing and catalogued in software documentation.

The Linux operating system provides the scalability and performance required to conduct the efficient analysis of large genetic datasets. Given this feature, many research groups conducting large genetic studies continue to develop and refine software that aid in the storage and analysis of genetic within the Linux operating system. Some groups have also created pipelines to help streamline genome-wide association studies. Such a pipeline has been created at Boston University for analysis of genetic data within the Framingham Heart Study and was used to conduct some of the genetic analyses in this project. As genetic datasets become larger with

rapid and inexpensive high-throughput sequencing techniques, the use and curation of these pipelines and analysis software will become even more important for the efficient conduct of genetic epidemiological studies.

3.2 Determination of SSB Exposure

The classification of SSB consumption in nutritional epidemiological studies varies, with some studies reporting SSB consumption in 2-6 categories (5–9), and others assessing it as a continuous variable (servings/day) (10,11). To inform SSB consumption classification in our analyses, the adjusted R^2 for models with different SSB consumption variable types were compared among Framingham Heart Study participants (**Table 3.1**). Beverage consumption categories were created that were similar to previous studies (5,6) and grouped a reasonable number of participants within each category (<1 serving/mo, 1-4 serving/mo, 1-2 serving/wk, 3-7 serving/wk, >1 serving/day). One serving of SSB and LCSB was defined as 12 fl. oz. and one serving of FJ was defined as 8 fl. oz. For the main outcomes in our gene-SSB interaction study (triglyceride and high-density lipoprotein cholesterol concentrations), a higher R^2 was observed when SSB consumption was modeled as a categorical variable (either as a numeric or a factor variable) for both outcomes. This indicated that the predictability of the categorical SSB consumption on our outcomes of interest was higher than that of the continuous SSB consumption variables (untransformed or log-transformed). Based on the outcome of this analysis, SSB exposure was grouped into five categories.

3.3 Selection of Methods for Discovery of Gene-SSB Interactions

Selection of Statistical Tests for SSB-Gene Interactions

It was originally proposed to assess the interaction between SSB intake and SNPs in the *CHREBP* region through a meta-analysis of multiplicative interactions terms (SNPxSSB), where SSB consumption was treated as a continuous exposure. Given our decision to focus on categories as described above, methods were explored to implement interaction analyses using SSB intake as a categorical variable. Emerging evidence from gene-environment interaction studies suggest that examination of genetic associations stratified according to environmental factors may provide evidence for gene-environment interactions (6,12,13). First, in a previous high-impact study, multiplicative interactions terms (SNPxSSB) treating SSB as an additive categorical variable were utilized to uncover SSB-gene interactions on obesity (6). Thus, confirming this would be a reasonable approach to apply in our study. Second, Recent meta-analyses have identified unique loci in sex-, age-, and BMI-stratified GWAS (14,15), further suggesting that it could be interesting to explore genetic analyses stratified by SSB intake. Systematic analyses have been conducted that compare approaches for identifying genetic effects that differ by strata in GWAS, and suggest they may be able to identify distinct types of interactions that are not discoverable with traditional multiplicative interaction models (12,16). We decided to focus on differences in SNP effects among the highest (>1 serving/day) and lowest (<1 serving/month) SSB consumers in order to focus on study participants that were least likely to have misclassified SSB intakes. The consequence of this approach is the tradeoff in losing the large sample size in the hope of gaining precision. Thus, we decided to pursue SNP

analyses stratified by category of SSB intake in parallel with multiplicative interaction analyses in the full sample to maximize our ability to detect interaction effects.

Selection of SNPs

In the original proposal, it was estimated that there are 27 distinct SNPs in the CHREBP region [100 kilobase pairs upstream and 190 kilobase pairs downstream of the transcription start site: chr7:72823661-73136661 (hg19)] using SNAP software (https://www.broadinstitute.org/snap/snap; no longer available), thus indicating that a Bonferroni-corrected $\alpha = 0.001$ (0.05/27) would be sufficient to account for multiple testing. However, when quantified using the simpleM method (17) with individuals in the 1000 genomes as a reference panel (18), it was estimated that there are 499 distinct SNPs in the *CHREBP* region, thus indicating that a more strict correction for multiple testing is necessary at a Bonferroni-corrected $\alpha = 0.0001$ (0.05/499). As new information about the structure of linkage disequilibrium in populations becomes available, it is important to utilize this new information through available software packages in order to properly account for multiple testing in genetic epidemiological studies.

Selection of Cohorts for Meta-Analysis

The Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium is a unique resource for conducting population-based studies in the area of cardiovascular genetics. This consortium welcomes graduate students to become involved and

fosters an environment of collaboration, career development, and the opportunity to obtain the large sample sizes required for genetic epidemiological studies. However, challenges exist in conducting standardized analyses across multiple institutions with multiple analysts with varying levels of engagement in projects. Recognizing the challenges associated with this, leaders in the CHARGE Consortium have created analysis pipelines to analyze the most recent genetic data across multiple CHARGE Consortium cohorts in a streamlined manner that can help circumvent some of these challenges [e.g. TopMed (https://www.nhlbiwgs.org/) and Analysis commons (http://analysiscommons.com/)]. In future studies, these resources should be explored to identify how they could be leveraged in future gene-diet interaction studies.

3.4 Statistical Power

Aim 1

Given the sample size of at least 3,000 participants in each analysis, we had 80% power at a 2-sided α=0.05 to detect effect sizes explaining 0.3% of the variance in the trait (R^2), or smaller for larger sample sizes (**Figure 3.1**).

Aim 2

Quanto (http://hydra.usc.edu/gxe/) was used to estimate power based on HDL-C concentrations with the following parameters: 2-sided, Bonferroni-corrected α=0.0001 (0.05/499 independent SNP tests) for allele frequencies ranging from 0.05 to 0.50. Using these parameters, we had 80% power to detect a SNP explaining 0.15% of the variance in the trait (R^2) with a sample size >16,000.

Aim 3

Given the greater than 60,000 participants in analyses of SSB consumption on lipid concentrations, we had 80% power at a 2-sided $\alpha=0.05$ to detect effect sizes explaining <0.01% of the variance in these traits (R^2) (**Figure 3.1**). Using similar specifications as in aim 2, and assuming the SNP and SSB consumption variables explain 0.1% of the variance in the trait (R^2), we had 80% power to detect an interaction effect explaining 0.1% of the variance in the trait (R^2) at a sample size >16,000.

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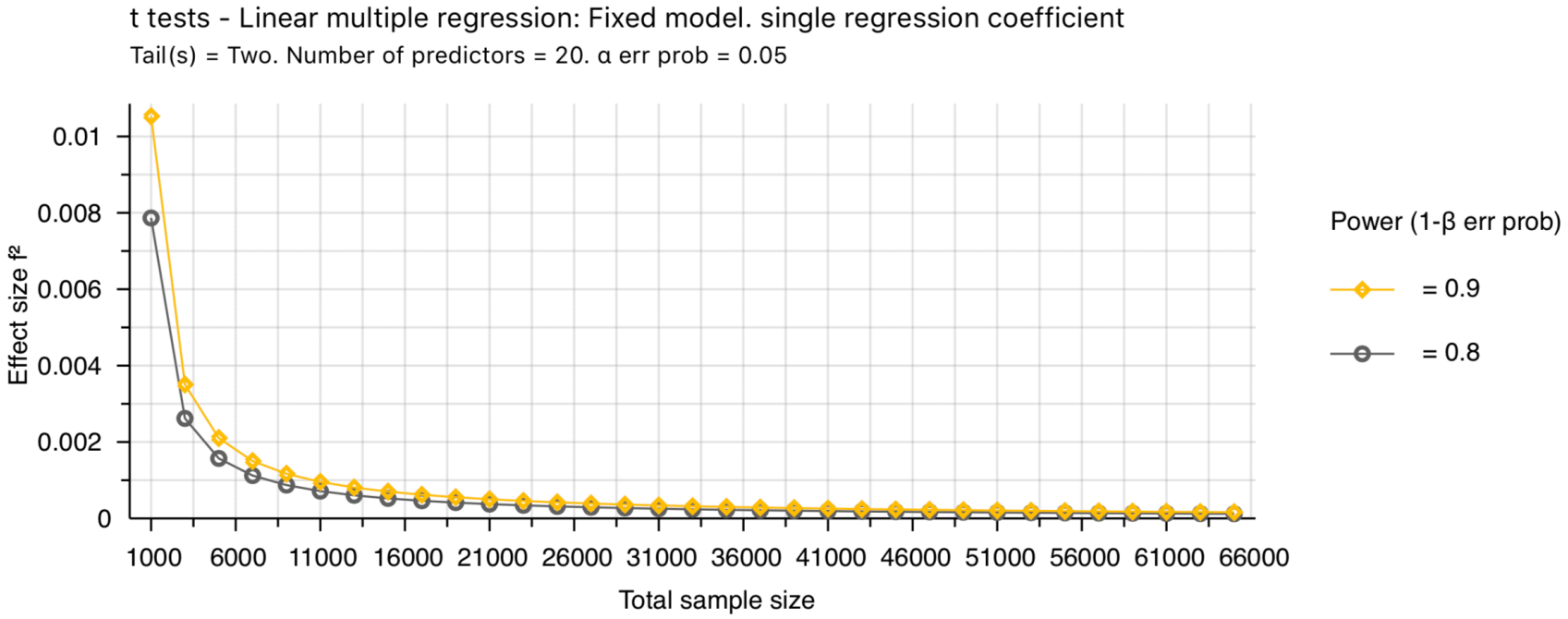
Table 3.1 Comparison of adjusted R^2 for models with different variable types for sugar-sweetened beverage consumption*

Variable Type	Adjusted R^2	
	HDL-C	ln(TG)
continuous	0.40	0.19
continuous (log-transformed)	0.40	0.30
categorical	0.46	0.30
additive categorical (values are 0-4)	0.46	0.30
additive categorical (values are median)	0.46	0.30

*All models were conducted among Framingham Heart Study participants using linear mixed effect regression models accounting for familial correlation and adjusted for age, cohort, sex, total energy, education, current smoking status, physical activity, alcohol intake, body mass index, percent energy from saturated fat, and servings per day of vegetables, total fruits, whole grains, nuts/seeds, and seafood. Continuous sugar-sweetened beverage consumption is estimated in servings/day. Categorical variables represent 5 categories of sugar-sweetened beverage consumption (<1 serving/month, 1-4 servings/month, 1-2 servings/week, 3-7 servings/week, >1 serving/day). Abbreviations: HDL-C, high-density lipoprotein cholesterol concentrations; ln(TG), log-transformed triglyceride concentrations.

Figure 3.1. Estimated effect size (quantified through $f^2 = R^2/[1- R^2]$) detected at 80% and 90% power for varying sample sizes



CHAPTER 4

Associations between sugar-sweetened beverage consumption and lipoprotein cholesterol, apolipoprotein, and lipoprotein particle size concentrations in U.S. adults

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4.1 Abstract

Background: Prospective cohort studies have found a relationship between sugar-sweetened beverage (SSB; sodas and fruit drinks) consumption and cardiometabolic diseases, such as cardiovascular disease and type 2 diabetes mellitus risk. Dyslipidemia, a condition which is traditionally characterized by high triglyceride (TG), high low-density lipoprotein (LDL) cholesterol and/or low high-density lipoprotein (HDL) cholesterol concentrations, is a common risk factor shared by these conditions. There is limited evidence linking SSB consumption to lipoprotein concentrations in population-based studies.

Objective: To examine the association between SSB consumption and lipoprotein cholesterol, apolipoprotein, and lipoprotein particle size concentrations among adults.

Design: We conducted an analysis of data from the Framingham Offspring Study (FOS) (1987-1995; n=3,047) and Women's Genome Health Study (1992; n=21,794). Plasma LDL-cholesterol, apolipoprotein (apo) B, HDL-cholesterol, apo A1, triglyceride (TG), non-HDL-cholesterol, total-cholesterol: HDL-cholesterol ratio, and apo B: apo A1 concentrations were quantified in both

cohorts, and apo E, apo C3, remnant-like particle (RLP) TG, and RLP cholesterol concentrations were quantified in the latter cohort. SSB consumption and lipoprotein particle sizes were also explored through measures of mean lipoprotein particle size and lipoprotein particle size concentrations [triglyceride-rich lipoprotein particles (TRL-P; very large, large, medium, small, and very small), LDL-particles (LDL-P; large, medium, and small), HDL-particles (HDL-P; large, medium, and small)]. Consumption of SSB was estimated using food frequency questionnaires. We examined the associations between SSB consumption and lipoprotein concentrations, adjusting for potential confounding factors, such as lifestyle and other dietary factors.

Results: SSB consumption was positively associated with LDL-cholesterol, apo B, TG, apo C3, RLP-TG, RLP-C, non-HDL-cholesterol concentrations and total:HDL cholesterol and apo B:apo A1 ratio, and negatively associated with HDL-C and apo A1 concentrations (p_{trend} ranges from <0.0001 to 0.008). After further adjustment for traditional risk factors and lipoprotein measures, SSB consumers had smaller LDL-P and HDL-P size, and lower concentrations of large LDL-P and medium HDL-P and higher concentrations of small LDL-P, small HDL-P, and large TRL-P (p_{trend} ranges from <0.0001 to 0.002).

Conclusions: Higher consumption of SSB was adversely associated with multiple measures of plasma lipoprotein concentrations that have been linked to higher cardiometabolic risk.

4.2 Introduction

Sugar-sweetened beverages (SSB) are the largest single source of added dietary sugars (1), contributing nearly 50% of added sugars in the U.S. diet (2). Consumption of SSB has been associated with increased risk for a variety of cardiometabolic disorders, including cardiovascular disease (CVD) (3–5), type 2 diabetes mellitus (T2D) (6), metabolic syndrome (7), and non-alcoholic fatty liver disease (8). A common risk factor shared by these conditions is dyslipidemia, a condition which is traditionally characterized by high triglyceride (TG), high low-density lipoprotein cholesterol (LDL-C), high non-high-density lipoprotein cholesterol (non-HDL-C) and/or low high-density lipoprotein cholesterol (HDL-C) concentrations. Even though dyslipidemia, by definition, encompasses a variety of lipid risk factors, lowering LDL-C concentrations is currently the primary target for health professionals (9). The role that HDL-C and TG concentrations play in the development of cardiometabolic disorders is less clear (10,11), thus understanding whether SSB is associated with "distinct" dyslipidemias is important. Although evidence from observational studies is mixed, the majority of studies have observed a significant adverse association between SSB consumption and dyslipidemia among adults (5,12–17).

In addition to these traditional markers of dyslipidemia, novel lipoprotein biomarkers exist that may be markers of "distinct" dyslipidemias, such as diabetic dyslipidemia (18,19) or atherogenic dyslipidemia (20). Diabetic dyslipidemia is characterized by the high concentrations of TG and small dense LDL, and low concentrations of HDL-C found in diabetics (18,19). Although there is currently no widely accepted definition for atherogenic dyslipidemia, it has been characterized by high TG, small low-density lipoprotein particles (LDL-P) and remnant-like particle (RLP) concentrations, and low HDL-C concentrations (20,21). RLP result during the

size reduction of TG-rich particles (TRL-P) through hydrolysis by lipoprotein lipase and remain in circulation due to the slow rate of removal. Elevated RLP concentrations have been associated with insulin resistance, T2D, and atherogenesis (22). Apolipoproteins are the main protein components of lipoprotein particles, and they include apolipoprotein A1 (apo A1), apolipoprotein B (apo B), apolipoprotein E (apo E) and apolipoprotein C3 (apo C3). Apolipoprotein concentrations reflect the functional properties of lipoprotein particles (23,24) and may also reflect aspects of the pathogenesis and risks associated with 'distinct' dyslipidemias. Plasma TG and cholesterol are also carried in lipoprotein particles of varying sizes, which include: TRL-P (very large, large, medium, small, and very small; these encompass chylomicrons, very-low-density lipoproteins, and intermediate density lipoproteins), LDL-P (large, medium, and small), and HDL-particles (HDL-P) (large, medium, and small) (25). Although the clinical utility of these additional lipoprotein measures is uncertain, specific lipoprotein profiles have been associated with increased cardiometabolic risk (26–32). To date, no observational studies have examined the association between SSB consumption and apolipoprotein and RLP or lipoprotein particle size concentrations. However, intervention studies suggest that consumption of SSB may promote dyslipidemia (33,34) and diets low in carbohydrate may lead to improvements in lipoprotein particle size profiles (35–39).

The objective of the present study was to examine the association between SSB consumption and plasma lipoprotein cholesterol, apolipoprotein, and lipoprotein particle size concentrations among participants from the Framingham Offspring Study (FOS) and Women's Genome Health Study (WGHS) to generate hypotheses regarding mechanisms by which SSB may influence plasma lipoprotein concentrations.

4.3 Methods

Subjects

The study population consisted of participants from both FOS and WGHS. The Framingham Heart Study is a long-standing, prospective cohort study in Framingham, Massachusetts that began in 1948. Data from the FOS (40) at exam 4 (1987-1991) (n=4,019) and exam 5 (1991–1995) (n=3,799) were used in the current study. WGHS is a prospective cohort of North American women aged ≥ 45 years that began in 1992 as part of the Women's Health Study (n=28,346) (41,42). Within each cohort, participants underwent a detailed medical history, physical examination, and standard laboratory tests. Participants also provided demographic, diet, lifestyle and medical history data via standard questionnaires. All participants provided written informed consent before study participation. All study protocols and procedures for the current study were approved by the institutional review board for human research at Boston University Medical Campus, Tufts University Health Sciences, and Brigham and Women's Hospital, Boston.

A total of 28,346 participants provided baseline data in WGHS. Participants were excluded if they provided invalid or did not provide dietary data (n=5,696), lipoprotein measurements were not available (n=106), or they were using lipid-lowering therapy (n=750), reducing the sample size to 21,794. This sample used for these analyses includes baseline blood samples collected from participants less than 8 hours after a meal. Thus, results are presented in the supplemental section among the 15,675 (72%) participants who provided a baseline blood sample at least 8 hours after a meal (fasting sub-sample). A total of 3,306 FOS participants provided dietary data (exam 5) and samples for lipoprotein measures (exam 4 and 5 FOS participants were excluded if provided invalid dietary data (n=9), lipoprotein measurements were

not available (exam 4: n=182; exam 5: n=9), or they were using lipid-lowering therapy at the time of the fasting blood draw (exam 4: n=116; exam 5: n=241), reducing the sample size to n=2,999 at exam 4 and 3,047 at exam 5.

Assessment of lipoprotein outcomes

Plasma samples from study participants in WGHS at baseline and FOS at exam 5 (unless otherwise noted) were used to measure HDL-C, TG, total cholesterol (TC), apo A1 (exam 4), and apo B (exam 4) concentrations using standard assays. As described above, all FOS participants included in this analysis provided fasting plasma samples, and among the 21,794 WGHS participants included in this analysis, a subsample of 15,675 participants provided fasting plasma samples. Among WGHS participants, LDL-C was determined directly using a standard assay. Among FOS participants, LDL-C concentrations were calculated according to the Friedewald equation (43) (LDL-C = TC – HDL-C – TG/5) and were reported as not available if TG ≥ 400 mg/dl. Non-HDL-C concentrations were calculated as TC minus HDL-C concentrations, TC: HDL-C ratio was calculated by dividing TC by HDL-C concentrations, and apo B: apo A1 ratio was calculated by dividing apo B by apo A1. Among FOS participants, apo C3 and apo E were measured at exam 5 using standard assays, along with RLP cholesterol (RLP-C) and RLP triglyceride (RLP-TG) concentrations at exam 4 using previously described assays (44–46).

Using proton NMR spectroscopy (47) the following lipoprotein particle size concentrations were measured: TRL-P (very large, large, medium, small, and very small); LDL-P (large, medium, and small); HDL-P (large, medium, and small). From this data, the NMR analysis software calculates TRL-P, LDL-P, and HDL-P mean size (nm diameter), which can be

used to assess provide an overall assessment of lipoprotein particle size profiles. Lipoprotein particle size profiles for FOS participants at exam 4 were measured with the LipoProtein-I assay (Liposcience Inc. Raleigh, NC), which provides slightly different size groupings (**Table 4.4**), less accuracy, and different units (mg/dL) compared to the LP4 assay used to measure lipoprotein particle size profiles for WGHS participants (nmol/L for TRL-P and LDL-P; μmol/L for HDL-P) (LP4 NMR *MetaboProfile*™ Analysis, Liposcience/LabCorp Global Research Services, Raleigh, NC).

Assessment of Dietary Intakes

Usual dietary intakes in the past year were estimated using the Harvard 126-item (FOS exam 5) and 61-item (WGHS) semi-quantitative food-frequency questionnaires (FFQ) (48,49). The FFQ consisted of a list of foods with standard serving sizes and a selection of 9 frequency categories ranging from none or <1 serving/month to ≥6 servings/day. Participants with implausible energy intake were excluded from the analysis based on cohort-specific cut-offs (FOS: <600 kcal/day for both men and women; ≥4,000 kcal/day for women or ≥4,200 kcal/day for men; and if >13 food items were left blank on the FFQ; WGHS: <600 and ≥3500 kcal/day). Both FFQ have been examined for reproducibility and validity for both nutrients and foods in women and men in various cohorts (48–51). Measures of apo A1, apo B, RLP, and lipoprotein particle size profiles were measured in FOS at exam 4, however, dietary intake data was not measured at this exam period. We used dietary data from exam 5 to estimate SSB intake, as based on other exam periods, SSB intake does not substantially change between consecutive exams (e.g. r^2 for SSB intake at exam 5 and 6 = 0.52), and estimates reflect approximate habitual

SSB consumption patterns. Thus, for these lipoprotein measures, exam 5 dietary intakes were used to approximate dietary intakes at exam 4 in FOS participants.

Estimates of SSB consumption in both cohorts included the following categories (1) Coke, Pepsi, or other cola beverages with sugar; (2) caffeine-free Coke, Pepsi, or other cola beverages with sugar; (3) other carbonated beverages with sugar (e.g., 7-Up, ginger ale); and (4) Hawaiian Punch, lemonade, or other non-carbonated fruit drinks. One serving of SSB is equivalent to 360 mL (12 fl oz.). Food groupings were based on the 2015 Dietary Guidelines for Americans and nutrient intakes were calculated from FFQ data by multiplying the frequency of consumption of a food item by the nutrient contents per standard serving size for the given food item.

Covariate Assessment

In each cohort, participants provided general demographic, lifestyle and medical history data via standard questionnaires. Education was assessed by asking the highest degree or level of education the participant had completed. Participants were grouped into categories (FOS: less than high school, graduated high school, some college, or graduated college; WGHS: less than Bachelor's degree, Bachelor's Degree, graduate degree). In FOS, participants were classified as diabetic if their fasting blood glucose was ≥ 126 mg/dL or their non-fasting blood glucose was ≥ 200 mg/dl, and in WGHS, participants self-reported whether they were diagnosed with diabetes. FOS participants completed a standardized physical examination, which included measurements of height and weight, and WGHS participants provided self-reported height and weight. Body mass index (BMI) was calculated as weight divided by height (kg/m^2). Alcohol intake was assessed by asking the number of alcoholic beverages consumed in a typical week in the previous year and expressed as grams per day. Current smokers were defined as participants

who reported currently smoking (WGHS) or smoking regularly in the past year (FOS). Physical activity was evaluated through standard questionnaires in FOS (at exam 5 only) (52) and WGHS (53). Potential confounding through other dietary components was explored through adjustment of individual dietary factors (percent energy from saturated fat and servings/day of fruit, vegetables, whole grains, fish, and nuts/seeds).

Statistical Analyses

Participants were grouped by categories of SSB consumption (<1 serving/month, 1-4 serving/month, 1-2 serving/week, 3-7 serving/week, >1 serving/day), similar to previous studies (54,55). Linear (WGHS) and linear mixed effects (FOS) regression models were used to examine the association between beverage consumption patterns and LDL-C, HDL-C, TG, non-HDL-C, and TC: HDL-C ratio. In the FOS cohort, familial correlation was accounted for by adding a random effect in the model with a covariance structure proportional to the kinship matrix as implemented in the *lmekin* function of the *coxme* R package (https://cran.r-project.org). A natural logarithmic transformation was applied to TG concentrations and quantile normalization was applied to RLP concentrations to approximate normal distributions. Due to differences in unit measures among FOS and WGHS participants and skewed distributions, lipoprotein particle size concentrations (TRL-P, LDL-P, and HDL-P measures) were harmonized through quantile normalization. Four models were performed. Model 1 adjusted for age (continuous), sex (M/F) (FOS only), and total energy intake (continuous). Model 2 adjusted for model 1 covariates plus education (FOS: less than high school, graduated high school, some college, or graduated college; WGHS: less than bachelor's degree, bachelor's degree, graduate degree), current smoking status (yes/no), physical activity (FOS: continuous index; WGHS: continuous metabolic equivalents), alcohol (grams/day), current diabetes (yes/no), servings per

day of vegetables, whole fruits, whole grains, nuts/seeds, and seafood, as well as percent energy from saturated fat (continuous). Model 3 adjusted for model 2 covariates plus BMI (continuous), which is a marker of adiposity and could be a mediator in the association between SSB consumption and lipoprotein measures. Model 4 adjusted for model 3 covariates plus total lipoprotein concentration (ln-TG, HDL-C, or LDL-C for TRL-P, HDL-P, and LDL-P measures, respectively) and was applied only for lipoprotein particle size measures. Model 4 allows us to examine the association of SSB with the lipoprotein particle size measures independent of the association between SSB and traditional lipoprotein measures. Models were run separately for WGHS and FOS cohorts, and regression coefficients and standard errors were combined through fixed-effects multivariate meta-analyses (56) using the *mvmeta* R package (https://cran.r-project.org). To assess for a linear trend across categories, SSB category was treated as a continuous variable, and regression coefficients and standard errors were combined through fixed-effects univariate meta-analysis using the *meta* R package (https://cran.r-project.org). The Cochrane Q statistic and the I^2 statistic were used to examine heterogeneity of the associations among the two cohorts.

Likelihood-ratio testing comparing models with and without multiplicative interaction terms were used to assess effect modification by sex (male/female; FOS only) and BMI (<25 kg/m^2; 25-29.9 kg/m^2; ≥30 kg/m^2). Among WGHS participants, sensitivity analyses were conducted among the fasting subsample. All statistical analyses were performed using either SAS (SAS Institute, Cary, NC; version 9.4 or higher) or R (version 3.1 or higher; https://cran.r-project.org) statistical software. All reported p-values are two-sided, and results were considered statistically significant at a global $p < 0.05$, corrected for multiple endpoints using Tukey's method (57).

4.4 Results

Table 4.1 presents the characteristics of participants within each cohort among the highest and lowest categories of SSB consumption. Full descriptive statistics stratified by all five categories of SSB intake are displayed in **Table 4.5** (FOS) and **Table 4.6** (WGHS). Among FOS participants, the highest SSB consumers were less likely to be women. In both cohorts, the highest SSB consumers were younger, more likely to smoke, less likely to have diabetes, and less physically active. Additionally, WGHS participants had achieved lower levels of education and had a higher BMI with increased SSB consumption, while FOS participants, had achieved higher levels of education with increased SSB consumption. Lipoprotein profiles were less favorable among the highest SSB consumers. Total energy intake and fruit juice consumption increased with increasing SSB consumption, while LCSB, whole fruit, vegetable, whole grain, and seafood consumption decreased with increasing SSB consumption. In addition, nuts/seeds consumption increased with increasing SSB consumption among FOS participants, and alcohol consumption decreased with increasing SSB consumption among WGHS participants.

Associations between SSB intake, and lipoprotein and apolipoprotein concentrations

Table 4.2 presents the associations of SSB intake with lipoprotein and apolipoprotein concentrations among categories of SSB intake (reference: <1 serving/month) in FOS, WGHS, and combined analyses. In combined analyses using the fully adjusted models (Model 3), participants in the highest category of SSB intake (>1 serving/day) had higher mean LDL-C (β ± SE: 2.6 ± 1.1 mg/dl), TG [β ± SE: 0.12 ± 0.02 [ln] mg/dl], non-HDL-C [β ± SE: 6.2 ± 1.2 mg/dl], TC:HDL-C ratio [β ± SE: 0.37 ± 0.04 mg/dl], apo B (β ± SE: 5.0 ± 0.8 mg/dl), and apo

B: apo A1 ratio [β ± SE: 0.05 ± 0.01 mg/dl], along with lower HDL-C (β ± SE: -3.3 ± 0.4 mg/dl) and apo A1 (β ± SE: -3.9 ± 0.8 mg/dl) concentrations compared to those in the lowest category of SSB intake (<1 serving/month). Trend analyses indicated a statistically significant linear trend across the five categories of SSB intake for all lipoprotein and apolipoprotein measures ($p_{trend} < 0.0001$). In analyses restricted to the FOS cohort, participants in the highest category of SSB intake (>1 serving/day) had higher mean RLP-TG (β ± SE: 0.21 ± 0.09 mg/dl; p_{trend} = 0.002) and RLP-C (β ± SE: 0.12 ± 0.08 mg/dl; $p_{trend} = 0.008$) concentrations. No significant associations were observed for apo E or apo C3 concentrations in the FOS cohort. No significant interactions ($p<0.05$) between SSB consumption and sex or BMI were observed. We observed similar results when analyses were limited to the fasting subsample of WGHS participants (**Table 4.7**).

Associations between SSB intake and lipoprotein particle size measures

Table 4.3 presents mean differences in lipoprotein particle size concentrations between the highest (>1 serving/day) and the lowest SSB consumers (<1 serving/month), along with a p_{trend} across the five categories of SSB intake in FOS, WGHS, and combined analyses. After adjustment for traditional lipoprotein measures and potential confounding factors (Model 4), the highest SSB consumers had a smaller LDL-P (β ± SE: -0.10 ± 0.01 nm; $p_{trend} < 0.0001$) and HDL-P (β ± SE: -0.02 ± 0.01 nm; $p_{trend} = 0.0005$) size, along with lower concentrations of large LDL-P (β ± SE: -0.18 ± 0.03; $p_{trend} < 0.0001$) and medium HDL-P (β ± SE: -0.10 ± 0.03; $p_{trend} <$ 0.0001) and higher concentrations of small LDL-P (β ± SE: 0.14 ± 0.03; $p_{trend} < 0.0001$) and small HDL-P (β ± SE: 0.16 ± 0.03; $p_{trend} < 0.0001$) compared to the lowest SSB consumers.

Among TRL-P measures, SSB was only associated with higher concentrations of large TRL-P (β ± SE: 0.06 ± 0.02; p_{trend} = 0.002). There was no evidence of heterogeneity among the two cohorts for these significant associations (p>0.05). However, significant heterogeneity was observed for medium LDL-P (p=0.0004), where SSB consumption was associated with higher concentrations of medium LDL-P among FOS participants (p_{trend} < 0.001), but a similar trend was not observed among WGHS participants (p_{trend} = 0.72). Similar results were observed when analyses were limited to the fasting subsample of WGHS participants (**Table 4.7**) and no significant interactions (p<0.05) were observed by sex or BMI.

4.5 Discussion

In this study of two large U.S. cohorts, SSB consumption was associated with concentrations of lipoprotein cholesterol, apolipoproteins, and lipoprotein particle size concentrations that have been linked to adverse cardiometabolic outcomes. We identified novel associations between SSB intake and higher non-HDL-C, RLP-TG and RLP-C concentrations, and smaller LDL-P and HDL-P size. We also replicated previously observed associations of SSB consumption with TC: HDL-C and apo B: apo A1 ratio, and HDL-C, LDL-C, TG, apo B, and apo A1 concentrations.

Our analysis is the largest to date that examines the associations between SSB consumption and a wide range of lipoprotein concentrations. These results are consistent with several other studies that have observed that higher SSB consumption is associated with lower HDL-C and apo A1 concentrations, and higher LDL-C and TG concentrations, and TC: HDL-C and apo B: apo A1 ratio (5,13,16,17,54,58). Although measurement of apo B and apo A1 concentrations are not currently recommended over lipoprotein measures in clinical settings

(32,59), there is some data to suggest that they can improve risk assessment and their elevation may confer high-risk (9,23). In addition to previously observed associations, our results indicate that SSB intake is associated with non-HDL-C, RLP-C, and RLP-TG concentrations, all of which are associated with increased cardiometabolic risk (9,22,32,60). RLP concentrations are another emerging cardiovascular risk factor (61–63), and to date, few interventions have considered whether dietary sugar or SSB influence RLP concentrations. One randomized controlled trial with 48 participants observed that consumption of SSB at 25% of total energy intake over a 2 week period resulted in significant increases in RLP-C and RLP-TG concentrations (33). Thus, reducing SSB consumption may be a promising target for reduction of RLP concentrations.

The results of this study also indicate that SSB consumption is significantly associated with decreased HDL-P and LDL-P size, independent of total HDL-C and LDL-C concentrations. The clinical implications of this observation are uncertain since the potential role of LDL-P and HDL-P size compared to traditional lipoprotein measures in relation to cardiometabolic disease is unclear. However, smaller HDL-P and LDL-P size have been associated with higher risk for T2D (30,64) and metabolic syndrome (65) in observational studies, independent of traditional lipoprotein measures. No previous observational studies have investigated the association between SSB consumption and lipoprotein particle size concentrations among adults. One cross-sectional study among 74 Swiss children observed that high total fructose intake, out of eleven categories of macronutrient intake, was the only significant predictor of LDL-P size (66). Two small (<50 participants) intervention studies comparing consumption of glucose-, fructose-, and sugar-sweetened beverages within ranges of normal intake over 2-3 week periods also observed that either consumption of fructose- or sugar-sweetened beverages led to lower LDL-P size

(33,34). We observed a significant association between SSB consumption and large TRL-P concentrations. Evidence regarding the clinical significance of this finding is sparse, but some large prospective studies have observed that greater concentrations of large TRL-P concentrations are associated with a higher risk for T2D compared to small TRL-P (30,64) and that large TRL-P concentrations were elevated among adults with non-alcoholic fatty liver disease (67). Interestingly, two small intervention studies that compared low-carbohydrate diets vs. low-fat weight loss diets for 3-6 months observed larger decreases in large TRL-P concentrations compared to small TRL-P (68,69) for the low-carbohydrate diet, suggesting a potential role of diets low in carbohydrates on TRL-P size. For many of these emerging lipoprotein risk factors, although statistically significant, the effect sizes are relatively small and further research is needed to determine the clinical relevance of these observed associations.

The strengths of our study include large sample size, the ability to adjust for multiple confounding factors, and ability to examine a wide range of lipoprotein concentrations among two independent cohorts. However, the research design also has some limitations. The design of this study limits our ability to infer causality between SSB consumption and the outcomes of interest, and the use of self-reported dietary data can potentially lead to misclassification of food and nutrient intakes. These FFQ did not include an exhaustive list of all potential sources of SSB, such as consumption of sweetened coffee/tea. However, estimates of beverage consumption during the time of data collection (1987-1995) suggest that consumption of coffee/tea (sweetening not captured) were low compared to consumption of SSB (70). Among FOS participants, measurements of apo A1, apo B, RLP and lipoprotein particle size concentrations were derived from blood draws at exam 4, whereas dietary intakes were estimated at exam 5. Thus, estimated dietary intakes may not reflect dietary intakes at the time of the blood

draw. However, consistency of the associations between SSB consumption and lipoprotein particle size concentrations in FOS and WGHS adds confidence that our estimates of dietary intakes are informative. Generalizability of our study is also limited by our sample of predominantly European-descent adults and by the inclusion of only women and health professionals, hence likely have a higher socioeconomic status than the general population, in WGHS.

In conclusion, our findings suggest that higher consumption of SSB is associated with multiple measures of plasma lipoprotein concentrations that have been linked to adverse cardiometabolic outcomes, including both traditional and emerging measures of lipoprotein cholesterol, apolipoprotein, and lipoprotein particle concentrations. These data suggest that differences in lipoprotein metabolism are a potential pathway by which SSB intake may increase risk for cardiometabolic diseases.

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Table 4.1 Characteristics of participants by cohort and category of sugar sweetened beverage intake*

	Framingham Offspring Study				Women's Genome Health Study			
	Overall	**<1 serving SSB/month**	**>1 serving SSB/day**		**Overall**	**<1 serving SSB/month**	**>1 serving SSB/day**	
n	**3,047**	**1,100**	**312**		**21,794**	**9,862**	**1,095**	
Median SSB (servings/week)		**0**	**14.0**	**p_{trend}**		**0**	**14.5**	**p_{trend}**
Sex (% women)	54.1	64.5	34.3	<0.0001	100	100	100	-
Age (yrs.)	54.3 (9.8)	55.9 (9.7)	51.1 (10.2)	<0.0001	54.5 (7.0)	54.7 (7.0)	52.5 (6.0)	<0.0001
Current Smoker (%)	19.4	15.7	30.1	<0.0001	11.6	10.2	20.0	<0.0001
Diabetics (%)	6.5	8.9	5.8	<0.0001	2.2	3.4	1.4	<0.0001
Education (% Bachelor's Degree)	33.6	31.2	33.7	<0.0001	43.2	48.3	34.2	<0.0001
BMI (kg/m^2)	27.3 (5.0)	27.6 (5.3)	27.5 (4.9)	0.35	25.9 (4.9)	25.9 (4.9)	26.3 (5.7)	0.003
Physical Activity** †	34.8 (6.1)	34.1 (5.5)	36.1 (7.3)	<0.0001	6.0 (17.6)	6.9 (18.2)	3.6 (13.9)	<0.0001
Traditional Lipoprotein Measures								
LDL-C (mg/dl)	126 (32)	125 (33)	124 (31)	0.44	124 (34)	123 (34)	125 (36)	<0.0001
HDL-C (mg/dl)	51 (15)	53 (16)	45 (13)	<0.0001	54 (15)	55 (16)	49 (14)	<0.0001
TG (mg/dl) †	122 (88)	119 (86)	134 (106)	<0.0001	122 (91)	119 (89)	137 (112)	<0.0001
non-HDL-C (mg/dl)	153 (38)	152 (38)	154 (37)	0.01	157 (41)	156 (40)	162 (44)	<0.0001
Ratio Total: HDL-C	4.4 (1.5)	4.2 (1.5)	4.8 (1.5)	<0.0001	4.2 (1.3)	4.1 (1.3)	4.6 (1.5)	<0.0001
Apolipoprotein Measures								
apo B (mg/dl)	97 (25)	97 (26)	99 (27)	0.03	95 (23)	94 (22)	97 (24)	<0.0001
apo A1 (mg/dl)	144 (30)	149 (33)	136 (24)	<0.0001	154 (30)	156 (30)	147 (30)	<0.0001
apo B: apo A1	0.70 (0.23)	0.67 (0.23)	0.75 (0.24)	<0.0001	0.71 (0.24)	0.69 (0.23)	0.77 (0.26)	<0.0001
apo E (mg/dl)	10.1 (4.8)	10.4 (4.9)	9.9 (4.2)	0.08	-	-	-	-
apo C3 (mg/dl)	16.3 (4.5)	16.5 (4.3)	16.2 (4.7)	0.27	-	-	-	-
Other Lipoprotein Measures								
RLP-TG (mg/dl)	18.0 (13.2)	17.6 (11.5)	21.3 (16.7)	<0.0001	-	-	-	-
RLP-C (mg/dl)	7.1 (2.2)	7.0 (2.0)	7.6 (2.8)	0.0005	-	-	-	-
TRL-P								
TRL-P mean size (nm)	46.2 (9.3)	45.9 (9.2)	47.9 (10.1)	<0.0001	44.1 (7.5)	44.1 (7.6)	45.3 (8)	<0.0001
TRL-P Very Large†‡	3.7 (6.5)	3.5 (5.5)	4.7 (9.9)	0.0001	0.1 (0.1)	0.1 (0.1)	0.1 (0.2)	0.36

TRL-P Large[†‡]	2.7 (25)	2.2 (21)	4.3 (33.9)	<0.0001	0.9 (4.0)	0.8 (3.8)	1.2 (4.7)	<0.0001
TRL-P Medium[†‡]	30.6 (37)	28.4 (34.3)	34.5 (39.9)	<0.0001	12.7 (16.7)	12.1 (16.6)	14.9 (18.3)	<0.0001
TRL-P Small[†‡]	14.1 (18.6)	13.6 (18.7)	14.0 (18.9)	0.79	47.5 (47.8)	46 (48.1)	47.7 (48.9)	<0.0001
TRL-P Very Small [†‡]	1.1 (6.6)	1.1 (6.8)	1.4 (5.9)	0.23	78.0 (55.2)	77.2 (54.8)	82.0 (58.6)	<0.0001
LDL-P								
LDL-P mean size (nm)	20.9 (0.6)	20.9 (0.5)	20.8 (0.6)	<0.0001	20.9 (0.5)	20.9 (0.4)	20.8 (0.5)	<0.0001
LDL-P Large [†‡]	53.2 (48.5)	55 (47.1)	43.5 (47.3)	<0.0001	184 (307)	197 (307)	124 (310)	<0.0001
LDL-P Medium [†‡]	20.6 (32.8)	19.9 (30.4)	25.3 (29.4)	0.0005	16.6 (348)	16.8 (339)	10.9 (341)	0.02
LDL-P Small[†‡]	13.7 (23.5)	12.5 (22.7)	17 (28.4)	<0.0001	926 (636)	914 (596)	1007 (750)	<0.0001
HDL-P								
HDL-P mean size (nm)	9.2 (0.5)	9.2 (0.5)	9.0 (0.4)	<0.0001	8.96 (0.38)	8.99 (0.39)	8.87 (0.36)	<0.0001
HDL-P Large [‡]	10.5 (7.1)	11.6 (7.9)	8.1 (5.6)	<0.0001	2.5 (1.5)	2.6 (1.6)	2.1 (1.4)	<0.0001
HDL-P Medium [‡]	22.9 (10.2)	24.6 (10.9)	19.8 (9.1)	<0.0001	5.8 (2.8)	6.0 (2.9)	5.1 (2.6)	<0.0001
HDL-P Small [‡]	17.8 (4.9)	17.3 (5.0)	18.1 (4.7)	0.003	16.4 (3.4)	16.2 (3.5)	16.8 (3.4)	<0.0001
Dietary Intakes								
Total Energy (kcal/d)	1869 (612)	1661 (541)	2368 (595)	<0.0001	1735 (525)	1616 (489)	2152 (547)	<0.0001
Saturated Fat (% total energy)	10.6 (2.8)	10.4 (3.0)	10.0 (2.5)	0.98	10.3 (2.5)	10.2 (2.6)	9.8 (2.4)	0.07
Fruits (serv/d) [†]	0.6 (1.1)	0.7 (1.1)	0.5 (1.0)	<0.0001	1.5 (1.5)	1.5 (1.4)	1.2 (1.5)	<0.0001
Vegetables (serv/d) [†]	1.7 (1.2)	1.8 (1.2)	1.6 (1.0)	0.0005	3.3 (2.6)	3.3 (2.7)	3.0 (2.6)	<0.0001
Whole Grain (serv/d) [†]	0.6 (1.3)	0.7 (1.4)	0.4 (1.1)	<0.0001	1.0 (1.4)	1.0 (1.3)	0.8 (1.1)	<0.0001
Nuts/Seeds (serv/d) [†]	0.2 (0.4)	0.1 (0.4)	0.2 (0.5)	<0.0001	0.1 (0.3)	0.1 (0.2)	0.1 (0.3)	0.26
Seafood (serv/d) [†]	0.3 (0.4)	0.3 (0.4)	0.2 (0.3)	0.05	0.2 (0.2)	0.2 (0.2)	0.2 (0.2)	<0.0001
Alcohol (g/d) [†]	1.2 (13.5)	1.1 (12.7)	0.6 (14.0)	0.14	0.3 (4.6)	0.4 (6.5)	0.1 (2.1)	<0.0001

[*] Values are means (standard deviations) unless otherwise noted. Maximum available observations, *n*, for analyses. All Framingham Offspring Study values are derived from exam 5 data except apo B, apo A1, apo B: apo A1 ratio, and all "Other Lipoprotein Measures", which were derived from exam 4 data. P_{trend} represents the p-value for regression coefficients where five categories of SSB intake (<1 serving/month, 1-4 servings/month, 1-2 servings/week, 3-7 servings/week, >1 serving/day) are treated as a continuous variable in unadjusted models. Descriptive statistics for all five categories of SSB intake are displayed in Supplemental Table S2 (FOS) and Supplemental Table S3 (WGHS).

[**] FHS: physical activity index; WGHS: metabolic equivalents (hours/week); [†] Geometric mean (IQR); [‡] FOS: cholesterol concentration in mg/dl; WGHS: particle concentration in nmol/L (TRL-P/LDL-P) or µmol/L (HDL-P)

Abbreviations: apo A1, apolipoprotein A1 concentrations; apo B, apolipoprotein B concentrations; apo C3, apolipoprotein C3 concentrations; apo E, apolipoprotein E concentrations; BMI, body mass index; HDL-C, high-density lipoprotein cholesterol concentrations; HDL-P, high-density lipoprotein particle concentrations; kcal, kilocalories; LDL-C, low-density lipoprotein cholesterol concentrations; LDL-P, low-density lipoprotein particle concentrations; RLP-C, remnant-like particle cholesterol concentrations; RLP-TG, remnant-like particle triglyceride concentrations; SSB, sugar-sweetened beverages; TC, total cholesterol concentrations; TG, triglyceride concentrations; TRL-P, triglyceride-rich lipoprotein particle concentrations.

Table 4.2 Associations between sugar-sweetened beverage intake and lipoprotein and apolipoprotein concentrations

	<1 serv SSB/month	1-4 serv SSB/month	1-2 serv SSB/week	3-7 serv SSB/week	>1 serv SSB/day	p_{trend}	Adjusted p_{trend}**	*P* for Heterogeneity
Framingham Offspring Study*								
No of Participants	1,100	710	289	635	312			
LDL-C (mg/dl)	Ref.	1.4 (1.6)	3 (2.2)	3 (1.7)	0.9 (2.5)	0.18	0.48	
HDL-C (mg/dl)	Ref.	-0.6 (0.6)	-1.3 (0.8)	-2.3 (0.7)	-1.6 (0.9)	0.0009	0.002	
TG (ln-mg/dl)	Ref.	0.04 (0.02)	0.04 (0.03)	0.09 (0.03)	0.13 (0.04)	0.0001	0.0001	
non-HDL-C (mg/dl)	Ref.	2.5 (1.7)	3.8 (2.4)	5.5 (1.9)	4.2 (2.7)	0.008	0.02	
TC: HDL-C	Ref.	0.02 (0.01)	0.05 (0.01)	0.04 (0.01)	0.04 (0.02)	0.0001	0.0001	
RLP-TG (mg/dl)	Ref.	-0.01 (0.05)	0.15 (0.07)	0.15 (0.06)	0.22 (0.08)	0.001	0.001	
RLP-C (mg/dl)	Ref.	-0.001 (0.05)	0.2 (0.07)	0.14 (0.05)	0.13 (0.08)	0.003	0.008	
apo B (mg/dl)	Ref.	1.1 (1.1)	6.3 (1.6)	2.6 (1.2)	4.1 (1.8)	0.003	0.008	
apo A1 (mg/dl)	Ref.	-2.3 (1.3)	-1.7 (1.8)	-4.1 (1.4)	-2.4 (2.1)	0.02	0.06	
apo B: apo A1	Ref.	0.07 (0.06)	0.14 (0.09)	0.26 (0.07)	0.23 (0.10)	0.0003	0.0005	
apo E (mg/dl)	Ref.	-0.4 (0.3)	-0.2 (0.4)	0.1 (0.3)	0.2 (0.4)	0.54	0.93	
apo C3 (mg/dl)	Ref.	0.2 (0.2)	0.3 (0.3)	0.4 (0.2)	0.5 (0.3)	0.07	0.20	
Women's Genome Health Study*								
No of Participants	7,430	3,642	2,168	2,516	864			
LDL-C (mg/dl)	Ref.	1.0 (0.6)	1.9 (0.7)	2.4 (0.7)	3.0 (1.2)	<0.0001	0.0001	
HDL-C (mg/dl)	Ref.	-1.0 (0.2)	-2.2 (0.3)	-2.2 (0.3)	-3.7 (0.5)	<0.0001	<0.0001	
TG (ln-mg/dl)	Ref.	0.02 (0.01)	0.05 (0.01)	0.06 (0.01)	0.12 (0.02)	<0.0001	<0.0001	
non-HDL-C (mg/dl)	Ref.	1.1 (0.7)	2.6 (0.9)	4.0 (0.8)	6.7 (1.4)	<0.0001	<0.0001	
TC: HDL-C	Ref.	0.08 (0.02)	0.17 (0.03)	0.22 (0.03)	0.40 (0.04)	<0.0001	<0.0001	
apo B (mg/dl)	Ref.	1.1 (0.47)	2.5 (0.6)	3.3 (0.6)	5.2 (0.9)	<0.0001	<0.0001	
apo A1 (mg/dl)	Ref.	-1.6 (0.4)	-3.1 (0.5)	-2.3 (0.5)	-4.1 (0.8)	<0.0001	<0.0001	
apo B: apo A1	Ref.	0.02 (0.004)	0.03 (0.005)	0.04 (0.005)	0.06 (0.008)	<0.0001	<0.0001	
Combined Results†								
LDL-C (mg/dl)	Ref.	1.0 (0.6)	2.0 (0.7)	2.5 (0.7)	2.6 (1.1)	<0.0001	<0.0001	0.93
HDL-C (mg/dl)	Ref.	-1.0 (0.2)	-2.1 (0.3)	-2.3 (0.3)	-3.3 (0.4)	<0.0001	<0.0001	0.21

TG (ln-mg/dl)	Ref.	0.02 (0.01)	0.05 (0.01)	0.07 (0.01)	0.12 (0.02)	<0.0001	<0.0001	0.79
non-HDL-C (mg/dl)	Ref.	1.3 (0.7)	2.7 (0.8)	4.2 (0.8)	6.2 (1.2)	<0.0001	<0.0001	0.86
TC: HDL-C	Ref.	0.08 (0.02)	0.17 (0.03)	0.22 (0.02)	0.37 (0.04)	<0.0001	<0.0001	0.46
apo B (mg/dl)	Ref.	1.1 (0.4)	3.0 (0.5)	3.1 (0.5)	5.0 (0.8)	<0.0001	<0.0001	0.08
apo A1 (mg/dl)	Ref.	-1.7 (0.4)	-3.0 (0.5)	-2.5 (0.5)	-3.9 (0.8)	<0.0001	<0.0001	0.39
apo B: apo A1	Ref.	0.02 (0.004)	0.03 (0.005)	0.04 (0.004)	0.05 (0.01)	<0.0001	<0.0001	0.39

[*] Values are regression coefficients for SSB intake in mixed effects models accounting for family structure (Framingham Offspring Study) and generalized linear models (Women's Genome Health Study) adjusted for age, sex (Framingham Offspring Study only), total energy intake, smoking status, education status, current diabetes status, physical activity, alcohol intake, body mass index, whole fruit intake, vegetable intake, whole grains intake, seafood intake, nuts/seeds intake, and saturated fatty acid intake (% of total energy).
[**]Adjusted for multiple endpoints using Tukey's method (Tukey et al. *Biometrics* 1985).
[†] Study estimates from the two cohorts were combined through fixed-effects meta-analyses

Abbreviations: apo A1, apolipoprotein A1 concentrations; apo B, apolipoprotein B concentrations; apo C3, apolipoprotein C3 concentrations; apo E, apolipoprotein E concentrations; HDL-C, high-density lipoprotein cholesterol concentrations; LDL-C, low-density lipoprotein cholesterol concentrations; RLP-C, remnant-like particle cholesterol concentrations; RLP-TG, remnant-like particle triglyceride concentrations; SSB, sugar-sweetened beverages; TC, total cholesterol concentrations; TG, triglyceride concentrations.

Table 4.3 Associations between sugar-sweetened beverage intake and lipoprotein particle size concentrations [*]

	FHS		WGHS		Combined Results[†]			
	β (SE)	P_{trend}	β (SE)	P_{trend}	β (SE)	P_{trend}	Adjusted P_{trend} [**]	*P* for Heterogeneity
TRL-P								
TRL-P mean size (nm)	0.57 (0.56)	0.39	0.41 (0.21)	0.37	0.43 (0.19)	0.27	0.64	0.48
TRL-P Very Large	0.06 (0.06)	0.28	0.004 (0.03)	0.30	0.02 (0.03)	0.60	0.96	0.60
TRL-P Large	0.06 (0.05)	0.21	0.06 (0.02)	0.002	0.06 (0.02)	0.0007	0.002	0.99
TRL-P Medium	-0.03 (0.05)	0.55	-0.002 (0.02)	0.65	-0.008 (0.02)	0.85	0.99	0.84
TRL-P Small	0.04 (0.08)	0.35	-0.04 (0.04)	0.29	-0.03 (0.03)	0.19	0.48	0.48
TRL-P Very Small	0.01 (0.08)	0.62	0.02 (0.03)	0.16	0.02 (0.03)	0.14	0.36	0.77
LDL-P								
LDL-P mean size (nm)	-0.08 (0.04)	0.02	-0.10 (0.02)	<0.0001	-0.10 (0.01)	<0.0001	<0.0001	0.78
LDL-P Large	0.08 (0.07)	0.23	-0.24 (0.03)	<0.0001	-0.18 (0.03)	<0.0001	<0.0001	0.52
LDL-P Medium	0.22 (0.07)	0.001	-0.06 (0.03)	0.36	-0.01 (0.03)	0.72	0.99	0.0004
LDL-P Small	-0.17 (0.08)	0.01	0.20 (0.03)	<0.0001	0.14 (0.03)	<0.0001	<0.0001	0.58
HDL-P								
HDL-P mean size (nm)	-0.06 (0.02)	0.05	-0.01 (0.01)	0.001	-0.02 (0.01)	0.0002	0.0005	0.40
HDL-P Large	-0.10 (0.04)	0.31	-0.03 (0.02)	0.15	-0.04 (0.02)	0.08	0.21	0.59
HDL-P Medium	-0.03 (0.05)	0.61	-0.12 (0.03)	<0.0001	-0.10 (0.03)	<0.0001	<0.0001	0.10
HDL-P Small	0.14 (0.08)	0.11	0.16 (0.03)	<0.0001	0.16 (0.03)	<0.0001	<0.0001	0.17

[*] Values are regression coefficients for highest category of SSB intake (>1 serving/day) compared to lowest category of SSB intake (<1 serving/month) on quantile normalized particle concentrations using mixed effects models accounting for family structure (FHS) and generalized linear models (WGHS) adjusted for age, sex (FHS only), total energy intake, smoking status, education status, current diabetes status, physical activity, alcohol intake, body mass index, whole fruit intake, vegetable intake, whole grains intake, seafood intake, nuts/seeds intake, saturated fatty acid intake (% of total energy) and total lipid measure (triglyceride, low-density lipoprotein cholesterol and high-density lipoprotein cholesterol concentrations for TRL-P, LDL-P, and HDL-P concentrations, respectively). P_{trend} represents the p-value for regression coefficients where the category of SSB intake (<1 serving/month, 1-4 servings/month, 1-2 servings/week, 3-7 servings/week, >1 serving/day) is treated as a continuous variable.

[**]Adjusted for multiple endpoints using Tukey's method (Tukey et al. *Biometrics* 1985).

[†] Study estimates from the two cohorts were combined through fixed-effects meta-analyses

Abbreviations: HDL-P, high-density lipoprotein particle concentration; LDL-P, low-density lipoprotein particle concentration; SSB, sugar-sweetened beverages; TRL-P, triglyceride-rich lipoprotein particle concentration.

Table 4.4 Range of lipoprotein particle sizes (nm) in classes of TRL-P, LDL-P, and HDL-P

Class	LipoProtein-I	LP4
TRL-P, Very Large	>80	90-240
TRL-P, Large	60-80	50-89
TRL-P, Medium	35-60	37-49
TRL-P, Small	27-35	30-36
TRL-P, Very Small	23-27	24-29
LDL-P, Large	21.3-22.7	21.5-23
LDP-P, Medium	19.8-21.2	20.5-21.4
LDL-P, Small	18.3-19.7	19-20.4
HDL-P, Large	10-13	10-13
HDL-P, Medium	8.2-10	9-10
HDL-P, Small	7.3-8.2	7-8

Abbreviations: HDL-P, high-density lipoprotein particle concentration; LDL-P, low-density lipoprotein particle concentration; TRL-P, triglyceride rich lipoprotein particle concentration.

Table 4.5 Characteristics of participants in the Framingham Offspring Study by category of sugar-sweetened beverage intake.

	Overall	**<1 serv SSB/mo**	**1-4 serv SSB/mo**	**1-2 serv SSB/wk**	**3-7 serv SSB/wk**	**>1 serv SSB/d**	
n	**3,047**	**1,100**	**710**	**289**	**635**	**312**	
Median SSB (serv†/wk)		**0**	**0.47**	**1.47**	**4.00**	**14**	**p-trend***
Age (yrs.)	54.3 (9.8)	55.9 (9.7)	54.6 (9.5)	52.5 (9.5)	53.5 (9.8)	51.1 (10.2)	<0.0001
Sex (% women)	54.1	64.5	60.4	43.9	43.3	34.3	<0.0001
Current Smoker (%)	19.4	15.7	18.5	17.6	22.2	30.1	<0.0001
Diabetics (%)	6.5	8.9	5.6	3.5	5.0	5.8	<0.0001
Education (% Bachelor's Degree)	33.6	31.2	32.4	40.5	35.9	33.7	<0.0001
BMI (kg/m^2)	27.3 (5.0)	27.6 (5.3)	27.0 (4.8)	26.8 (4.2)	27.2 (4.9)	27.5 (4.9)	0.35
Physical Activity Index	34.8 (6.1)	34.1 (5.5)	34.4 (5.6)	35.3 (6.2)	35.4 (6.8)	36.1 (7.3)	<0.0001
Traditional Lipoprotein Measures							
LDL-C (mg/dl)	126 (32)	125 (33)	126 (31)	126 (32)	127 (33)	124 (31)	0.44
HDL-C (mg/dl)	51 (15)	53 (16)	53 (15)	50 (14)	48 (14)	45 (13)	<0.0001
TG (mg/dl)	122 (88)	119 (86)	119 (86)	116 (86)	128 (91)	134 (106)	<0.0001
non-HDL-C (mg/dl)	153 (38)	152 (38)	152 (37)	152 (36)	157 (38)	154 (37)	0.01
Ratio Total: HDL-C	4.4 (1.5)	4.2 (1.5)	4.2 (1.4)	4.4 (1.4)	4.6 (1.6)	4.8 (1.5)	<0.0001
Apolipoprotein Measures							
apo B (mg/dl)	97 (25)	97 (26)	96 (25)	100 (25)	98 (24)	99 (27)	0.03
apo A1 (mg/dl)	144 (30)	149 (33)	146 (31)	143 (26)	140 (27)	136 (24)	<0.0001
apo B: apo A1	0.70 (0.23)	0.67 (0.23)	0.68 (0.22)	0.72 (0.24)	0.73 (0.23)	0.75 (0.24)	<0.0001
apo E (mg/dl)	10.1 (4.8)	10.4 (4.9)	9.9 (4.8)	9.9 (4.8)	9.9 (4.7)	9.9 (4.2)	0.08
apo C3 (mg/dl)	16.3 (4.5)	16.5 (4.3)	16.3 (4.3)	16.1 (4.9)	16.3 (4.6)	16.2 (4.7)	0.27
Other Lipoprotein Measures							
RLP-TG (mg/dl)	18.0 (13.2)	17.6 (11.5)	16.3 (11.8)	18.3 (14.8)	19.1 (15.3)	21.3 (16.7)	<0.0001
RLP-C (mg/dl)	7.1 (2.2)	7.0 (2.0)	6.8 (1.8)	7.2 (2.7)	7.2 (2.6)	7.6 (2.8)	0.0005
TRL-P							
TRL-P mean size (nm)	46.2 (9.3)	45.9 (9.2)	45.3 (8.8)	46 (8.6)	47 (9.6)	47.9 (10.1)	<0.0001
TRL-P Very Large†‡	3.7 (6.5)	3.5 (5.5)	3.5 (5.1)	3.7 (7.0)	4.1 (8.0)	4.7 (9.9)	0.0001
TRL-P Large†‡	2.7 (25)	2.2 (21)	2.1 (22)	2.8 (33)	3.7 (29)	4.3 (33.9)	<0.0001

TRL-P Medium†‡	30.6 (37)	28.4 (34.3)	29.4 (34.1)	32.6 (41.8)	32.9 (37.6)	34.5 (39.9)	<0.0001
TRL-P Small†‡	14.1 (18.6)	13.6 (18.7)	14.1 (18.1)	15.5 (18.9)	14.6 (19.4)	14.0 (18.9)	0.79
TRL-P Very Small †‡	1.1 (6.6)	1.1 (6.8)	0.9 (6.4)	1.1 (6.9)	1.4 (6.9)	1.4 (5.9)	0.23
LDL-P							
LDL-P mean size (nm)	20.9 (0.6)	20.9 (0.5)	21 (0.5)	20.9 (0.6)	20.9 (0.6)	20.8 (0.6)	<0.0001
LDL-P Large †‡	53.2 (48.5)	55 (47.1)	58.8 (48)	54.5 (52.4)	49.2 (47.8)	43.5 (47.3)	<0.0001
LDL-P Medium †‡	20.6 (32.8)	19.9 (30.4)	18.4 (30.8)	23.9 (37)	20.7 (37.2)	25.3 (29.4)	0.0005
LDL-P Small†‡	13.7 (23.5)	12.5 (22.7)	12.8 (21.5)	14.8 (24.5)	14.7 (23.6)	17 (28.4)	<0.0001
HDL-P							
HDL-P mean size (nm)	9.2 (0.5)	9.2 (0.5)	9.2 (0.5)	9.1 (0.5)	9.1 (0.5)	9 (0.4)	<0.0001
HDL-P Large ‡	10.5 (7.1)	11.6 (7.9)	11.2 (7.4)	9.8 (6.3)	9.3 (5.8)	8.1 (5.6)	<0.0001
HDL-P Medium ‡	22.9 (10.2)	24.6 (10.9)	23.8 (9.9)	21.6 (9.6)	21.3 (9.7)	19.8 (9.1)	<0.0001
HDL-P Small ‡	17.8 (4.9)	17.3 (5.0)	17.9 (4.9)	18.1 (4.7)	18 (5.0)	18.1 (4.7)	0.003
Dietary Intakes							
Total Energy (kcal/d)	1869 (612)	1661 (541)	1814 (553)	1983 (618)	1995 (616)	2368 (595)	<0.0001
Saturated Fat (% total energy)	10.6 (2.8)	10.4 (3)	10.7 (2.8)	11.2 (2.9)	10.7 (2.8)	10 (2.5)	0.98
Fruits (serv/d) †	0.6 (1.1)	0.7 (1.1)	0.7 (1.0)	0.6 (1.0)	0.6 (1.1)	0.5 (1.0)	<0.0001
Vegetables (serv/d) †	1.7 (1.2)	1.8 (1.2)	1.8 (1.3)	1.7 (1.2)	1.7 (1.1)	1.6 (1.0)	0.0005
Whole Grain (serv/d) †	0.6 (1.3)	0.7 (1.4)	0.7 (1.3)	0.7 (1.4)	0.6 (1.2)	0.4 (1.1)	<0.0001
Nuts/Seeds (serv/d) †	0.2 (0.4)	0.1 (0.4)	0.2 (0.4)	0.2 (0.4)	0.2 (0.4)	0.2 (0.5)	<0.0001
Seafood (serv/d) †	0.3 (0.4)	0.3 (0.4)	0.3 (0.4)	0.3 (0.3)	0.3 (0.4)	0.2 (0.3)	0.05
Alcohol (g/d) †	1.2 (13.5)	1.1 (12.7)	1.5 (14)	1.7 (12.4)	1.4 (13.9)	0.6 (14)	0.14

Maximum available observations, *n*, for analyses; Values are means (standard deviations) unless otherwise noted. All Framingham Offspring Study values are derived from exam 5 data except apo B, apo A1, apo B: apo A1 ratio, and all "Other Lipoprotein Measures", which were derived from exam 4 data. Values are means (standard deviations) unless otherwise noted.

*P_{trend} represents the p-value for regression coefficients where category of SSB intake (<1 serving/month, 1-4 servings/month, 1-2 servings/week, 3-7 servings/week, >1 serving/day) is treated as a continuous variable in unadjusted models. † Geometric mean (IQR) ‡ FHS: cholesterol concentration in mg/dl

Abbreviations: apo A1, apolipoprotein A1 concentrations; apo B, apolipoprotein B concentrations; apo C3, apolipoprotein C3 concentrations; apo E, apolipoprotein E concentrations; BMI, body mass index; HDL-C, high-density lipoprotein cholesterol concentrations; HDL-P, high-density lipoprotein particle concentrations; kcal, kilocalories; LDL-C, low-density lipoprotein cholesterol concentrations; LDL-P, low-density lipoprotein particle concentrations; RLP-C, remnant-like particle cholesterol concentrations; RLP-TG, remnant-like particle triglyceride concentrations; SSB, sugar-sweetened beverages; TC, total cholesterol concentrations; TG, triglyceride concentrations; TRL-P, triglyceride-rich lipoprotein particle concentrations.

Table 4.6 Characteristics of participants in the Women's Genome Health Study by category of sugar-sweetened beverage intake.

	Overall	**<1 serv SSB/mo**	**1-4 serv SSB/mo**	**1-2 serv SSB/wk**	**3-7 serv SSB/wk**	**>1 serv SSB/d**	
n	**21,794**	**9,862**	**4,761**	**2,816**	**3,260**	**1,095**	
Median SSB (serv†/wk)		**0**	**0.47**	**1.47**	**4.00**	**14**	**p-trend***
Age (yrs.)	54.5 (7)	54.7 (7)	55 (7.3)	54.4 (7)	54.1 (7)	52.5 (6)	<0.0001
Current Smoker (%)	11.6	10.2	10.9	11.5	14.2	20.0	<0.0001
Diabetics (%)	2.2	3.4	1.2	1.3	1.4	1.4	<0.0001
Education (% Bachelor's Degree)	43.2	48.3	44.5	42.7	40.5	34.2	<0.0001
BMI (kg/m^2)	25.9 (4.9)	25.9 (4.9)	25.7 (4.7)	25.8 (4.9)	25.8 (5)	26.3 (5.7)	0.003
Metabolic equivalents (hrs/wk)	6 (17.6)	6.9 (18.2)	6.2 (17.1)	5.7 (17)	4.8 (16.2)	3.6 (13.9)	<0.0001
Traditional Lipoprotein Measures							
LDL cholesterol (mg/dl)	124 (34)	123 (33.6)	124 (34.2)	125 (34)	125 (34)	125 (36.1)	<0.0001
HDL cholesterol (mg/dl)	53.9 (15.1)	55.3 (15.5)	54.1 (14.9)	52.7 (14.1)	52.2 (14.5)	49.2 (13.9)	<0.0001
TG (mg/dl)	121.8 (91)	119 (89)	120 (88)	123.4 (90)	126 (94)	137 (112)	<0.0001
non-HDL cholesterol (mg/dl)	157 (41)	156 (40)	157 (41)	158 (41)	160 (41)	162 (44)	<0.0001
Ratio Total: HDL Cholesterol	4.18 (1.31)	4.07 (1.27)	4.15 (1.3)	4.25 (1.32)	4.33 (1.36)	4.6 (1.5)	<0.0001
Apolipoprotein Measures							
apo B (mg/dl)	94.5 (22.7)	93.6 (22.3)	94.5 (22.8)	95 (22.8)	95.7 (22.9)	96.8 (24.3)	<0.0001
apo A1 (mg/dl)	154 (30.2)	156 (30.4)	154 (30.1)	152 (29.6)	151 (29.6)	147 (29.7)	<0.0001
apo B: apo A1							<0.0001
Other Lipoprotein Measures							
TRL-P							
TRL-P mean size (nm)	44.1 (7.5)	44.1 (7.6)	44 (7.5)	43.8 (7.1)	44.4 (7.6)	45.3 (8)	<0.0001
TRL-P Very Large†‡	0.1 (0.1)	0.1 (0.1)	0.1 (0.1)	0.1 (0.1)	0.1 (0.1)	0.11 (0.2)	0.36
TRL-P Large†‡	0.88 (4)	0.81 (3.8)	0.85 (4)	0.92 (3.9)	1 (4.1)	1.22 (4.7)	<0.0001
TRL-P Medium†‡	12.7 (16.7)	12.1 (16.6)	13 (16.6)	13.1 (16.3)	13.5 (17)	14.9 (18.3)	<0.0001
TRL-P Small†‡	47.5 (47.8)	46 (48.1)	48 (47.5)	49.8 (48.7)	49.5 (47)	47.7 (48.9)	<0.0001
TRL-P Very Small †‡	78.02 (55.15)	77.2 (54.8)	77.71 (55.23)	77.81 (55)	79.9 (55.3)	81.96 (58.55)	<0.0001
LDL-P							
LDL-P mean size (nm)	20.9 (0.45)	20.9 (0.44)	20.9 (0.44)	20.9 (0.46)	20.8 (0.45)	20.8 (0.47)	<0.0001

LDL-P Large †‡	184 (307)	197 (307)	193 (298)	177 (320)	165 (309)	124 (310)	<0.0001
LDL-P Medium †‡	16.6 (348)	16.8 (339)	18 (347)	17 (347)	17 (359)	10.9 (341)	0.02
LDL-P Small†‡	926 (636)	914 (596)	918 (629)	926 (682)	952 (680)	1007 (750)	<0.0001
HDL-P							
HDL-P mean size (nm)	8.96 (0.38)	8.99 (0.39)	8.96 (0.38)	8.93 (0.37)	8.92 (0.38)	8.87 (0.36)	<0.0001
HDL-P Large ‡	2.5 (1.5)	2.6 (1.6)	2.5 (1.5)	2.4 (1.5)	2.3 (1.5)	2.1 (1.4)	<0.0001
HDL-P Medium ‡	5.8 (2.8)	6 (2.9)	5.8 (2.8)	5.6 (2.7)	5.5 (2.7)	5.1 (2.6)	<0.0001
HDL-P Small ‡	16.4 (3.4)	16.2 (3.5)	16.3 (3.3)	16.5 (3.3)	16.6 (3.3)	16.8 (3.4)	<0.0001
Dietary Intakes							
Total Energy (kcal/d)	1735 (525)	1616 (489)	1739 (506)	1818 (525)	1878 (529)	2152 (547)	<0.0001
Saturated Fat (% total energy)	10.3 (2.5)	10.2 (2.6)	10.4 (2.4)	10.5 (2.3)	10.3 (2.4)	9.8 (2.4)	0.07
Fruits (serv/d) †	1.48 (1.46)	1.48 (1.44)	1.57 (1.45)	1.57 (1.5)	1.43 (1.5)	1.19 (1.5)	<0.0001
Vegetables (serv/d) †	3.28 (2.63)	3.31 (2.74)	3.31 (2.48)	3.3 (2.54)	3.21 (2.62)	2.98 (2.61)	<0.0001
Whole Grain (serv/d) †	0.99 (1.35)	1 (1.3)	1.03 (1.29)	1.03 (1.29)	0.93 (1.22)	0.75 (1.14)	<0.0001
Nuts/Seeds (serv/d) †	0.11 (0.29)	0.09 (0.22)	0.13 (0.36)	0.13 (0.36)	0.12 (0.36)	0.12 (0.29)	0.26
Seafood (serv/d) †	0.17 (0.21)	0.16 (0.21)	0.17 (0.21)	0.18 (0.21)	0.16 (0.15)	0.16 (0.15)	<0.0001
Alcohol (g/d) †	0.3 (4.6)	0.38 (6.5)	0.3 (4.6)	0.24 (3.6)	0.2 (3.6)	0.1 (2.1)	<0.0001

Maximum available observations, *n*, for analyses; Values are means (standard deviations) unless otherwise noted.
*P_{trend} represents the p-value for regression coefficients where the category of SSB intake (<1 serving/month, 1-4 servings/month, 1-2 servings/week, 3-7 servings/week, >1 serving/day) is treated as a continuous variable in unadjusted models.
† Geometric mean (IQR)
‡ particle concentration in nmol/L (TRL-P/LDL-P) or μmol/L (HDL-P)
Abbreviations: apo A1, apolipoprotein A1 concentrations; apo B, apolipoprotein B concentrations; BMI, body mass index; HDL-C, high-density lipoprotein cholesterol concentrations; HDL-P, high-density lipoprotein particle concentrations; kcal, kilocalories; LDL-C, low-density lipoprotein cholesterol concentrations; LDL-P, low-density lipoprotein particle concentrations; RLP-C, remnant-like particle cholesterol concentrations; RLP-TG, remnant-like particle triglyceride concentrations; SSB, sugar-sweetened beverages; TC, total cholesterol concentrations; TG, triglyceride concentrations; TRL-P, triglyceride-rich lipoprotein concentrations.

Table 4.7 Associations between sugar-sweetened beverage intake and lipoprotein cholesterol, apolipoprotein, and lipoprotein particle size concentrations among fasting sub-sample of WGHS participants. *

	β (SE)	P_{trend}	Adjusted P_{trend} **
Lipoprotein Cholesterol Concentrations			
LDL-C (mg/dl)	3.7 (1.4)	0.0002	0.0005
HDL-C (mg/dl)	-3.4 (0.6)	<0.0001	<0.0001
TG (ln-mg/dl)	0.11 (0.02)	<0.0001	<0.0001
non-HDL-C (mg/dl)	7.1 (1.6)	<0.0001	<0.0001
TC: HDL-C	0.37 (0.05)	<0.0001	<0.0001
Apolipoprotein Concentrations			
apo B (mg/dl)	4.9 (1.1)	<0.0001	<0.0001
apo A1 (mg/dl)	-3.7 (1.0)	<0.0001	<0.0001
apo B: apo A1	0.05 (0.01)	<0.0001	<0.0001
Lipoprotein Particle Size Measures			
TRL-P			
TRL-P mean size (nm)	0.34 (0.24)	0.72	0.99
TRL-P Very Large	0.001 (0.04)	0.55	0.95
TRL-P Large	0.05 (0.03)	0.007	0.02
TRL-P Medium	-0.01 (0.03)	0.23	0.55
TRL-P Small	-0.01 (0.04)	0.18	0.43
TRL-P Very Small	0.01 (0.04)	0.42	0.85
LDL-P			
LDL-P mean size (nm)	-0.08 (0.02)	<0.0001	<0.0001
LDL-P Large	-0.19 (0.04)	<0.0001	<0.0001
LDL-P Medium	-0.05 (0.04)	0.27	0.65
LDL-P Small	0.17 (0.04)	<0.0001	<0.0001
HDL-P			
HDL-P mean size (nm)	-0.01 (0.01)	0.005	0.01
HDL-P Large	-0.02 (0.03)	0.22	0.53
HDL-P Medium	-0.12 (0.04)	0.002	0.004
HDL-P Small	0.16 (0.04)	<0.0001	<0.0001

* Values are regression coefficients for highest category of SSB intake (>1 serving/day) compared to lowest category of SSB intake (<1 serving/month) on quantile normalized particle concentrations using generalized linear models adjusted for age, total energy intake, smoking status, education status, current diabetes status, physical activity, alcohol intake, body mass index, whole fruit intake, vegetable intake, whole grains intake, seafood intake, nuts/seeds intake, saturated fatty acid intake (% of total energy) and total lipid measure (for lipoprotein particle size concentrations only: TG, LDL-C and HDL-C concentrations for TRL-P, LDL-P, and HDL-P concentrations, respectively).

**Adjusted for multiple endpoints using Tukey's method (Tukey et al. *Biometrics* 1985).

Abbreviations: apo A1, apolipoprotein A1 concentrations; apo B, apolipoprotein B concentrations; HDL-C, high-density lipoprotein cholesterol concentrations; HDL-P, high-density lipoprotein particle concentration; LDL-C, low-density lipoprotein cholesterol concentrations; LDL-P, low-density lipoprotein particle concentration; SSB, sugar-sweetened beverages; TC, total cholesterol concentrations; TG, triglyceride concentrations; TRL-P, triglyceride rich lipoprotein particle concentration.

CHAPTER 5

Beverage consumption and longitudinal changes in lipoprotein concentrations and incident dyslipidemia in U.S. adults: the Framingham Heart Study

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5.1 Abstract

Background: Limited data are available on the prospective relationship between beverage consumption and plasma lipid or lipoprotein concentrations in population-based studies. Two major sources of sugar in the US diet are sugar-sweetened beverages (SSB; sodas and fruit drinks) and 100% fruit juices (FJ). Low-calorie sweetened beverages (LCSB) are common replacements.

Methods: Fasting plasma concentrations of total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) were measured at up to 5 exams in Framingham Heart Study Offspring (FOS) (1991-2014; N=3,146) and up to 2 exams in the Generation Three (GEN3) (2002-2011; N=3,584) cohorts. Intake was estimated from food frequency questionnaires. Mixed-effect linear regression models were used to examine 4-year changes in lipoprotein measures, and Cox proportional hazard models were used to estimate hazard ratios (HR) for incident dyslipidemia, adjusting for potential confounding factors.

Results: We found that SSB intake was associated with smaller mean 4-year changes in HDL-C [highest (>1 serving/day) vs. lowest intake category (<1 serving/month) (H vs. L): $\beta \pm SE$: -1.0 ± 0.3 mg/dl, p

trend = 0.0001] and greater mean 4-year changes in TG (H vs. L: β ± SE: 5.6 ± 2.1 mg/dl, p trend = 0.0004), along with a higher incidence of low HDL-C [H vs. L HR (95% CI): 1.98 (1.20-3.28); p = 0.007; p for trend = 0.01)], and high TG [HR (95% CI): 1.53 (1.01-2.31); p=0.05; p for trend = 0.004)]. LCSB intake was associated with a higher incidence of high non-HDL-C [H vs. L HR (95% CI): 1.40 (1.17-1.69); p = 0.007; p for trend = 0.0002)] and LDL-C [H vs. L HR (95% CI): 1.27 (1.05-1.53); p = 0.01; p for trend = 0.01)]. No other significant associations between beverage consumption and lipids were observed.

Conclusions: SSB intake was associated with adverse changes in HDL-C and TG concentrations, along with a higher risk of incident dyslipidemia, indicating that increased SSB consumption may contribute to the development of dyslipidemia.

5.2 Introduction

An estimated 40-50% of adults in the United States (U.S.) can be classified as dyslipidemic (1), characterized by high triglyceride (TG), high low-density lipoprotein cholesterol (LDL-C) and/or low high-density lipoprotein cholesterol (HDL-C) concentrations, predisposing them to increased risk for cardiovascular disease (CVD) (2). Thus, managing patients' blood lipid concentrations is a major focus for health professionals (3). Dietary modification offers a promising strategy to both prevent and treat dyslipidemia (4).

Evidence from observational studies suggest there is a positive association between added sugar intake and CVD risk (5,6), particularly in the form of sugar-sweetened beverages (SSB) (7). SSB, such as sodas, fruit-flavored drinks, sports drinks, and presweetened coffees and teas, are a significant source of added dietary sugars in diets of U.S. adults and a major contributor to excess energy intake (8). One

potential mechanism by which SSB may increase the risk for CVD is through the development of dyslipidemia. Animal and human intervention trials suggest that consumption of very large amounts of sugar, particularly those high in fructose, can rapidly induce dyslipidemia (9–11). Several cross-sectional studies have observed that that higher SSB consumption is adversely associated with lipid concentrations (12–16). In the Framingham Heart Study, a higher incidence of hypertriglyceridemia and low HDL-C concentrations was observed among men with higher soft drink consumption (17). In that report, soft drinks included both SSB and low-calorie sweetened beverages (LCSB).

One hundred percent fruit juices (FJ) and LCSB are commonly used as alternative "healthier" beverages to SSB (18,19). Evidence from observational studies is mixed, but the current body of research seems to suggest there is no significant association between LCSB or FJ consumption and CVD (16,20,21). Results from randomized controlled trials indicate no significant effect of FJ or LCSB on plasma lipid or lipoprotein concentrations (20–24).

The objective of the present study was to examine the association of SSB, LCSB, and FJ consumption with longitudinal changes in concentrations of TG, LDL-C, HDL-C and non-HDL-C in the Framingham Offspring and Generation Three cohorts. We hypothesized that greater SSB consumption will be associated with unfavorable longitudinal changes in lipoprotein concentrations and incident dyslipidemia, and to a greater extent than LCSB and FJ.

5.3 Methods

Subjects

The Framingham Heart Study (FHS) is a long-standing, prospective cohort study in Framingham, Massachusetts that began in 1948. Data from the latter two cohorts, Framingham Offspring (FOS) (25) at exam 5 (1991–1995; n=3,799), exam 6 (1995-1998; n=3,532), exam 7 (1998-2001; n=3,539), exam 8 (2005-2008; n=3,021), and exam 9 (2011-2014; n=2,430) and Framingham

Third Generation (GEN3) (26) at exams 1 (2002–2005; n=4,095) and 2 (2008–2011; n=3,411), were used in the current study for up to 23 years of follow-up (mean follow-up = 12.5 years). In each cohort and at each examination cycle within FHS, participants underwent a detailed medical history, physical examination, and standard laboratory tests. Participants also provided demographic, diet, lifestyle and medical history data via standard questionnaires. All participants provided written informed consent before study participation. All study protocols and procedures were approved by the institutional review boards for human research at Boston University Medical Campus and Tufts University Health Sciences.

Assessment of lipid outcomes

Fasting blood samples from FHS participants were used to measure plasma HDL-C (mg/dl), TG (mg/dl), and total cholesterol (TC) (mg/dl) concentrations using standard assays at each exam. LDL-C concentrations were calculated according to the Friedewald equation (LDL-C = TC - HDL-C - TG/5), and set to missing if TG concentrations ≥ 400 mg/dl (27). Non-HDL-C concentrations were calculated as TC minus HDL-C concentrations. Non-HDL-C concentrations were explored because observational studies have shown them to be more predictive of cardiovascular events than individual lipid concentrations alone (28,29). Changes in each of these lipoprotein concentrations were calculated as the difference between two consecutive exams. To correct for unequal time intervals between exams, changes in lipoprotein concentrations were normalized to 4-year changes.

Four dyslipidemia outcomes were defined as: LDL-C concentrations ≥160 mg/dl or use of LDL-lowering medications; HDL-C concentrations <40 mg/dl in men or <50 mg/dl in women; TG concentrations ≥175 mg/dl; and non-HDL-C concentrations ≥190 mg/dl or use of LDL-lowering medications. The cut-points for LDL-C, HDL-C, TG, and non-HDL-C concentrations are established cut-offs for CVD risk-enhancing factors in the 2018 Guideline on the Management of Blood Cholesterol (4).

A total of 3,146 FOS and 3,584 GEN3 participants provided diet and lipid measures at baseline (exam 5 in FOS and exam 1 in GEN3 in this study). For analysis of changes in lipid measures, 3,182 FOS and 2,805 GEN3 participants provided diet and lipid measures for at least two consecutive examination periods from exams 5-9 in FOS and 1-2 in GEN3. FOS participants contributed multiple observations if diet and lipid measures were provided at more than two consecutive examination periods. Participants were excluded if the change in lipoprotein concentrations was not within 4 standard deviations of the mean 4-year change. A total of 100 FOS and 5 GEN3 participants were excluded because they were missing covariate data, reducing the sample size to 3,082 in the FOS cohort and 2,800 in the GEN3 cohort for analysis of change in lipoprotein concentrations. These criteria resulted in different sample sizes for each lipoprotein outcome: LDL-C 8,584 observations among 3,030 FOS participants and 2,736 GEN3 participants), TG (8,858 observations among 3,082 FOS participants and 2,800 GEN3 participants), and HDL-C and non-HDL-C (7,273 observations among 2,897 FOS participants and 2,384 GEN3 participants). For analysis of the development of dyslipidemia, participants were excluded for the following reasons: prevalent dyslipidemia at baseline; use of LDL-lowering medications (for lipid outcomes that include LDL-C concentrations); lack of follow-up data, or missing covariate data. After these exclusions, the sample sizes were as follows for the development of dyslipidemia: FOS cohort based on LDL-C (n=2,161), HDL-C (n=1,703), TG (n=2,116), and non-HDL-C (n=2,205); GEN3 cohort based on LDL-C (n=2,377), HDL-C (n=2,084), TG (n=2,426), and non-HDL-C (n=2,400).

Beverage Consumption

Usual dietary intakes in the past year were estimated at each exam using the Harvard 126-item semi-quantitative food-frequency questionnaire (FFQ) (30). The FFQ was mailed to participants to be completed at home and returned at the study appointment. The FFQ consisted of a list of foods with standard serving sizes and a selection of 9 frequency categories ranging from none or <1 serving/month

to ≥6 servings/day. Dietary information was considered valid only if reported energy intake was as follows: 600 kcal/day for both men and women; 4,000 kcal/d for women; 4,200 kcal/day for men; and if ≤ 13 food items were left blank on the FFQ. The relative validity of the FFQ in FHS has been examined for both nutrients and foods in men and women in other cohorts (30,31).

Estimates of SSB consumption included the following categories (1) Coke, Pepsi, or other cola with sugar; (2) caffeine-free Coke, Pepsi, or other cola with sugar; (3) other carbonated beverage with sugar (e.g., 7-Up, ginger ale); and (4) Hawaiian Punch, lemonade, or other non-carbonated fruit drinks. Estimates of FJ consumption included the following categories (1) orange juice; (2) grapefruit juice; (3) apple juice or cider; and (4) other 100% fruit juices. Estimates of LCSB consumption included the following categories (1) low-calorie cola, e.g. Tab with caffeine; (2) low-calorie caffeine-free cola, e.g. Pepsi Free; and (3) other low-calorie carbonated beverage, e.g., Fresca, Diet 7-Up, diet ginger ale. One serving of SSB or LCSB is equivalent to 12 fl oz., and one serving FJ is equivalent to 8 fl oz. For analysis of the development of dyslipidemia, we estimated both "recent" beverage intake and "cumulative" average beverage intake. We assessed "recent" beverage intake as the association between dyslipidemia in one exam with beverage intake in the prior exam. We assessed "cumulative" beverage intake as the association between dyslipidemia in one exam with average beverage intake over the prior two exams.

Covariate Assessment

Education was assessed by asking the highest degree or level of school the participant had completed (obtained in FOS at exam 8 and GEN3 at exam 1), and participants were grouped into four categories (less than high school, high school, some college, graduated college). Participants self-reported whether they had taken medication for high blood cholesterol since their last exam, and participants were classified as diabetic if their fasting blood glucose was ≥ 126 mg/dL or they were

under current treatment for diabetes. In the GEN3 cohort, an additional criterion was applied where participants were classified as diabetic if their non-fasting blood glucose was ≥ 200 mg/dL. Participants also completed a standardized physical examination, which included measurements of height, weight, and waist circumference (measured at the level of the umbilicus in a standing position). Body mass index (BMI) was calculated as weight divided by height (kg/m^2). Alcohol intake was assessed by asking the number of alcoholic beverages consumed in a typical week in the previous year and expressed as grams per day. Current smokers were defined as participants who reported smoking regularly in the past year. Physical activity was evaluated through a standard exercise questionnaire (32). Physical activity was not assessed at exam 6, so the physical activity estimates from exam 5 and exam 7 were used to estimate physical activity during the intervals of exam 5 to 6 and exam 6 to 7, respectively.

Nutrient intakes were calculated from FFQ data by multiplying the frequency of consumption of a food item by the nutrient contents per standard serving size for the given food item. Potential confounding through other dietary components was explored through adjustment of individual dietary factors (percent energy from saturated fat and servings/day of fruit, vegetables, whole grains, fish, and nuts/seeds), as well as through a composite diet quality score: the Dietary Guidelines Adherence Index-2015 (DGAI-2015) (33), which reflects adherence to key recommendations based on the 2015 Dietary Guidelines for Americans.

Statistical Analyses

Beverage consumption was explored using 5 categories of intake (<1 serving/mo, 1-4 serving/mo, 1-2 serving/wk, 3-7 serving/wk, >1 serving/day), similar to previous studies (16,34). Linear mixed effects regression models were used to examine the association between beverage consumption patterns and 4-year changes in fasting LDL-C, HDL-C, TG, and non-HDL-C using the *pedigreemm* and *lmekin* R packages (https://cran.r-project.org). Familial correlation and multiple observations per person

were accounted for by adding a random effects term in the model with a covariance structure proportional to the kinship matrix and a random effects term for individual, respectively. To estimate usual dietary intakes and covariate data within each exam interval, the average of all measurements within the exam intervals was computed. Three models were performed. Model 1 adjusted for age (continuous), sex (M/F), total energy intake (continuous), baseline lipoprotein concentrations (continuous), education (less than high school, graduated high school, some college, or graduated college), current smoking status (yes/no), current diabetes (yes/no), physical activity index (continuous), alcohol (grams/day), use of LDL-lowing medication (yes/no; where applicable); Model 2 adjusted for model 1 covariates plus servings per day of vegetables, whole fruits, whole grains, nuts/seeds, and seafood, as well as percent energy from saturated fat (continuous) and mutual adjustment for SSB, LCSB, and FJ (categorical as continuous). Model 3 adjusted for model 2 covariates plus the change in waist circumference (WC) (continuous), which is a marker of abdominal adiposity and could be in the causal pathway between beverage consumption and development of dyslipidemia. Covariate adjustment did not drastically change the results, so fully adjusted models are presented (Model 3). Models were run separately for FOS and GEN3 cohorts, and then data were combined in a pooled analysis (adjusting for cohort). For the presentation of the cross-stratified association of SSB and LCSB on lipoprotein concentrations, intakes were grouped as follows: low intake (<1 serving/month), medium intake (1-10 servings/month), and high intake (>3 servings/week). The joint association of SSB and LCSB was modeled as the interaction of the low/medium/high categories of intake among participants and the models were compared using likelihood-ratio testing with and without multiplicative interaction terms.

We additionally used Cox proportional hazards models with time-varying covariates and follow-up time as the underlying time scale to estimate hazard ratios and 95% confidence intervals of dyslipidemia for beverage consumption using the *survival* R package (https://cran.r-project.org). Family structure and multiple observations were accounted for using a robust standard error and clustering on family and individual, respectively. We tested the proportional hazard assumption by examining the

scaled Schoenfeld residuals over time, and the assumption was unlikely violated. We examined the linear trend by modeling beverage consumption categories as a continuous variable. Recent beverage consumption from repeated FFQs was categorized as described above. Multivariable Cox proportional hazards models were adjusted for potential confounders, which were updated at each exam cycle. Models were adjusted for age (continuous), sex (Male/Female), total energy (continuous), education (less than high school, graduated high school, some college, or graduated college), current smoking status (yes/no), current diabetes status (yes/no), physical activity index (continuous), alcohol (grams/day), WC (continuous), servings per day of vegetables, whole fruits, whole grains, nuts/seeds, and seafood, as well as percent energy from saturated fat (continuous) and mutual adjustment for SSB, LCSB, and FJ (categorical as continuous). Given that the GEN3 participants were younger and followed for only one exam, main analyses were performed in the FOS cohort and validation was performed in the GEN3 cohort.

Secondary Analyses

For both analyses, likelihood-ratio testing comparing models with and without multiplicative interaction terms were used to assess effect modification by sex (male/female) and BMI (<25 kg/m^2; 25-29.9 kg/m^2; ≥30 kg/m^2). No significant interactions were observed; thus, data are not stratified in the main analysis. Several sensitivity analyses were conducted to examine the consistency of the associations. To minimize reverse-causation, analyses were performed eliminating those who developed diabetes or began taking lipid-lowering medications. We also substituted recent beverage intake for cumulative averages of beverage consumption. To evaluate whether overall diet quality was adequately controlled for in our models, the dietary covariates (vegetables, whole fruits, whole grains, nuts/seeds, seafood, and saturated fat) were replaced with DGAI-2015 (33). Furthermore, we substituted adjustment for WC with BMI (continuous) to consider whether overall adiposity, compared to abdominal adiposity, changed the reported associations.

All statistical analyses were performed using either SAS (SAS Institute, Cary, NC; version 9.4 or higher) or R (version 3.1 or higher; https://cran.r-project.org) statistical software. All reported p-values are two-sided, and results were considered statistically significant at a Bonferroni-corrected $p < 0.01$ (0.05/4 outcomes).

4.4 Results

Table 5.1 shows the characteristics of participants for each cohort and examination cycle. Mean age (± standard deviation [SD]) at baseline was 54.8 (± 9.8) years among FOS participants and 40.3 (± 8.8) years among GEN3 participants. Participants smoked less, had achieved more education, had increased BMI and WC, and a higher percent was classified as having diabetes and taking LDL-lowering medications with each subsequent examination cycle. Lipoprotein concentrations (LDL-C, HDL-C, TG, and non-HDL-C) improved across exam cycles and use of LDL-C-lowering medications increased. Differences in dietary intakes across exams were not substantial (<0.5 servings/day), but statistically significant *p* for trends were observed. We observed a slight decrease in consumption of SSB, FJ and LCSB among both FOS (1991-2008) and GEN3 (2002-2011) participants across exam cycles. Among FOS participants, mean intakes of total energy, vegetables, and seafood remained similar through the exam cycles, whereas increases were observed for energy from saturated fat (%), alcohol (g) and servings of whole fruits, whole grains, and nuts/seeds. Trends were different among GEN3 participants where decreases were observed in total energy intake, % energy from saturated fat, and consumption of alcohol, whole fruits, vegetables, whole grains, nuts/seeds, and seafood.

Table 5.2 shows that after multivariable adjustment for potential confounding factors and change in abdominal adiposity (covariate model 3), participants in the highest category of SSB intake (>1 serving/day) had mean 4-year change in HDL-C concentrations 0.9 mg/dl lower [β ± SE: -1.0 ± 0.3 mg/dl; p for trend = 0.0001] and TG concentrations 5.7 mg/dl higher [β ± SE: 5.6 ± 2.1 mg/dl; p for trend = 0.0004] than those in the lowest category of SSB intake (<1 serving/month). SSB consumption

was not significantly associated with mean 4-year changes in LDL-C or non-HDL-C concentrations. LCSB and FJ consumption were not significantly associated with mean 4-year changes in lipid concentrations in fully adjusted models. There was no evidence of heterogeneity among the FOS and GEN3 cohorts for these models. No significant interactions were observed between beverage intakes and sex or BMI. Similar results were observed in sensitivity analyses eliminating those who were diabetic or on LDL-C-lowering medications, when food groups were substituted for the DGAI-2015, and when the change in WC was replaced with the change in BMI. Additional analyses of the joint effects of SSB and LCSB revealed that the highest categories of both SSB and LCSB intakes had the largest mean 4-year changes in HDL-C and TG concentrations [HDL-C: $\beta \pm$ SE: -1.0 ± 0.3 mg/dl; p = 0.0001; TG: $\beta \pm$ SE: 6.7 ± 2.2 mg/dl; p = 0.003] compared to the non-consumers, but not significant interaction was observed ($p > 0.01$) (Figure 5.1 and Table 5.4).

During a mean 12.5 years of follow-up in the FOS cohort, incident cases of dyslipidemia were as follows: 961 cases of high LDL-C, 318 cases of low HDL-C, 456 cases of high TG, and 974 cases of high non-HDL-C. Multivariable-adjusted hazard ratios for the highest category of beverage consumption (>1 serving/day) compared to the lowest category (<1 serving/month), estimated as both recent and cumulative average intakes are presented in Figure 2. After adjustment for potential confounders, in the FOS cohort the highest recent SSB consumers had 98% higher incidence of low HDL-C [HR (95% CI): 1.98 (1.20-3.28); p = 0.007; p for trend = 0.01)] and 53% higher incidence of high TG [HR (95% CI): 1.53 (1.01-2.31); p=0.05; p for trend = 0.004)] compared to the lowest SSB consumers. The highest recent LCSB consumers had 40% higher incidence of high non-HDL-C [HR (95% CI): 1.40 (1.17-1.69); p = 0.007; p for trend = 0.0002)] and 27% higher incidence of high LDL-C [H vs. L HR (95% CI): 1.27 (1.05-1.53); p = 0.01; p for trend = 0.01)] compared to the lowest LCSB consumers. However, in secondary analyses using cumulative average intake of SSB and LCSB intakes, these associations were attenuated to non-significant, suggesting that recent intake has more of an effect on incident dyslipidemia than cumulative intake. No other significant differences in incidences of

dyslipidemia by category of beverage consumption were observed among FOS participants. Multivariable-adjusted hazard ratios for additional categories of beverage consumption are presented in Tables 5.5 and 5.6. During a mean follow-up of 6.1 years in the GEN3 cohort, results for the association between recent SSB and LCSB consumption were attenuated to non-significant, suggesting that beverage consumption may play less of a role in the development of dyslipidemia in this younger, lower risk cohort (Table 5.7). No significant interactions were observed between beverage intakes and sex or BMI in either cohort. Similar results were observed in sensitivity analyses removing individuals with diabetes, when food groups were substituted for the DGAI-2015, and when WC was replaced with BMI.

4.5 Discussion

In this population-based, prospective cohort study among U.S. adults, greater consumption of SSB was associated with adverse changes in lipoprotein concentrations over time and development of dyslipidemia. Consumption of SSB was adversely associated with mean 4-year changes in HDL-C and TG concentration, along with increased incidence of low HDL-C and high TG. Mixed results were observed for LCSB consumption, where consumption of LCSB was not associated with significant changes in lipoprotein concentrations but was associated with an increased incidence of high non-HDL-C and LDL-C concentrations. FJ consumption was not significantly associated with lipoprotein concentrations or the development of dyslipidemia.

Several cross-sectional studies have observed that higher SSB consumption is associated with lower HDL-C concentrations and higher TG concentrations (13,15,16). In the only other prospective analysis conducted to date among adults, Dhingra et al. observed a higher incidence of hypertriglyceridemia and low HDL-C concentrations among adults with higher soft drink consumption (SSB + LCSB) in the FOS cohort during a follow-up period of about 4 years (17). Our study strengthens this evidence-base by providing prospective data that SSB alone is associated with 4-year changes in TG

and HDL-C concentrations, along with the incidence of dyslipidemia over up to 23 years of follow-up. These results also agree with shorter prospective studies among children and young adults that observed a positive association between higher SSB intake and increases in TG and decreases in HDL-C concentrations (35–37). Animal and human intervention trials corroborate these observational studies and provide evidence that SSB may influence lipoprotein concentrations (9–11).

We also assessed the association of LCSB consumption and changes in lipoprotein concentrations and development of dyslipidemia. Conflicting results were observed. Although no significant association was observed between LCSB consumption and 4-year changes in lipoprotein concentrations, LCSB consumption was associated with an increased incidence of dyslipidemia. Several cross-sectional and short term randomized control trials indicate no association between LCSB intake and lipoprotein concentrations (15,21,38,39), but a recent meta-analysis concluded that LCSB intake was associated with increased risk of metabolic syndrome and cardiovascular events (40). However, these results have a high risk of reverse causality given that higher consumers of LCSB may choose to consume these products because they are at a higher risk for disease (21,23). Consistent with this supposition, no significant association was observed between LCSB consumption and changes in lipoprotein concentrations. Additional data examining the joint association of SSB and LCSB consumption indicate that the association between SSB consumption and mean 4-year changes in TG and HDL-C concentrations is not dependent on LCSB consumption, further supporting the notion that LCSB consumption is not associated with mean changes in lipoprotein concentrations in our data. Thus, we cannot rule out the possibility that the significant association observed between LCSB consumption and incident high non-HDL-C and LDL-C, only observed among recent consumers, may be more influenced by reverse causality than LCSB intakes.

The results from our study did not identify a significant relationship between FJ consumption and changes in plasma lipoprotein concentrations or the risk of dyslipidemia among adults. This is in contrast with prior work that has identified both positive and negative associations between FJ

consumption and risk for cardiometabolic diseases (41–45) and changes in plasma lipoprotein concentrations (46–48). Dietary patterns high in FJ consumption may be more likely to associate with positive dietary behaviors than dietary patterns high in SSB consumption (49). Additionally, FJ may contain other beneficial nutrients not contained in SSB or the way people consume FJ may differ from that of SSB (whether it is consumed with meals or not and/or time of consumption). These factors may mitigate potential adverse effects of sugar from FJ on lipids and other cardiometabolic outcomes.

As with all research designs, the proposed study has limitations. The use of self-reported dietary data from FFQ to infer dietary intakes could potentially lead to misclassification of food and nutrient intakes. While FFQ are able to provide rough estimates of absolute dietary intakes, they are more suited to ranking individuals on relative dietary intakes. Thus, in this study, we categorized individuals based on estimates of beverage consumption. These FFQ did not include an exhaustive list of all potential sources of SSB, such as consumption of presweetened coffee/tea. Thus, we are not able to capture added sugar intake from these sources in our study. Individuals diagnosed as having high plasma cholesterol concentrations may be advised to change their diet in order to help improve lipid profiles. Thus, this potential reverse-causality makes it difficult to infer underlying mechanisms based on results from this study. Even for longitudinal analyses in prospective cohort studies adjusting for a variety of potential demographic, lifestyle, and dietary confounding factors, residual confounding cannot be ruled out. Long-term, randomized controlled intervention studies would be necessary to infer causal mechanisms for how differing beverage consumption patterns might be influencing plasma lipoprotein concentrations. Our findings are only generalizable to European-descent adults who are middle-aged or older. It is possible that our findings may be biased because of the differences in age and health status between participants who were excluded from the analyses and those who remained in the study.

The strengths of the present study include its large sample size, repeated assessments of dietary intakes and covariates, long follow-up period, and prospective design. High quality observational studies are necessary to inform whether it would be cost-effective to conduct a long-term randomized

control trial. We were able to account for important lifestyle variables that could confound the association between beverage consumption and lipids such as overall diet quality, physical activity, and alcohol intake. We have also used two different types of longitudinal analyses in this study. Because models for the development of dyslipidemia may be biased by reverse causality, the ability to additionally assess the change in lipoprotein concentrations in a larger subset of individuals by beverage consumption category strengthen our findings. Few studies directly compare the health effects of both FJ and LCSB to those of SSB (50), and this comparison can be useful when making recommendations for changes in dietary patterns.

Conclusions

Our findings suggest that lower consumption of SSB is associated with lower risk of dyslipidemia, along with smaller changes in HDL-C concentrations and greater changes in TG concentrations. LCSB intake may be associated with an increased risk of dyslipidemia, but it is likely this finding suffers from bias due to reverse causation. Our study provides no evidence that FJ consumption influences lipid concentrations or risk of dyslipidemia. These findings are consistent with current recommendations to limit SSB consumption.

4.6 References

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Table 5.1 Characteristics of participants by cohort and exam cycle

	Offspring Cohort						Generation 3 Cohort		
	Exam 5: 1991-95	**Exam 6: 1995-98**	**Exam 7: 1998-2001**	**Exam 8: 2005-08**	**Exam 9: 2011-14**	***P*-trend***	**Exam 1: 2002-05**	**Exam 2: 2008-11**	***P*-value***
n	3,146	3,008	2,697	2,529	2,204		3,584	3,132	
Age (yrs.)	54.8 (9.8)	58.7 (9.6)	61 (9.4)	66.3 (8.9)	71.2 (8.7)	<0.0001	40.3 (8.8)	46.6 (8.7)	<0.0001
Sex (% women)	53.1	53.6	54.3	55.2	54.9	0.09	54.3	54.1	0.88
Current Smoker (%)	19.1	15.1	12.0	8.4	5.8	0.002	14.5	9.9	<0.0001
Education (% Some College)	60.5	62.3	64.6	66.3	68.9	<0.0001	85.3	84.5	0.75
BMI (kg/m^2)	27.4 (5.0)	27.9 (5.1)	28.2 (5.3)	28.2 (5.4)	28.4 (5.4)	<0.0001	26.8 (5.5)	28.0 (5.8)	<0.0001
Waist Circumference (in.)	36.5 (5.6)	38.4 (5.3)	39.3 (5.5)	39.9 (5.7)	40.1 (5.5)	<0.0001	36.5 (5.9)	38.1 (6.0)	<0.0001
Physical Activity Index	34.7 (6.1)	NA	37.9 (6.3)	35.3 (5.4)	34.8 (5.8)	0.08	37.3 (7.7)	36.4 (6.6)	<0.0001
LDL-C (mg/dl)	126 (33)	127 (33)	120 (33)	105 (31)	99 (31)	<0.0001	112 (32)	104 (31)	<0.0001
HDL-C (mg/dl)	50 (15)	51 (16)	54 (17)	58 (18)	62 (19)	<0.0001	55 (16)	60 (18)	<0.0001
TG (mg/dl) ‡	125 (92)	119 (89)	117 (87)	104 (69)	103 (63)	<0.0001	97 (73)	97 (66)	0.16
non-HDL-C	262 (81)	258 (78)	250 (77)	220 (68)	211 (63)	<0.0001	220 (76)	212 (71)	<0.0001
Diabetics (%)	7.1	9.5	10.8	13.2	14.5	<0.0001	2.9	4.7	<0.0001
LDL-lowering medication users (%)	7.3	13.0	20.7	42.9	50.1	<0.0001	6.8	16.2	<0.0001
Dietary Intakes									
Total Energy (kcal/day)	1862 (612)	1846 (607)	1827 (591)	1866 (626)	NA	0.33	2055 (667)	1990 (628)	<0.0001
Saturated Fat (% total energy)	10.4 (2.9)	10.1 (2.8)	10.7 (2.9)	11.1 (2.7)	NA	<0.0001	11.5 (2.9)	10.5 (2.5)	<0.0001
Alcohol (grams/day) ‡	1.24 (13.3)	1.19 (13)	1.14 (12.9)	1.20 (13.9)	NA	0.001	1.9 (12.8)	2.3 (14.5)	0.006
Whole Fruits (servings/day) ‡	0.66 (1.09)	0.77 (1.12)	0.79 (1.13)	0.78 (1.13)	NA	<0.0001	0.64 (1.06)	0.54 (0.92)	<0.0001
Vegetables (servings/day) ‡	1.74 (1.22)	1.75 (1.23)	1.75 (1.26)	1.75 (1.24)	NA	0.28	1.77 (1.37)	1.60 (1.20)	<0.0001
Whole Grain (servings/day) ‡	0.62 (1.31)	0.62 (1.25)	0.65 (1.34)	0.83 (1.40)	NA	<0.0001	0.71 (1.21)	0.17 (0.44)	<0.0001
Nuts/Seeds (servings/day) ‡	0.16 (0.36)	0.14 (0.36)	0.18 (0.50)	0.36 (0.86)	NA	<0.0001	0.28 (0.64)	0.11 (0.36)	<0.0001
Seafood (servings/day) ‡	0.29 (0.37)	0.27 (0.33)	0.29 (0.37)	0.30 (0.37)	NA	0.68	0.26 (0.38)	0.21 (0.28)	<0.0001
SSB (servings/day) †‡	0.09 (0.49)	0.09 (0.44)	0.08 (0.42)	0.05 (0.20)	NA	<0.0001	0.12 (0.56)	0.08 (0.42)	<0.0001
100% Fruit Juice (servings/day) †‡	0.36 (0.93)	0.37 (0.99)	0.37 (0.92)	0.23 (0.93)	NA	<0.0001	0.31 (0.87)	0.21 (0.79)	<0.0001
LCSB (servings/day) †‡	0.10 (0.85)	0.09 (0.78)	0.08 (0.70)	0.07 (0.49)	NA	<0.0001	0.08 (0.70)	0.06 (0.42)	<0.0001

Abbreviations: BMI, body mass index; DGAI, Dietary Guidelines Adherence Index; HDL-C, high density lipoprotein cholesterol concentrations; LCSB, low-calorie sweetened beverages; LDL-C, low-density lipoprotein cholesterol concentrations; mg/dl, milligrams per deciliter; SSB, sugar-sweetened beverages; TC, total cholesterol concentrations; TG, triglyceride concentrations.

Maximum available observations, *n*, for analyses; Values are means (standard deviations) unless otherwise noted. Means are compared with the use of a general linear model.

* P-trend (Offspring Cohort) or P-value (Generation 3 Cohort) for exam follow-up number.

† One serving SSB or LCSB is equivalent to 12 fl oz. and one serving 100% fruit juice is equivalent to 8 fl oz.

‡ Geometric mean (IQR)

Table 5.2 Mean difference in 4-year changes in lipoprotein concentrations across beverage consumption groups*

	Beverage Consumption Groups					
	<1 serving/ month	**1-4 servings/ month**	**1-2 servings/ week**	**3-7 servings/ week**	**>1 serving/ day**	***P* for trend**
Sugar-Sweetened Beverage Intake						
Offspring Cohort						
No of Observations	3,488	1,667	1,321	1,698	684	
LDL-C (mg/dl)	Reference	-0.01 (0.5)	0.9 (0.5)	0.5 (0.5)	0.00 (0.7)	0.31
HDL-C (mg/dl)	Reference	-0.1 (0.2)	-0.03 (0.2)	-0.3 (0.2)	-1.0 (0.3)	0.008
TG (mg/dl)	Reference	1.1 (1.5)	0.7 (1.7)	3.9 (1.7)	2.2 (2.5)	0.05
Non-HDL-C (mg/dl)	Reference	0.00 (0.3)	-0.1 (0.4)	0.4 (0.4)	0.8 (0.5)	0.07
Generation 3 Cohort						
No of Observations	867	549	483	576	325	
LDL-C	Reference	0.1 (0.8)	-0.2 (0.9)	0.8 (0.9)	1.8 (1.2)	0.20
HDL-C (mg/dl)	Reference	0.1 (0.4)	-0.6 (0.4)	-1.0 (0.4)	-0.7 (0.6)	0.02
TG (mg/dl)	Reference	5.3 (2.5)	3.7 (2.7)	8.0 (2.7)	12.3 (3.6)	0.0006
Non-HDL-C (mg/dl)	Reference	-0.03 (0.7)	-0.7 (0.8)	0.5 (0.8)	2.1 (1.1)	0.22
Pooled Results†						
LDL-C	Reference	-0.04 (0.4)	0.6 (0.4)	0.5 (0.4)	0.6 (0.6)	0.18
HDL-C (mg/dl)	Reference	-0.05 (0.2)	-0.2 (0.2)	-0.5 (0.2)	-1.0 (0.3)	0.0001
TG (mg/dl)	Reference	2.0 (1.3)	1.5 (1.5)	5.1 (1.4)	5.6 (2.1)	0.0004
Non-HDL-C (mg/dl)	Reference	-0.3 (0.3)	-0.3 (0.3)	0.2 (0.3)	1.0 (0.5)	0.10
Low-Calorie Sweetened Beverage Intake						
Offspring Cohort						
No of Observations	3,792	1,100	805	1,894	1,265	
LDL-C (mg/dl)	Reference	-0.3 (0.5)	0.3 (0.6)	-0.21 (0.4)	-0.6 (0.5)	0.37
HDL-C (mg/dl)	Reference	-0.3 (0.2)	-0.1 (0.2)	-0.2 (0.2)	-0.1 (0.2)	0.33
TG (mg/dl)	Reference	-0.4 (1.7)	-0.7 (2.0)	1.7 (1.5)	-0.4 (1.7)	0.63
Non-HDL-C (mg/dl)	Reference	0.4 (0.4)	0.2 (0.4)	0.3 (0.3)	-0.3 (0.4)	0.97
Generation 3 Cohort						
No of Observations	1,242	351	287	506	414	
LDL-C	Reference	0.4 (0.9)	0.2 (0.9)	-0.09 (0.8)	-1.8 (0.8)	0.11
HDL-C (mg/dl)	Reference	0.6 (0.4)	-0.4 (0.4)	-0.3 (0.4)	-0.4 (0.4)	0.18
TG (mg/dl)	Reference	4.6 (2.7)	4.1 (2.9)	4.2 (2.3)	-0.2 (2.6)	0.41
Non-HDL-C (mg/dl)	Reference	0.3 (0.8)	0.00 (0.8)	-0.1 (0.7)	-1.1 (0.8)	0.24
Pooled Results†						
LDL-C	Reference	-0.2 (0.4)	0.2 (0.5)	-0.2 (0.4)	-0.7 (0.4)	0.20
HDL-C (mg/dl)	Reference	-0.2 (0.2)	-0.2 (0.2)	-0.3 (0.2)	-0.3 (0.2)	0.03
TG (mg/dl)	Reference	0.9 (1.5)	0.3 (1.6)	2.2 (1.3)	-0.3 (1.5)	0.47
Non-HDL-C (mg/dl)	Reference	0.3 (0.3)	0.2 (0.4)	0.3 (0.3)	-0.3 (0.3)	0.99
100% Fruit Juice Intake						
Offspring Cohort						
No of Observations	1,096	981	1,013	3,663	2,105	
LDL-C (mg/dl)	Reference	0.8 (0.7)	0.2 (0.7)	-0.5 (0.5)	-0.7 (0.6)	0.03
HDL-C (mg/dl)	Reference	-0.2 (0.2)	-0.4 (0.2)	-0.3 (0.2)	-0.1 (0.2)	0.49
TG (mg/dl)	Reference	1.5 (2.2)	2.3 (2.2)	1.4 (1.8)	-1.8 (2)	0.40
Non-HDL-C (mg/dl)	Reference	0.6 (0.5)	0.4 (0.5)	0.2 (0.4)	-0.2 (0.4)	0.39
Generation 3 Cohort						
No of Observations	307	377	547	1,066	503	
LDL-C	Reference	0.2 (1.1)	0.4 (1)	-0.6 (1)	-0.2 (1.1)	0.47
HDL-C (mg/dl)	Reference	0.06 (0.5)	0.1 (0.5)	0.6 (0.5)	0.6 (0.5)	0.11
TG (mg/dl)	Reference	0.5 (3.4)	-2.6 (3.2)	-2.8 (3)	-4.8 (3.4)	0.09

Non-HDL-C (mg/dl)	Reference	0.8 (1)	0.7 (0.9)	-0.01 (0.9)	0.7 (1)	1.00
Pooled Results[†]						
LDL-C	Reference	0.6 (0.6)	0.3 (0.6)	-0.6 (0.5)	-0.5 (0.5)	0.04
HDL-C (mg/dl)	Reference	-0.1 (0.2)	-0.3 (0.2)	-0.1 (0.2)	-0.06 (0.2)	0.83
TG (mg/dl)	Reference	1.3 (1.9)	1.1 (1.8)	0.7 (1.5)	-2.3 (1.8)	0.17
Non-HDL-C (mg/dl)	Reference	0.8 (0.4)	0.6 (0.4)	0.4 (0.4)	0.4 (0.4)	0.82

Abbreviations: HDL-C, high density lipoprotein cholesterol concentrations; LDL-C, low-density lipoprotein cholesterol concentrations; mg/dl, milligrams per deciliter; TC, total cholesterol concentrations; TG, triglyceride concentrations.

[*]Framingham Offspring Cohort: 8,858 observations from 3,082 participants; Framingham Generation 3 Cohort: 2,800 participants. Values are beta-coefficients for beverage intake in multivariate mixed effects models accounting for family structure and adjusted for age (continuous), sex (M/F), total energy (continuous), baseline for lipoprotein concentrations (continuous), education (less than high school, graduated high school, some college, or graduated college), current smoking status (yes/no), current diabetes status (yes/no), physical activity index (continuous), alcohol (grams), use of LDL-lowing medication (yes/no; where applicable), servings per day of vegetables, whole fruits, whole grains, nuts/seeds, and seafood, as well as percent energy from saturated fat (continuous), change in waist circumference, and mutual adjustment for SSB, LCSB, and 100% fruit juice (categorical as continuous).

Figure 5.1 Relationship between cross-stratified SSB and LCSB intakes for mean 4-year changes in HDL-C and TG concentrations among Framingham cohorts (pooled data)

Participants in the highest categories of both SSB and LCSB intakes had mean 4-year changes in TG concentrations 6.8 mg/dl larger [β ± SE: 6.7 ± 2.2 mg/dl; p = 0.003] and mean 4-year changes in HDL-C concentrations 0.8 mg/dl smaller [β ± SE: -1.0 ± 0.3 mg/dl; p = 0.0001] compared to those in the lowest categories of both SSB and LCSB intakes. There was little evidence of a significant interaction between SSB and LCSB intake (P>0.01 for the interaction). All changes in lipoprotein concentrations were adjusted for age, cohort, sex, total energy, baseline lipoprotein concentration, education, current smoking status, current diabetes status, physical activity index, alcohol intake, percent energy from saturated fat, change in waist circumference, and servings per day of vegetables, whole fruits, 100% fruit juice, whole grains, nuts/seeds, and seafood. Multivariate adjusted beta estimates for additional comparisons are presented in Supplemental Table S1. Vertical error bars indicate standard errors for regression coefficients.

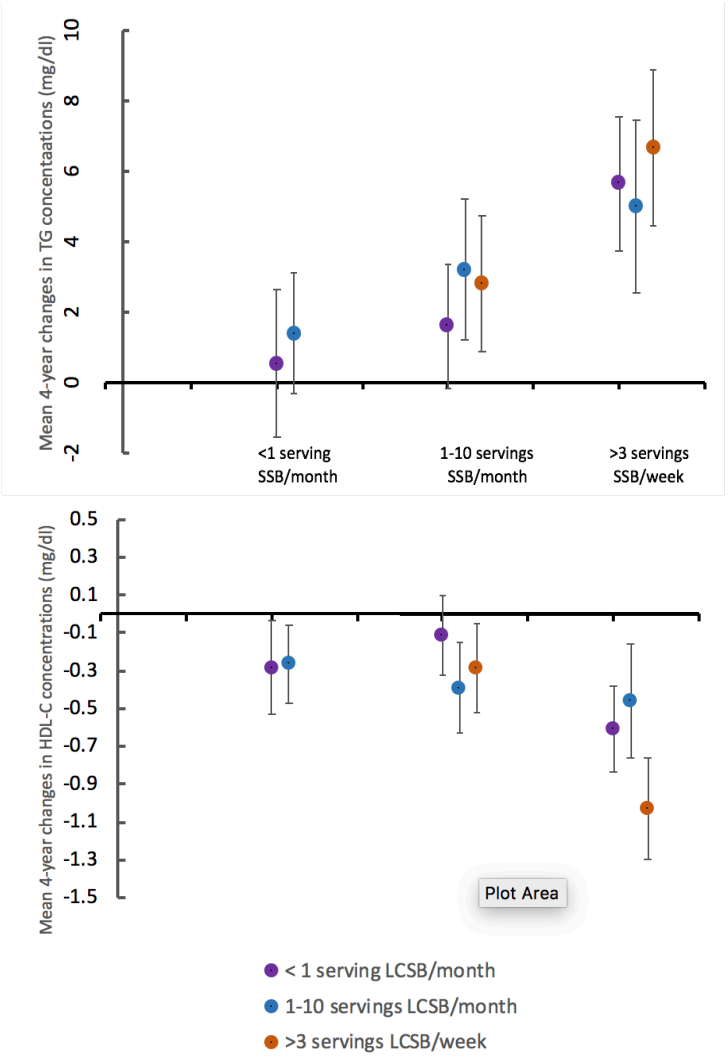

Figure 5.2 Hazard ratios for development of dyslipidemia among highest beverage consumers compared to lowest beverage consumers indicated by recent beverage consumption versus the cumulative average of beverage consumption among Framingham Offspring cohort

Participants were followed for mean of 12.5 years and were free of dyslipidemia at baseline (according to each definition). Thus, sample sizes and case numbers were as follows LDL-C (n=2,161; 961 cases), HDL-C (n=1,703; 318 cases), TG (n=2,116; 456 cases), non-HDL-C (n=2,205; 974 cases). All hazard ratios are adjusted for age, sex, total energy, education, current smoking status, current diabetes status, physical activity index, body mass index, alcohol intake, percent energy from saturated fat, and servings per day of vegetables, whole fruits, 100% fruit juice, whole grains, nuts/seeds, and seafood. Horizontal bars indicate 95% confidence intervals.

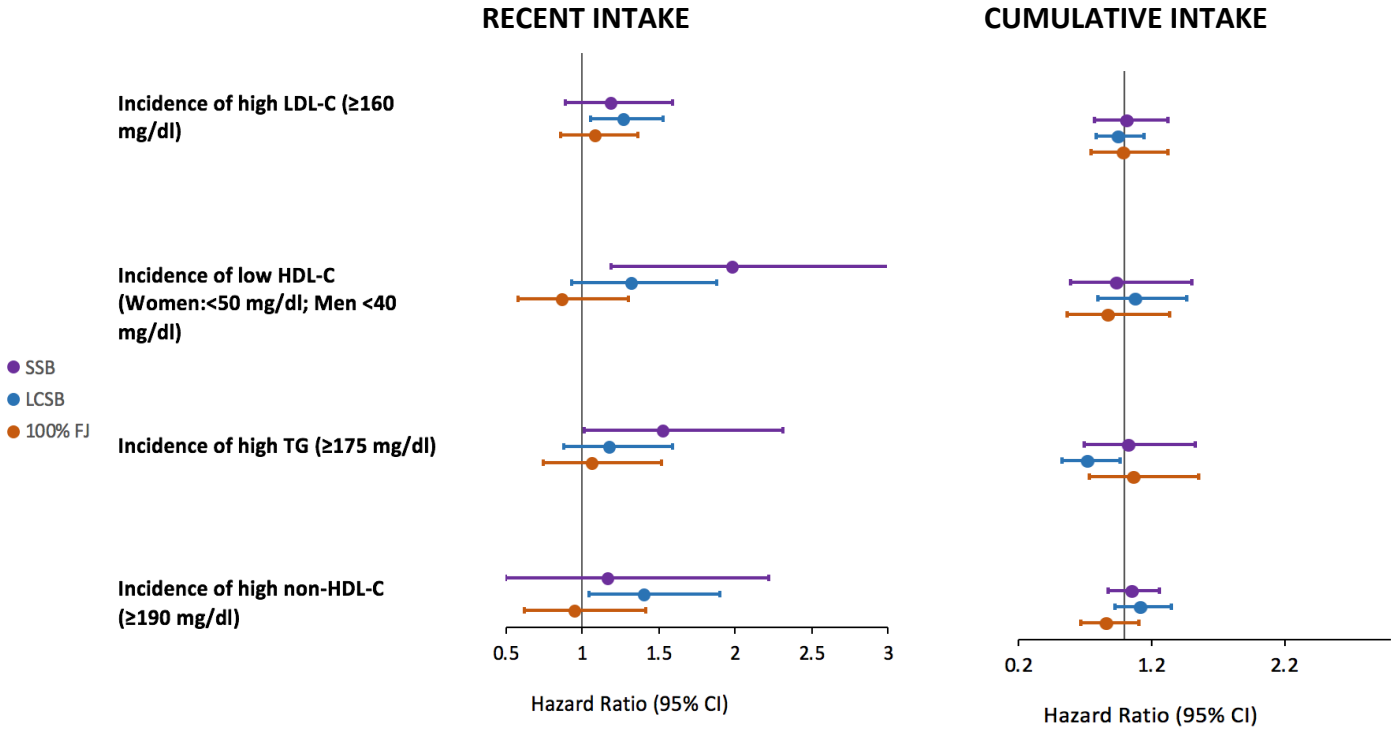

Table 5.3 Mean difference in 4-year changes in lipoprotein concentrations across beverage consumption groups (Offspring: 8,858 observations from 3,082 participants; Generation 3: 2,800 participants)[1]

	Offspring Cohort			Generation 3 Cohort		
	Beverage Consumption Groups			Beverage Consumption Groups		
	<1 serving/ month	>1 serving/ day	*P* for Trend	<1 serving/ month	>1 serving/ day	*P* for Trend
Sugar-Sweetened Beverage Intake						
n	3,488	685		867	325	
LDL-C (mg/dl)						
Model 1	Ref.	-0.1 (0.7)	0.40	Ref.	1.7 (1.1)	0.15
Model 2	Ref.	-0.1 (0.8)	0.31	Ref.	2.0 (1.2)	0.14
Model 3	Ref.	0.00 (0.7)	0.31	Ref.	1.8 (1.2)	0.20
HDL-C (mg/dl)						
Model 1	Ref.	-0.8 (0.3)	0.004	Ref.	-1.1 (0.5)	0.001
Model 2	Ref.	-0.9 (0.3)	0.005	Ref.	-0.8 (0.6)	0.01
Model 3	Ref.	-1.0 (0.3)	0.008	Ref.	-0.7 (0.6)	0.02
TG (mg/dl)						
Model 1	Ref.	3.6 (2.3)	0.005	Ref.	10.2 (3.3)	0.001
Model 2	Ref.	1.7 (2.5)	0.06	Ref.	12.8 (3.7)	0.0003
Model 3	Ref.	2.2 (2.5)	0.05	Ref.	12.3 (3.6)	0.0006
Non-HDL-C (mg/dl)						
Model 1	Ref.	0.8 (0.5)	0.04	Ref.	1.7 (1.0)	0.22
Model 2	Ref.	0.8 (0.5)	0.06	Ref.	2.5 (1.1)	0.11
Model 3	Ref.	0.8 (0.5)	0.07	Ref.	2.1 (1.1)	0.22
Low-Calorie Sweetened Beverage Intake						
n	3,792	1,265		1,894	1,265	
LDL-C (mg/dl)						
Model 1	Ref.	-0.3 (0.5)	0.67	Ref.	-1.5 (0.8)	0.14
Model 2	Ref.	-0.3 (0.5)	0.76	Ref.	-1.3 (0.9)	0.22
Model 3	Ref.	-0.6 (0.5)	0.37	Ref.	-1.8 (0.8)	0.11
HDL-C (mg/dl)						
Model 1	Ref.	-0.1 (0.2)	0.39	Ref.	-0.7 (0.4)	0.05
Model 2	Ref.	-0.2 (0.2)	0.18	Ref.	-0.6 (0.4)	0.09
Model 3	Ref.	-0.1 (0.2)	0.33	Ref.	-0.4 (0.4)	0.18
TG (mg/dl)						
Model 1	Ref.	-0.5 (1.7)	0.63	Ref.	0.8 (2.6)	0.37
Model 2	Ref.	0.2 (1.7)	0.37	Ref.	1.2 (2.6)	0.28
Model 3	Ref.	-0.4 (1.7)	0.63	Ref.	-0.2 (2.6)	0.41
Non-HDL-C (mg/dl)						
Model 1	Ref.	-0.2 (0.4)	0.99	Ref.	-0.7 (0.8)	0.46
Model 2	Ref.	-0.1 (0.4)	0.74	Ref.	-0.6 (0.8)	0.54
Model 3	Ref.	-0.3 (0.4)	0.97	Ref.	-1.1 (0.8)	0.24
100% Fruit Juice Intake						
n	1,096	2,105		3,663	2,105	
LDL-C (mg/dl)						
Model 1	Ref.	-0.4 (0.6)	0.07	Ref.	0.3 (1.1)	0.88
Model 2	Ref.	-0.4 (0.6)	0.07	Ref.	-0.1 (1.2)	0.76
Model 3	Ref.	-0.7 (0.6)	0.03	Ref.	-0.2 (1.1)	0.47
HDL-C (mg/dl)						
Model 1	Ref.	-0.3 (0.2)	0.19	Ref.	0.7 (0.5)	0.18
Model 2	Ref.	-0.2 (0.2)	0.34	Ref.	0.6 (0.6)	0.30
Model 3	Ref.	-0.1 (0.2)	0.49	Ref.	0.6 (0.5)	0.11
TG (mg/dl)						
Model 1	Ref.	-0.1 (2.0)	0.98	Ref.	-4.8 (3.4)	0.12
Model 2	Ref.	-1.5 (2.0)	0.49	Ref.	-4.5 (3.5)	0.18
Model 3	Ref.	-1.8 (2.0)	0.40	Ref.	-4.8 (3.4)	0.09
Non-HDL-C (mg/dl)						

Model 1	Ref.	0.1 (0.4)	0.87	Ref.	0.5 (1.0)	0.73
Model 2	Ref.	-0.1 (0.4)	0.53	Ref.	0.6 (1.1)	0.65
Model 3	Ref.	-0.2 (0.4)	0.39	Ref.	0.7 (1.0)	1.00

Abbreviations: HDL-C, high density lipoprotein cholesterol concentrations; LDL-C, low-density lipoprotein cholesterol concentrations; mg/dl, milligrams per deciliter; TC, total cholesterol concentrations; TG, triglyceride concentrations.

Values are beta-coefficients for beverage intake in multivariate mixed effects models accounting for family structure and multiple observations (Offspring only).

[1]**Model 1** adjusted for age (continuous), sex (M/F), total energy (continuous), baseline lipoprotein concentration (continuous), education (less than high school, graduated high school, some college, or graduated college), current smoking status (yes/no), current diabetes status (yes/no), physical activity index (continuous), alcohol (grams), use of LDL-lowing medication (yes/no; where applicable); **Model 2** adjusted for model 1 covariates plus servings per day of vegetables, whole fruits, whole grains, nuts/seeds, and seafood, as well as percent energy from saturated fat (continuous) and mutual adjustment for SSB, LCSB, and 100% fruit juice (categorical as continuous). **Model 3** adjusted for model 2 covariates plus change in waist circumference.

Table 5.4 Mean difference in 4-year changes in lipoprotein concentrations by SSB and LCSB category*

					HDL-C (mg/dl)		TG (mg/dl)	
SSB Category	**LCSB Category**	**Median SSB Intake (servings/week)**	**Medan LCSB Intake (servings/week)**	***n***	**β ± SE**	***p***	**β ± SE**	***p***
<1 serv/ month	**<1 serv/ month**	0.0	0.0	1,563	**Reference**		**Reference**	
	1-10 serv/month	0.0	1.0	882	-0.3 (0.2)	0.25	0.5 (2.1)	0.80
	>3 serv/week	0.0	7.0	1,909	-0.3 (0.2)	0.21	1.4 (1.7)	0.42
1-10 serv/month	**<1 serv/ month**	1.0	0.0	1,738	-0.1 (0.2)	0.60	1.6 (1.8)	0.36
	1-10 serv/month	1.0	1.0	1,029	-0.4 (0.2)	0.10	3.2 (2.0)	0.11
	>3 serv/week	1.0	6.5	1,253	-0.3 (0.2)	0.22	2.8 (1.9)	0.15
>3 serv/week	**<1 serv/ month**	5.7	0.0	1,733	-0.6 (0.2)	0.008	5.7 (1.9)	0.003
	1-10 serv/month	4.7	1.0	632	-0.5 (0.3)	0.13	5.0 (2.5)	0.04
	>3 serv/week	4.7	6.3	917	-1.0 (0.3)	0.0001	6.7 (2.2)	0.002

Abbreviations: HDL-C, high density lipoprotein cholesterol concentrations; LDL-C, low-density lipoprotein cholesterol concentrations; mg/dl, milligrams per deciliter; serv, serving(s); TC, total cholesterol concentrations; TG, triglyceride concentrations.

*Framingham Offspring Cohort: 8,858 observations from 3,082 participants; Framingham Generation 3 Cohort: 2,800 participants. Values are pooled beta-coefficients for beverage intake in multivariate mixed effects models accounting for family structure and adjusted for age (continuous), sex (M/F), total energy (continuous), baseline lipoprotein concentration (continuous), education (less than high school, graduated high school, some college, or graduated college), current smoking status (yes/no), current diabetes status (yes/no), physical activity index (continuous), alcohol (grams), use of LDL-lowing medication (yes/no; where applicable), change in waist circumference (continuous), servings per day of vegetables, whole fruits, whole grains, nuts/seeds, and seafood, as well as percent energy from saturated fat (continuous) and mutual adjustment for SSB, LCSB, and 100% fruit juice (categorical as continuous).

Table 5.5 HRs (95% CIs) for incident dyslipidemia according to recent beverage consumption category (Framingham Offspring Cohort)[1]

	SSB Intake			LCSB Intake			100% Fruit Juice Intake		
	Incident Cases	**Person-Years**	**HR (95% CI)**	**Incident Cases**	**Person-Years**	**HR (95% CI)**	**Incident Cases**	**Person-Years**	**HR (95% CI)**
Incidence of high LDL-C (≥160 mg/dl)									
<1 serving/month	394	9956	Reference	419	11653	Reference	129	3290	Reference
1-4 servings/month	216	6190	0.99 (0.84-1.17)	138	3830	1.00 (0.83-1.20)	162	4011	1.13 (0.90-1.40)
1-2 servings/week	70	2239	0.98 (0.76-1.28)	42	1336	0.85 (0.63-1.15)	67	2009	0.94 (0.70-1.26)
3-7 servings/week	206	5010	1.22 (1.01-1.47)	216	5162	1.17 (0.99-1.39)	376	10091	0.98 (0.81-1.20)
>1 serving/day	75	2044	1.19 (0.88-1.59)	144	3428	1.27 (1.05-1.53)	227	6033	1.08 (0.86-1.36)
			p_{trend}=0.05			**p_{trend}=0.01**			p_{trend}=0.94
Incidence of low HDL-C (Women:<50 mg/dl; Men <40 mg/dl)									
<1 serving/month	108	9784	Reference	124	10481	Reference	45	3221	Reference
1-4 servings/month	90	5610	1.47 (1.11-1.95)	51	3495	1.18 (0.85-1.63)	45	3579	0.83 (0.55-1.25)
1-2 servings/week	29	2030	1.24 (0.80-1.92)	9	1240	0.54 (0.25-1.13)	20	1804	0.63 (0.38-1.07)
3-7 servings/week	63	4064	1.43 (1.02-2.01)	85	4823	1.38 (1.04-1.84)	135	9064	0.96 (0.67-1.37)
>1 serving/day	28	1396	**1.98 (1.20-3.28)**	49	2809	1.32 (0.92-1.88)	74	5215	0.87 (0.58-1.30)
			p_{trend}=0.01			p_{trend}=0.05			p_{trend}=0.98
Incidence of high TG (≥175 mg/dl)									
<1 serving/month	167	11515	Reference	194	12684	Reference	59	3971	Reference
1-4 servings/month	99	6784	1.03 (0.80-1.31)	65	4263	1.04 (0.77-1.39)	75	4398	1.15 (0.82-1.63)
1-2 servings/week	49	2427	1.50 (1.07-2.10)	35	1534	1.43 (0.99-2.04)	44	2184	1.24 (0.83-1.84)
3-7 servings/week	98	5097	1.42 (1.07-1.88)	93	5724	1.07 (0.83-1.37)	179	10823	1.14 (0.83-1.56)
>1 serving/day	43	1937	1.53 (1.01-2.31)	68	3534	1.18 (0.88-1.59)	99	6384	1.06 (0.74-1.52)
			p_{trend}=0.004			p_{trend}=0.23			p_{trend}=0.84
Incidence of high Non-HDL-C (≥190 mg/dl)									
<1 serving/month	402	10351	Reference	404	11864	Reference	145	3435	Reference
1-4 servings/month	222	6298	1.02 (0.87-1.20)	144	3895	1.12 (0.93-1.34)	159	4110	0.99 (0.81-1.23)
1-2 servings/week	82	2367	1.11 (0.87-1.42)	47	1402	0.98 (0.73-1.30)	65	2080	0.84 (0.63-1.12)
3-7 servings/week	193	5021	1.16 (0.96-1.41)	223	5352	**1.26 (1.07-1.49)**	386	10335	0.92 (0.77-1.12)
>1 serving/day	75	2065	1.17 (0.87-1.57)	155	3565	**1.40 (1.17-1.69)**	220	6141	0.95 (0.77-1.18)
			p_{trend}=0.10			**p_{trend}=0.0002**			p_{trend}=0.49

Abbreviations: HDL-C, high density lipoprotein cholesterol concentrations; LDL-C, low-density lipoprotein cholesterol concentrations; mg/dl, milligrams per deciliter; TC, total cholesterol concentrations; TG, triglyceride concentrations.

Participants were free of dyslipidemia at baseline (according to each definition), thus sample sizes were as follows LDL-C (n=2,161), HDL-C (n=1,703), TG (n=2,116), and non-HDL-C (n=2,205).

[1]Models adjusted for age (continuous), sex (M/F), total energy (continuous), education (less than high school, graduated high school, some college, or graduated college), current smoking status (yes/no), physical activity index (continuous), body mass index (BMI), alcohol (grams), servings per day of vegetables, whole fruits, whole grains, nuts/seeds, and seafood, as well as percent energy from saturated fat (continuous) and mutual adjustment for SSB, LCSB, and 100% fruit juice (categorical as continuous).

Table 5.6 HRs (95% CIs) for incident dyslipidemia according to recent beverage consumption category (Framingham Generation 3 Cohort)[1]

	SSB Intake			LCSB Intake			100% Fruit Juice Intake		
	Incident Cases	Person-Years	HR (95% CI)	Incident Cases	Person-Years	HR (95% CI)	Incident Cases	Person-Years	HR (95% CI)
Incidence of high LDL-C (≥160 mg/dl)									
<1 serving/month	81	4261	Reference	118	6909	Reference	33	1627	Reference
1-4 servings/month	47	3613	0.76 (0.53-1.09)	31	2275	0.84 (0.57-1.26)	52	2667	0.82 (0.52-1.32)
1-2 servings/week	30	1764	1.15 (0.72-1.83)	12	705	1.14 (0.58-2.22)	27	2078	0.72 (0.42-1.24)
3-7 servings/week	56	3046	1.18 (0.80-1.73)	50	2597	1.17 (0.83-1.64)	77	5081	0.69 (0.44-1.08)
>1 serving/day	30	1872	1.04 (0.61-1.76)	33	2064	0.82 (0.53-1.28)	55	3102	0.87 (0.53-1.44)
Incidence of low HDL-C (Women:<50 mg/dl; Men <40 mg/dl)			p_{trend}=0.32			p_{trend}=0.92			p_{trend}=0.45
<1 serving/month	25	3851	Reference	44	6010	Reference	14	1495	Reference
1-4 servings/month	27	3316	1.15 (0.64-2.05)	13	1930	0.87 (0.44-1.70)	17	2322	0.67 (0.30-1.47)
1-2 servings/week	15	1555	1.15 (0.54-2.46)	5	617	1.27 (0.44-3.64)	24	1806	1.42 (0.69-2.93)
3-7 servings/week	28	2548	1.55 (0.81-2.95)	25	2324	1.33 (0.78-2.28)	35	4363	0.88 (0.43-1.79)
>1 serving/day	14	1461	1.07 (0.42-2.72)	23	1851	1.55 (0.89-2.72)	20	2753	0.85 (0.39-1.86)
Incidence of high TG (≥175 mg/dl)			p_{trend}=0.44			p_{trend}=0.09			p_{trend}=0.88
<1 serving/month	48	4394	Reference	73	7026	Reference	21	1739	Reference
1-4 servings/month	35	3690	0.89 (0.56-1.43)	32	2267	1.24 (0.81-1.90)	39	2767	1.01 (0.56-1.81)
1-2 servings/week	21	1806	0.92 (0.53-1.62)	10	765	1.41 (0.72-2.77)	27	2091	1.08 (0.59-1.97)
3-7 servings/week	40	3090	1.04 (0.63-1.72)	31	2579	1.18 (0.75-1.86)	52	5111	0.80 (0.46-1.42)
>1 serving/day	32	1843	1.49 (0.83-2.69)	30	2187	1.00 (0.62-1.61)	37	3121	1.00 (0.55-1.83)
Incidence of high Non-HDL-C (≥190 mg/dl)			p_{trend}=0.30			p_{trend}=0.77			p_{trend}=0.64
<1 serving/month	76	4269	Reference	117	6988	Reference	31	1659	Reference
1-4 servings/month	48	3648	0.86 (0.59-1.25)	31	2267	0.91 (0.61-1.35)	54	2675	0.92 (0.57-1.47)
1-2 servings/week	29	1784	1.28 (0.80-2.04)	12	697	1.51 (0.80-2.85)	27	2102	0.72 (0.42-1.23)
3-7 servings/week	54	3077	1.16 (0.78-1.72)	50	2633	1.25 (0.88-1.76)	85	5168	0.76 (0.49-1.18)
>1 serving/day	43	1909	1.49 (0.92-2.42)	40	2103	1.01 (0.66-1.54)	53	3091	0.80 (0.49-1.31)
			p_{trend}=0.09			p_{trend}=0.46			p_{trend}=0.25

Abbreviations: HDL-C, high density lipoprotein cholesterol concentrations; LDL-C, low-density lipoprotein cholesterol concentrations; mg/dl, milligrams per deciliter; TC, total cholesterol concentrations; TG, triglyceride concentrations.

Participants were free of dyslipidemia at baseline (according to each definition), thus sample sizes were as follows LDL-C (n=2,377), HDL-C (n=2,084), TG (n=2,426), and non-HDL-C (n=2,400).

[1]Models adjusted for age (continuous), sex (M/F), total energy (continuous), education (less than high school, graduated high school, some college, or graduated college), current smoking status (yes/no), physical activity index (continuous), body mass index (BMI), alcohol (grams), servings per day of vegetables, whole fruits, whole grains, nuts/seeds, and seafood, as well as percent energy from saturated fat (continuous) and mutual adjustment for SSB, LCSB, and 100% fruit juice (categorical as continuous).

Table 5.7 HRs (95% CIs) for incident dyslipidemia according to cumulative beverage consumption category (Framingham Offspring Cohort)[1]

	SSB Intake			LCSB Intake			100% Fruit Juice Intake		
	Incident Cases	**Person-Years**	**HR (95% CI)**	**Incident Cases**	**Person-Years**	**HR (95% CI)**	**Incident Cases**	**Person-Years**	**HR (95% CI)**
Incidence of high LDL-C (≥160 mg/dl)									
<1 serving/month	267	7810	Reference	341	8891	Reference	54	1580	Reference
1-4 servings/month	141	3765	1.03 (0.84-1.25)	115	2880	1.13 (0.92-1.37)	69	1937	0.88 (0.62-1.23)
1-2 servings/week	212	5387	1.05 (0.88-1.26)	135	3252	1.02 (0.84-1.24)	108	3383	0.80 (0.58-1.10)
3-7 servings/week	262	6364	1.05 (0.88-1.26)	223	6237	0.93 (0.80-1.10)	425	10722	0.97 (0.73-1.29)
>1 serving/day	79	2116	1.01 (0.77-1.32)	147	4183	0.95 (0.78-1.15)	305	7820	0.99 (0.74-1.31)
Incidence of low HDL-C (Women:<50 mg/dl; Men <40 mg/dl)			p_{trend}=0.69			p_{trend}=0.30			p_{trend}=0.34
<1 serving/month	113	7732	Reference	114	7334	Reference	27	1716	Reference
1-4 servings/month	46	3687	0.76 (0.54-1.07)	36	2756	0.85 (0.59-1.23)	24	1758	0.90 (0.52-1.56)
1-2 servings/week	72	4606	1.01 (0.75-1.36)	32	2753	0.79 (0.54-1.15)	48	3454	0.94 (0.59-1.49)
3-7 servings/week	60	5327	0.63 (0.45-0.89)	73	6353	0.76 (0.57-1.01)	121	9238	0.84 (0.55-1.27)
>1 serving/day	28	1536	0.94 (0.59-1.50)	64	3691	1.08 (0.80-1.46)	99	6722	0.87 (0.57-1.35)
Incidence of high TG (≥175 mg/dl)			p_{trend}=0.13			p_{trend}=0.70			p_{trend}=0.51
<1 serving/month	117	8680	Reference	175	9662	Reference	34	2370	Reference
1-4 servings/month	83	4317	1.23 (0.93-1.63)	57	3244	0.94 (0.69-1.28)	30	2193	0.87 (0.53-1.41)
1-2 servings/week	99	6258	1.05 (0.81-1.37)	56	3386	0.91 (0.68-1.23)	59	3740	1.09 (0.73-1.64)
3-7 servings/week	120	6551	1.13 (0.87-1.48)	113	7074	0.91 (0.71-1.16)	179	11386	0.98 (0.68-1.41)
>1 serving/day	38	1961	1.03 (0.69-1.53)	56	4402	0.72 (0.53-0.97)	155	8078	1.07 (0.74-1.56)
Incidence of high Non-HDL-C (≥3.5)			p_{trend}=0.73			p_{trend}=0.05			p_{trend}=0.54
<1 serving/month	301	8106	Reference	318	9008	Reference	70	1755	Reference
1-4 servings/month	161	3993	1.05 (0.87-1.26)	119	3042	1.19 (0.98-1.44)	87	1945	0.92 (0.68-1.24)
1-2 servings/week	182	5445	0.89 (0.74-1.06)	136	3232	1.11 (0.92-1.34)	115	3444	0.75 (0.57-1.00)
3-7 servings/week	244	6446	0.98 (0.82-1.17)	238	6554	1.06 (0.90-1.24)	404	10932	0.82 (0.64-1.05)
>1 serving/day	87	2118	1.14 (0.87-1.49)	164	4273	1.12 (0.93-1.35)	299	8033	0.86 (0.67-1.10)
			p_{trend}=0.96			p_{trend}=0.35			p_{trend}=0.41

Abbreviations: HDL-C, high density lipoprotein cholesterol concentrations; LDL-C, low-density lipoprotein cholesterol concentrations; mg/dl, milligrams per deciliter; TC, total cholesterol concentrations; TG, triglyceride concentrations.

Participants were free of dyslipidemia at baseline (according to each definition), thus sample sizes were as follows LDL-C (n=2,161), HDL-C (n=1,703), TG (n=2,116), and non-HDL-C (n=2,205).

[1]Models adjusted for age (continuous), sex (M/F), total energy (continuous), education (less than high school, graduated high school, some college, or graduated college), current smoking status (yes/no), physical activity index (continuous), body mass index (BMI), alcohol (grams), servings per day of vegetables, whole fruits, whole grains, nuts/seeds, and seafood, as well as percent energy from saturated fat (continuous) and mutual adjustment for SSB, LCSB, and 100% fruit juice (categorical as continuous).

CHAPTER 6

Associations between genetic variants near the *CHREBP* locus and lipoprotein concentrations may be modified by sugar-sweetened beverage consumption

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6.1 Abstract

Background: Genetic determinants of triglyceride (TG) and high-density lipoprotein cholesterol (HDL-C) concentrations have been identified in genome-wide association studies (GWAS), but the loci discovered only account for a small fraction of the estimated heritability. Carbohydrate responsive element binding protein (ChREBP) is a transcription factor that responds to sugar consumption. Consumption of sugar-sweetened beverages (SSB) and genetic variants at the *CHREBP* (also known as *MLXIPL*) locus have separately been linked to dyslipidemia. We hypothesized that SSB consumption may modify the associations of *CHREBP* variants and HDL-C and TG concentrations.

Methods: We conducted a cross-sectional analysis of data from 11 Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) consortium cohorts (N=63,599). A total of 1,606 single-nucleotide polymorphisms (SNPs) were identified within or near the *CHREBP* locus. SSB consumption (sodas, fruit punches, lemonades, or other fruit drinks) was estimated from food-frequency questionnaires. Participants were grouped by category of SSB intake (<1 serving/month, 1-4 serving/month, 1-2 serving/week, 3-7 serving/week, >1 serving/day). Inverse-variance weighted fixed- and random-effect meta-analyses were used to quantify the following associations: 1) SSB consumption and HDL-C and TG concentrations; 2) *CHREBP* locus SNPs and HDL-C and TG concentrations; and 3) interactions between SSB consumption and *CHREBP* locus SNPs, and HDL-C and TG concentrations.

Results: SSB consumption was inversely associated with HDL-C and positively associated with TG concentrations (p_{trend} <0.0001). We first replicated previously observed GWAS associations between one SNP on HDL-C (rs71556736) and two distinct SNPs (rs71556736 and rs13225660) on TG concentrations (Bonferroni-corrected p<0.0001). Additionally, we identified two distinct novel SNP associations with TG concentrations (rs42124 and rs10245965) in this locus. One distinct SNP displayed a statistically significant difference in effect by category of SSB consumption with HDL-C (rs71556729), and additional SNPs displayed a suggestive difference for both HDL-C and TG concentrations.

Conclusions: Our results indicate that high SSB consumption may modify the association between genetic variants within or near the *CHREBP* locus and HDL-C and TG concentrations.

6.2 Introduction

Dyslipidemia is a risk factor for cardiovascular disease (CVD) (1,2) that is influenced by both genes and environment (3–6). While elevated low-density lipoprotein cholesterol is an established risk factor for CVD, mounting evidence also suggests a role of reduced high-density lipoprotein cholesterol (HDL-C) and elevated triglyceride (TG) concentrations in development of CVD (7,8). Genetic determinants of these plasma lipid concentrations have been identified in genome-wide association studies (GWAS) (9–12), but the loci discovered thus far only account for a small fraction of the estimated heritability (5,6,13,14). It is possible that the interaction between environmental factors, particularly dietary components, and genetic predisposition are important contributors to this unexplained heritability. High sugar-sweetened beverage (SSB) consumption has been positively associated CVD risk (15,16), negatively associated with high-density lipoprotein cholesterol (HDL-C), and positively associated with triglyceride (TG) concentrations (15,17–19). Although reduction of SSB consumption is a worldwide public health initiative (20), determining whether SSB consumption interacts with genetic variants to influence lipid concentrations could provide important insights for understanding mechanisms contributing to CVD risk.

Carbohydrate Responsive Element Binding Protein (ChREBP) is a transcription factor that regulates glucose and lipid metabolism in response to sugar consumption, including sugar from SSB (21,22). GWAS have consistently observed an association between single nucleotide polymorphisms (SNPs) in the *CHREBP* locus (also known as *MLXIPL*) and HDL-C and TG concentrations (9,10,23–25), suggesting that variants in this gene are clinically relevant. In animal studies, hepatic ChREBP is robustly activated by consumption of fructose, a major

component of dietary sugar and hepatic ChREBP potentiates hepatic lipogenesis and TG secretion (22,26–29). Thus, this biological, epidemiological, and genetic evidence suggests that ChREBP may mediate effects of SSB consumption on HDL-C and TG concentrations. Thus, SNPs within the *CHREBP* locus present promising candidates for gene-diet interactions on HDL-C and TG concentrations.

The present study examines whether SSB consumption modifies the association of genetic variants within the *CHREBP* locus on HDL-C and TG concentrations in aggregated data from cohorts who are part of the Cohorts for Heart and Aging Research in Genetic Epidemiology (CHARGE) consortium.

6.3 Methods

Subjects

The study population consisted of up to 63,599 study participants of European ancestry from 11 well-characterized, large population-based cohorts participating in the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) consortium's Nutrition Working Group: Western Australian Birth Cohort Study (Raine) (Australia), Atherosclerosis Risk in Communities Study (ARIC) (USA), Framingham Heart Study (FHS) (USA), Netherlands Epidemiology in Obesity Study (NEO) (The Netherlands), The Fenland Study (Fenland) (United Kingdom), Young Finns Study (YFS) (Finland), Women's Genome Health Study (WGHS) (USA), Women's Health Initiative (WHI) (USA), Multi-Ethnic Study of Atherosclerosis (MESA) (USA), Cardiovascular Health Study (CHS), and the Rotterdam Study (RS) (The Netherlands). A full description of each of the 11 studies is presented in **Table 6.1**. All study

participants provided written informed consent, and approval for all study protocols was granted by local institutional review boards and/or oversight committee.

Dietary Assessment

Dietary intakes for all cohorts were estimated using validated food frequency questionnaires (FFQs) (**Table 6.2**). Food intakes were estimated from FFQ data using cohort-specific groupings and nutrient intakes were calculated from these data by multiplying the frequency of consumption of a food item by the respective nutrient content per standard serving size. Participants were excluded if they had implausible dietary data based on cohort-specific cut-points. Estimates of SSB consumption included the following cohort-specific categories (1) regular cola/soda with sugar; (2) caffeine free cola/soda with sugar; (3) carbonated non-soda beverages with sugar (e.g., 7-Up, ginger ale); and (4) fruit-flavored drinks (e.g. lemonade, Tang, Hawaiian Punch, squash). One serving of SSB was equivalent to 360 mL (12 fl oz.).

Genotyping and Imputation

Genotyping of individuals was carried out using Affymetrix and Illumina genotyping platforms and additional genotypes were imputed using algorithms that were implemented by IMPUTE, MACH or the Haplotype Reference Consortium (HRC) server, as described in **Table 6.3**. A total of 1,606 SNPs within the region 100 kilobases upstream and 190 kilobases downstream of the transcription start site of the *CHREBP* gene [chr7:72823661-73136661(hg19)] were examined. Genotyped SNPs were excluded on the basis of low minor allele count (allele frequency*sample size <20), low imputation quality (R^2 <0.3), and cohort-specific low call rates (minimally <90%) or departure from Hardy-Weinberg equilibrium

(minimally $p < 1x10^{-4}$). Individuals were excluded if they were missing data for all genotypes, age or sex.

Phenotype Definitions and Methods

HDL-C and TG concentrations were measured by standard enzymatic methods from venous blood collected after ≥8 hours of fasting. BMI was calculated from measured or self-reported weight (kg) divided by height squared (m^2). Description of cohort-specific methodologies for all relevant variables are described in **Table 6.4**.

Cohort-Specific Statistical Analyses

All CHARGE consortium cohorts followed a uniform, pre-specified analysis plan. Participants were grouped by category of SSB intake (<1 serving/month, 1-4 serving/month, 1-2 serving/week, 3-7 serving/week, >1 serving/day) and a natural logarithmic transformation was applied to TG concentrations to approximate a normal distribution. Linear regression models (and mixed effect linear regression for FHS) using an additive genetic effect were used to examine the associations between SSB consumption, SNPs, and the interaction between SSB consumption and SNPs on HDL-C and TG concentrations. Four models were assessed for the association between SSB consumption and lipid traits. Model 1 adjusted for age (continuous), sex (M/F) (where applicable), total energy intake (continuous), principal components of ancestry (where applicable for genetic analyses only), and study site for multi-centered cohorts. Model 2 adjusted model 1 covariates plus education (cohort-specific definition), smoking status (cohort-specific definition), physical activity (cohort-specific definition), and alcohol intake (grams/day) (Table 6.4). Model 3 adjusted for model 2 covariates plus BMI (continuous). Model 4 adjusted

for model 3 covariates plus servings per day of vegetables, total fruit, whole grains, nuts/seeds, and seafood, as well as % energy from saturated fat (continuous). In single-SNP analyses on lipid traits, only one model was performed (Model 1). In SNP analyses stratified by category of SSB intake, Model 1 adjusted for Model 1 covariates listed above and Model 2 adjusted for Model 3 covariates listed above. These same covariate models were used to model a multiplicative interaction term using an additive SSB category effect (categorical SNPxSSB) on lipid traits. Robust standard errors were reported in multiplicative interaction models to reduce risk of spurious type-I error inflation in SNPxSSB interaction analyses (30).

Meta-Analyses

For associations between SSB intake on lipid outcomes, beta coefficients were combined through inverse-variance weighted, random effects meta-analyses. When SSB intake was assessed as a categorical variable, multivariate meta-analyses (31) were conducted using the *mvmeta* R package (https://cran.r-project.org). When SSB intake was assessed using an additive model (per increase in category), univariate meta-analyses were conducted using the *meta* R package (https://cran.r-project.org). Results from analyses between SSB intake and lipid traits were considered statistically significant at $p < 0.05$.

For all SNP association and interaction tests on lipid outcomes, we excluded associations with high heterogeneity ($p < 1 \times 10^{-4}$) and low number of contributing cohorts (<3). In the full sample, regression (β) coefficients for the additive genetic effect of SNPs on lipid outcomes were combined through inverse-variance weighted, fixed-effect meta-analyses using METAL (version released on 2011-03-25). We undertook single SNP analyses and then implemented

conditional and joint association (COJO) analysis with GCTA software (32) to select top distinct signals [to account for SNPs that may be in high linkage disequilibrium (LD)] from the summary statistics (using the FHS cohort as a reference sample). The simpleM (33) method estimated 499 effective independent tests within and near the *CHREBP* locus among individuals in the 1000 genomes reference panel (34). Thus, results were considered statistically significant at a Bonferroni-corrected $p < 0.0001$ (0.05/499 independent tests).

To identify a statistically significant interaction between SNPs and SSB consumption, two separate methods were used: 1) a difference test; and 2) a multiplicative interaction test. For the difference test, an inverse-variance weighted fixed-effect meta-analysis was conducted using METAL for SNP effects on lipid outcomes stratified by category of SSB intake. The difference test for stratum-specific regression coefficients in the lowest (<1 serving SSB per month) and highest (>1 servings SSB per day) categories of SSB intake was used for each SNP. We set statistical significance for interactions at a Bonferroni-corrected $P_{Diff}<0.0001$, and a suggestive interaction was observed when $P_{Diff}<0.005$. For the multiplicative interaction test, beta coefficients for multiplicative interactions terms (categorical SNPxSSB) were combined through inverse-variance weighted, random-effect meta-analyses using METAL. A statistically significant multiplicative interaction was observed when $P_{interaction} < 0.0001$ and a suggestive multiplicative interaction was observed when $P_{interaction}<0.01$. For all interaction tests, we excluded SNPs with a minor allele frequency < 1%. To select top distinct signals from the summary statistics in interaction analyses, LD-based result clumping was implemented with PLINK software (version 1.90 released 21 May 2017) (http://pngu.mgh.harvard.edu/purcell/plink/) (35) using the FHS cohort as a reference sample.

The Cochrane Q statistic (36) and the I^2 statistic (37) were used to examine heterogeneity among the cohorts. Analyses with moderate-to-high heterogeneity ($I^2 > 30\%$) were further assessed for potential sources of heterogeneity by conducting meta-regression and sensitivity analyses. Meta-regression analyses were conducted using the R *meta* package to assess the effect of potential moderator variables on heterogeneity of associations/interactions. Mean effect sizes were compared in meta-analyses stratified by geographical region, age, BMI, SSB intake, study date, proportion of current smokers, and sample size. A statistically significant moderator was defined at $p_{\text{interaction}} < 0.007$ (0.05/7 potential moderators). The influence of individual cohorts on the meta-analyzed estimates was examined by removing one cohort at a time for statistically significant associations.

6.4 Results

Study Characteristics

General characteristics and mean dietary intakes for the eleven CHARGE consortium cohorts are shown in **Table 6.5**. The cohorts are ordered from highest to lowest mean SSB consumption. The mean age ranged from 20 to 72 years, and mean BMI ranged from 24.5 to 30.0 kg/m^2. Women comprised 52-61% of participants in each cohort, except WGHS and WHI, which included only women. Some variation in mean fasting HDL-C and TG concentrations was observed, ranging from 51 to 59 mg/dl and 85 to 156 mg/dl, respectively. Mean SSB intakes ranged from 0.1 to 0.7 servings/day, with two cohorts (CHS and RS) estimating <1% of participants consumed >1 serving of SSB per day and another cohort (Raine) estimating that 19% of its participants consumed >1 serving of SSB per day. Mean total energy intake ranged

from 1,644 kcal/day (ARIC) to 2,381 kcal/day (YFS). Mean dietary intakes in food and nutrient groups exhibited wide ranges across the cohorts.

Associations between SSB Consumption and Lipid Traits

Figures 6.1 and 6.2 represent forest plots of regression coefficients in multivariate inverse-variance weighted random-effects meta-analyses of SSB intake on lipid traits in fully adjusted models (Model 4), and **Figures 6.3 and 6.4** represent forest plots additionally including cohort-specific association estimates**.** Participants in the highest category of SSB intake (>1 serving/day) had lower mean HDL-C concentrations [β (95% CI):-2.1 (-2.9, -1.2) mg/dl] and higher mean TG concentrations [β (95% CI): 0.06 (0.03, 0.09) ln-mg/dl] compared to those in the lowest category of SSB intake (<1 serving/month). Covariate adjustment did not change the directionality or significance of results, but effect sizes tended to attenuate as covariates were added to the models (**Table 6.6**). Moderate to high heterogeneity was observed in both analyses (HDL-C: I^2=38%; TG: I^2=40%). **Figures 6.5 and 6.6** represent forest plots of regression coefficients in individual cohorts and in univariate random-effects meta-analyses for the association of categorical SSB intake (additive) on lipid traits in fully adjusted models (Model 4). Each increase in SSB intake category was associated with lower mean HDL-C concentrations [β (95% CI) : -0.56 (-0.75, -0.36) mg/dl; p_{trend} = 0.002] and higher mean TG concentrations [β (95% CI): 0.02 (0.01, 0.02) ln-mg/dl; p_{trend} < 0.0001]. Although the direction of effect sizes was consistent for both HDL-C (negative for 11/11 cohorts) and TG (positive for 10/11 cohorts) concentrations, high heterogeneity was also observed in these analyses (HDL-C: I^2=70; TG: I^2=62%).

We conducted sensitivity analyses to investigate potential sources of heterogeneity among the cohorts. Results were similar when each cohort was removed from the meta-analysis individually, indicating the observed heterogeneity could not be attributed to a single cohort. Stratified analyses of the association between SSB intake (additive) on HDL-C concentrations indicated that geographic region of study [USA (N studies=6), Australia (N studies=1), or Europe (N studies=4)] was a significant moderator ($p_{interaction} < 0.0001$) that accounted for 100% of the observed heterogeneity among the cohorts (**Table 6.7** and **Figure 6.7**). The effect size for additive SSB intake on HDL-C concentrations in the USA cohorts was larger [β (95% CI) : -0.77 (-0.89, -0.65) mg/dl] compared to that of the European cohorts [β (95% CI) : -0.25 (-0.41, -0.25) mg/dl], and the effect size for the Australian cohort was between the two [β (95% CI) : -0.41 (-1.18, 0.37) mg/dl]. Stratified analyses of the association between additive SSB intake on TG concentrations indicated that study mean age [< 40 years (N studies=2); 40-60 years (N studies=5); >60 years (N studies=4)] was a significant moderator ($p_{interaction} = 0.004$) that accounted for 83% of the observed heterogeneity among the cohorts (**Figure 6.8**). The effect size of additive SSB intake on TG concentrations among cohorts with a mean age between 40 – 60 years was larger [β (95% CI) : 0.02 (0.02, 0.03) ln-mg/dl] compared to that of cohorts with mean ages < 40 years [β (95% CI) : 0.01 (-0.01, 0.02) ln-mg/dl] or > 60 years [β (95% CI) : 0.01 (0.001, 0.01) ln-mg/dl]. However, additional heterogeneity remained among the cohorts with a mean age between 40 – 60 years ($I^2 = 55\%$), which could be attributed to the largest effect size observed in the FHS cohort ($I^2 = 0\%$ when FHS cohort removed). Similar moderators accounted for heterogeneity observed in multivariate meta-analyses (data not shown).

Associations of SNPs on lipid traits

Main effect associations of *CHREBP* locus SNPs on lipids traits are presented in **Table 6.8**. We identified 98 SNPs that reached statistical significance with one distinct signal (*CHREBP*-rs71556736) associated with HDL-C concentrations ($p<0.0001$). We identified 294 SNPs that reached statistical significance with four distinct signals (*CHREBP*-rs71556736, *FZD9*-rs42124, *CHREBP*-rs13225660, and *VPS37D*-rs10245965) associated with TG concentrations ($p<0.0001$). Consistent direction of effect was observed in the single SNP analyses for most cohorts, and heterogeneity was low for all SNPs except *VPS37D*-rs10245965 ($I^2 = 72.7$). The direction of the observed effect for *VPS37D*-rs10245965 was consistent in 9/10 cohorts, but the effect size was opposite in the WGHS cohort. All of the top SNPs were significantly associated with lipid concentrations in the single SNP analyses ($p<0.0001$) except for *VPS37D*-rs10245965 on TG concentrations ($p=0.07$), suggesting that the single SNP analysis of *VPS37D*-rs10245965 underestimated its effect size on TG concentrations. Forest plots for top distinct signals on lipid traits are presented in **Figures 6.9-6.13**.

Interactions between SSB consumption and SNPs on lipid traits

One SNP (*TBL2*- rs71556729) displayed a statistically significant difference in effect by category of SSB intake on HDL-C concentrations in minimally adjusted models (Model 1; P_{Diff} =0.0001) and a suggestive difference in effect in fully adjusted models (Model 2; P_{Diff}=0.0003) (**Table 6.9** and **Figure 6.14**). The association between the minor allele at *TBL2*- rs71556729 and HDL-C concentrations was stronger among the highest SSB consumers (>1 serving/day), compared to the lowest SSB consumers (<1 serving/month). In Model 1, each additional minor allele at *TBL2*- rs71556729 was associated with higher mean concentrations of HDL-C [β (SE): 4.47 (1.10), p=5.0E-05] among the highest SSB consumers (> 1 serving/day), but was not

associated with mean HDL-C concentrations among the lowest SSB consumers (<1 serving/month; p=0.31). In Model 2, results were similar, but the interaction was non-significant after accounting for multiple testing. The effect sizes of these SNPs among the highest SSB consumers were consistent across all the cohorts where available. We additionally identified 21 SNPs and six distinct signals that displayed a suggestive difference in effect by category of SSB consumption on HDL-C concentrations (P_{Diff} <0.005) in either covariate model. There was low heterogeneity (I^2 < 20%) observed for all of the top signals (statistically significant and suggestive) among the highest (>1 serving/day) and lowest (<1 serving/month) SSB consumers.

No statistically significant difference in effect by category of SSB intake on TG concentrations was observed (P_{Diff} >0.0001 for all SNPs). Two SNPs that were part of one distinct signal (*CHREBP*-rs799157) displayed a suggestive difference in effect by category of SSB intake on TG concentrations in minimally adjusted models (Model 1; P_{Diff} =0.002) and fully adjusted models (Model 2; P_{Diff} =0.003) (**Table 6.9**). In Model 1, each additional minor allele at *CHREBP*-rs799157 was associated with higher mean TG concentrations among the highest SSB consumers (> 1 serving/day) [β (SE): 0.11 (0.03) ln-mg/dl, p=0.002], but this association was attenuated among the lowest SSB consumers ([β (SE): 0.01 (0.01) ln-mg/dl, p=0.45; P_{Diff} = 3.7E-05] (**Figure 6.15**). The effect sizes of these SNPs among the highest SSB consumers were consistent across all the cohorts where these SNPs were available. There was low heterogeneity observed for the top signals in the association between SNPs and TG concentrations among the highest SSB consumers (>1 serving/day). The high heterogeneity in the lowest SSB consumers (<1 serving/month) could be partially attributed to the higher effect size observed in the FHS and ARIC cohorts (I^2=30% after removal of both cohorts from meta-analysis).

No statistically significant interactions between SNPs and SSB consumption were observed when applying the multiplicative interaction test ($p_{interaction}$>0.0001). Thus, suggestive findings are presented for the multiplicative interaction test in **Table 6.10** ($p_{interaction}$<0.01). We observed a suggestive interaction for five SNPS that were part of four distinct signals on HDL-C concentrations in either covariate model, and were suggestive of an interaction with one SNP and TG concentrations in Model 1. Two distinct SNPs that reached either a statistically significant or suggestive difference in effect by category of SSB consumption when applying the difference test (P_{Diff}<0.005) on HDL-C concentrations also displayed a suggestive SNPxSSB interaction on HDL-C concentrations [*TBL2*-rs71556729: Model 1 $\beta_{interaction}$ (SE): 0.74 (0.22), $p_{interaction}$ =0.001; *CHREBP*-rs13240662: Model 1 $\beta_{interaction}$ (SE): 0.42 (0.16), $p_{interaction}$ =0.008]. A suggestive SNPxSSB interaction was also observed for two SNPs that did not display a suggestive difference in effect by category of SSB consumption on HDL-C concentrations [CHREBP-rs79578725: Model 1 $\beta_{interaction}$ (SE): -0.51 (0.18), $p_{interaction}$ =0.006, P_{Diff} =0.05; BAZB1-rs17145717: Model 2 $\beta_{interaction}$ (SE): 0.37 (0.15), $p_{interaction}$ =0.01, P_{Diff} =0.65] and one SNP on TG concentrations [*CHREBP*-rs72649028: Model 1 $\beta_{interaction}$ (SE): 0.03 (0.01), $p_{interaction}$ =0.009; P_{Diff} = not available]. Forest plots for top distinct signals in SSBxSNP interaction analyses on lipid traits are presented in **Figures 6.16** and **6.17**.

6.5 Discussion

In this study, we observed a significant relationship between SSB consumption and dyslipidemia, characterized by an inverse association of SSB and fasting HDL-C and positive association with TG concentrations in a meta-analysis of data from 11 participating CHARGE consortium cohorts. We identified novel SNPs near the CHREBP locus that were significantly

associated with HDL-C and TG concentrations, and also replicated previously observed associations in this region. We also found evidence that SSB consumption modifies the association between SNPs near the *CHREBP* locus on HDL-C concentrations with suggestive interactions on TG concentrations.

This is the largest study to date assessing the association between SSB consumption and lipid concentrations among adults from multiple geographic areas and varying ranges of SSB consumption. Our results are consistent with several smaller studies that have observed higher SSB consumption is associated with lower HDL-C and higher of TG (15,38–41) concentrations. Evidence from meta-analysis of randomized-controlled trials, most of relatively small size and short duration, indicate a similar positive association between high sugar consumption and TG concentrations, but also a small positive association between high sugar consumption and HDL-C concentrations. This contrasts with the negative association between SSB consumption and HDL-C concentrations seen in this and other observational studies (42). Reasons for this discrepancy are unclear but may be due to differences in long-term versus short-term effects, the type of sugar exposure, or could be due to residual confounding. Secondary analyses of the heterogeneity of the association between SSB intake on these lipid traits indicated that the estimated effect of SSB on HDL-C concentrations was larger among USA-based cohorts compared to Australian and European cohorts and that the estimated effect of SSB on TG concentrations was larger among cohorts with a mean age of 40-60 years compared to cohorts with a mean age either less than 40 years or greater than 60 years. We cannot determine the source of these heterogeneous associations from our study-design, but this observation could be due to a number of factors, including, but not limited to differences in the information elicited by

the questionnaires, societal/generational norms, differences in SSB consumption patterns, and/or difference in the effect of SSB on lipid traits in these groups.

Although many studies have reported a significant association between SNPs in the *CHREBP* region on HDL-C and TG concentrations (9–12), this is the first study to conduct SNP analyses in this region adjusted for one another to identify distinct signals within this region. We observed one distinct SNP significantly associated with HDL-C concentrations and four distinct SNPs significantly associated with TG concentrations in the *CHREBP* region. Two SNPs significantly associated with TG concentrations (*FZD9*-rs42124 and *VPS37D*-rs10245965) were novel associations that were not observed in previous GWAS. Another SNP that was significantly associated with TG concentrations in this study and in a previous a gene-centric association analysis (CHREBP-rs13225660) (43) is in a high LD region that includes a SNP within the 3'UTR of *CHREBP*. We also replicated the top signal from previous GWAS for both HDL-C and TG concentrations (*CHREBP*- rs3812316), which is a missense SNP in a high LD region of *CHREBP*.

Our study provides evidence that SSB consumption may modify the association between SNPs in the *CHREBP* region on HDL-C and TG concentrations. One distinct SNP (*TBL2*-rs71556729) reached statistical significance in interaction analyses, where participants with the minor allele at *TBL2*- rs71556729 who consumed >1 serving SSB/day had higher mean HDL-C concentrations compared to those with the major allele who also consumed >1 serving SSB/day. This could suggest that participants with the minor allele *TBL2*- rs71556729 may be protected against SSB-induced low HDL-C concentrations, but since the lipoprotein concentration and

SSB consumption data were observational, this observation should be treated as hypothesis-generating for further studies. After accounting for lifestyle factors (education, smoking status, physical activity, alcohol intake, and BMI), the interaction became statistically non-significant, but a suggestive interaction remained. This suggests that this SSB interaction effect may be partially mediated through lifestyle factors or BMI.

This interaction was the top signal when testing for interactions using the difference test and the multiplicative interaction test on HDL-C concentrations. However, when applying the multiplicative interaction test, the interaction p-value was an order of magnitude larger compared to the p-value when applying the difference test. This is likely due to the large heterogeneity observed in the association between *TBL2*- rs71556729 and HDL-C concentrations among those reporting low (1-4 servings/month) to moderate (1-2 and 3-7 servings/week) SSB consumption (**Figure 6.14**). These data suggest that the difference test may be a useful method for identifying gene-diet interactions in observational studies, and this could be due to a reduction in misclassification of SSB intake and the potential to detect non-linear interaction effects.

Additional SNPs in the *CHREBP* region displayed a similar, yet statistically non-significant, differences in effect size stratified by category of SSB intake on lipid traits. Most of the SNPs displaying a suggestive difference in their effect on HDL-C concentrations showed a similar larger mean increase in HDL-C concentrations among the highest (>1 serving/day) compared to the lowest (<1 serving/month) SSB consumers. However, one distinct SNP (*VPS3D*-rs941294) displayed an opposite response, suggestive of a difference in effect where participants with the minor allele at *VPS3D*-rs941294 who consumed <1 serving SSB/month had higher mean HDL-C concentrations compared to those with the major allele. These data may suggest that high SSB consumption could mask the potential HDL-C lowering effect of *VPS3D*-

rs941294. For TG concentrations, participants with the minor allele at *CHREBP*- rs799157 or *CHREBP*-rs72649028 who consumed >1 serving SSB/day had higher mean TG concentrations compared to those with the major allele at these locations who also consumed >1 serving SSB/day. Although suggestive, these data would support the notion that SSB consumption may have the potential to exacerbate genetic effects of SNPs within *CHREBP* on TG concentrations.

There is a limited body of evidence describing how genes implicated in various diseases may interact with SSB consumption to modify cardiometabolic health and chronic disease risk (44). One large prospective cohort study among Swedish adults examined whether genetic risk for dyslipidemia (using a weighted genetic risk score) interacted with SSB consumption to influence plasma lipid concentrations, but no significant interactions were observed (45). Although genetic risk scores can be useful for translation, a weakness of genetic risk scores is that aggregation of multiple SNPs from across the genome does not allow inclusion of potential interacting SNPs that may not be associated with the outcome in overall analyses. In addition, SNP effects may be diluted by the null effects of other SNPs included in the genetic risk score. The candidate gene approach in the current study allows for the potential to generate hypotheses of the mechanisms underlying the interaction that could be tested using animal and human models in future studies.

No previous studies have examined the interaction between SNPs in the *CHREBP* region and SSB consumption on lipid concentrations. We previously investigated how selected SNPs in the ChREBP-FGF21 pathway interacted with SSB consumption to influence fasting insulin and glucose measures among 34,748 adults from CHARGE consortium cohorts, but we did not

identify a significant multiplicative interaction that was consistent among the discovery and replication phases of this study (46). In the current study, we applied a more comprehensive approach that tested a wide range of SNPs in the *CHREBP* region that were not necessarily identified in GWAS. Given that our suggestive interaction results do not include any SNPs that were statistically significant in the overall SNP analyses, our data suggest that there may be additional SNPs contributing to the heritability of HDL-C and TG concentrations, but their contribution is influenced by SSB consumption.

The strengths of our study include the large sample size attained through meta-analysis of multiple independent cohorts, the ability to standardize the analyses conducted in all cohorts through a collaborative approach, and the use of multiple methods to screen for potential interactions between SSB consumption and over 1,500 SNPs in the *CHREBP* region on HDL-C and TG concentrations. Application of modern analysis techniques revealed novel SNPs that may contribute to unexplained heritability of HDL-C and TG concentrations. Limitations of this study include the cross-sectional design of this study that limits our ability to infer causality, the sample of European-descent adults that limits generalizability, and the use of self-reported dietary data from FFQ that may lead to misclassification of food and nutrient intakes. Our focus on the comparison of the highest SSB consumers to the lowest SSB consumers helps minimize this potential misclassification, but the conclusions we can draw are also limited by the modest sample size among the highest SSB consumers compared to the lowest SSB consumers. We are additionally limited by the models used to conduct the interaction analyses. The second model that accounted for lifestyle factors also accounted for BMI, which could have been a potential mediator in the association between these SNPs and the lipid traits. Validation of these results in

an independent cohort with a larger number of participants consuming >1 serving SSB/day utilizing additional covariate models is necessary.

In conclusion, our findings suggest that higher SSB consumption is associated with lower HDL-C and higher TG concentrations among adults. We confirmed that one distinct SNP in the CHREBP region is associated with HDL-C concentrations and multiple distinct SNPs within the *CHREBP* region are associated with TG. Our results suggest that some SNPs in the *CHREBP* region may help protect against the potential adverse consequences of SSB consumption on HDL-C and TG concentrations, while other genetic variants may exacerbate the negative health effects of daily SSB consumption.

6.6 References

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Table 6.1. Descriptions and acknowledgements of participating CHARGE cohorts.

Cohort	Study Description and Acknowledgements	Relevant References
Atherosclerosis Risk In Communities (ARIC) Study **USA**	The ARIC study is a population-based cohort study designed to study new and established risk factors for atherosclerosis and community trends in coronary heart disease. In 1987-89, baseline data was collected on 15,792 adults, aged 45–64 y, living in four U.S. communities (Forsyth County, NC; Jackson, MS; northwest Minneapolis suburbs, MN; Washington County, MD). The baseline exam was conducted in 1987-89 and information was collected on African Americans, Caucasians, and a few adults of other ethnicities, aged 45–64 y. After providing informed consent, 15,792 adults were enrolled (8,710 women and 7,082 men). Up to 10,924, Caucasian adults with available DNA, valid dietary information, and consent to share genetic data were eligible for the current analysis The Atherosclerosis Risk In Communities (ARIC) Study is carried out as a collaborative study supported by National Heart, Lung, and Blood Institute contracts (HHSN268201100005C, HHSN268201100006C, HHSN268201100007C, HHSN268201100008C, HHSN268201100009C, HHSN268201100010C, HHSN268201100011C, and HHSN268201100012C), R01HL087641, R01HL59367 and R01HL086694; National Human Genome Research Institute contract U01HG004402; and National Institutes of Health contract HHSN268200625226C. The authors thank the staff and participants of the ARIC study for their important contributions. Infrastructure was partly supported by Grant Number UL1RR025005, a component of the National Institutes of Health and NIH Roadmap for Medical Research.	https://www2.cscc.unc.edu/aric/ The ARIC Investigators. The Atherosclerosis Risk in Communities (ARIC) Study: design and objectives. Am J Epidemiol. 1989 Apr;129(4):687-702.
Cardiovascular Health Study (CHS) **USA**	The Cardiovascular Health Study (CHS) is a population-based cohort study of risk factors for coronary heart disease and stroke in adults≥65 conducted across four field centers. The originally, predominantly European ancestry cohort of 5,201 persons was recruited in 1989-1990 from random samples of the Medicare eligibility lists; subsequently, an additional predominantly African-American cohort of 687 persons was enrolled in 1992-1993 for a total sample of 5,888. Blood samples were drawn from all participants at their baseline examination and DNA was subsequently extracted from available samples. Genotyping was performed at the General Clinical Research Center's Phenotyping/Genotyping Laboratory at Cedars-Sinai among CHS participants who consented to genetic testing and had DNA available using the Illumina 370CNV BeadChip system (for European ancestry participants, in 2007). The current analysis uses diet data collected on the first cohort in 1989-1990 and is restricted to individuals of European ancestry. CHS was approved by institutional review committees at each field center and individuals in the present analysis had available DNA and gave informed consent including consent to use of genetic information for the study of cardiovascular disease. This CHS research was supported by NHLBI contracts HHSN268201200036C, HHSN268200800007C, HHSN268200960009C, N01HC55222, N01HC85079, N01HC85080, N01HC85081, N01HC85082, N01HC85083, N01HC85086; and NHLBI grants U01HL080295, R01HL087652, R01HL105756, R01HL103612, R01HL120393, and R01HL130114 with additional contribution from the National Institute of Neurological Disorders and Stroke (NINDS). Additional support was provided through R01AG023629 from the National Institute on Aging (NIA). A full list of principal CHS investigators and institutions can be found at CHS-NHLBI.org. The provision of genotyping data was supported in part by the National Center for Advancing Translational Sciences, CTSI grant UL1TR000124, and the National Institute of Diabetes and Digestive and Kidney Disease Diabetes Research Center (DRC) grant DK063491 to the Southern California Diabetes Endocrinology Research Center. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.	http://www.chs-nhlbi.org/ Ann Epidemiol. 1(3): 263-276, 1991 Fried, L.P. et al. The Cardiovascular Health Study: design and rationale. Ann Epidemiol 1, 263-76 (1991).

Cohort	Study Description and Acknowledgements	Relevant References
Fenland Study (Fenland) **United Kingdom**	The Fenland Study is a population-based observational study in the East of England (Cambridgeshire), the United Kingdom. Participants born between 1950 and 1975 were recruited from general practice lists near and around Cambridge, Ely, and Wisbech. In total, 12,434 participants were enrolled between 2005 and 2015. Exclusion criteria of the Fenland study included pregnancy, physician-diagnosed diabetes, inability towalk unaided, psychosis, or terminal illness. Participants were excluded for this study if participants reported non-white or uncertain race/ethnic status; provided no data on habitual diet, HDL or trig, or genetic variants; reported energy intake <500 or >3500 kcal/day for women or <800 or >4200 kcal/day for men; or classified as outliers in the genetic variables for population admixture. The authors are grateful to all the Fenland Study volunteers for their time and to the general practitioners and practice staff for help with recruitment. The authors thank the Functional groups of the MRC Epidemiology Unit, including the Fenland Study Co- ordination team, the Field Epidemiology team, the data management team and the laboratory team. The authors also thank Kate Westgate and Stefanie Hollidge for assistance with physical activity data processing. Biochemical assays were performed by the National Institute for Health Research, Cambridge Biomedical Research Centre, Core Biochemistry Assay Laboratory and the Cambridge University Hospitals NHS Foundation Trust, Department of Clinical Biochemistry. The Fenland Study was funded by the Wellcome Trust and the Medical Research Council. Support from Medical Research Council programmes MC_UU_12015/1 and MC_UU_12015/5 is acknowledged.	http://www.mrc-epid.cam.ac.uk/research/studies/ fenland/
Framingham Heart Study (FHS) **USA**	The Framingham Offspring Study is a community-based longitudinal study designed to examine CVD risk in the offspring of the Original Cohort participants of the Framingham Heart Study and their spouses. In 1971, 5,124 individuals were enrolled; since then, the Offspring Cohort has been examined periodically. Between 1998 and 2001, during the 7th examination cycle, 3,539 adults, with a mean age of 61y, underwent a standardized medical history and physical examination. Beginning in 2002, 4,095 Gen III participants, who had at least one parent in the offspring cohort, were enrolled in the Framingham Heart Study. At the first cycle of the Gen III study, 4,095 individuals with a mean age of 40 y, underwent the standard clinic examination. For the present study both cohorts were combined for the analysis. A total of 6,382 adults with available DNA, valid dietary information, and consent to share genetic data were eligible for the current study. This research was conducted in part using data and resources from the Framingham Heart Study of the National Heart Lung and Blood Institute of the National Institutes of Health and Boston University School of Medicine. The analyses reflect intellectual input and resource development from the Framingham Heart Study investigators participating in the SNP Health Association Resource (SHARe) project. This work was partially supported by the National Heart, Lung and Blood Institute's Framingham Heart Study (Contract No. N01-HC-25195 & HHSN268201500001I) and its contract with Affymetrix, Inc for genotyping services (Contract No. N02-HL-6-4278). Dr. McKeown is partially supported by the USDA Agricultural Research Service (agreement 58-1950-0-014).	Dawber TR, Kannel WB, Lyell LP. An approach to longitudinal studies in a community: the Framingham Study. Ann N Y Acad Sci. 1963;107:539-556. PMID 14025561 Feinleib m, Kannel WB, Garrison RJ, McNamara PM, Castelli WP. The Framingham Offspring Study. Design and preliminary data. Prev Med. 1975;4:518-525. PMID 1208363 Splansky GL, Corey D, Yang Q et al. The Third Generation Cohort of the National Heart, Lung, and Blood Institute's Framingham Heart Study: design, recruitment, and initial examination. Am J Epidemiol. 2007;165:1328-1335. PMID 17372189

Cohort	Study Description and Acknowledgements	Relevant References
Multi-Ethnic Study of Atherosclerosis (MESA) **USA**	The Multi-Ethnic Study of Atherosclerosis (MESA) is a study of the characteristics of subclinical cardiovascular disease (disease detected non-invasively before it has produced clinical signs and symptoms) and the risk factors that predict progression to clinically overt cardiovascular disease or progression of the subclinical disease. MESA researchers study a diverse, population-based sample of 6,814 asymptomatic men and women aged 45-84 (38 percent of the recruited participants are white, 28 percent African-American, 22 percent Hispanic, and 12 percent Asian, predominantly of Chinese descent), as well as 2,128 additional individuals from 594 families recruited through MESA Family by utilizing the existing MESA framework, yielding 3,026 sibpairs divided between African Americans and Hispanic-Americans. Participants were recruited from six field centers across the United States: Wake Forest University, Columbia University, Johns Hopkins University, University of Minnesota, Northwestern University and University of California - Los Angeles. The authors thank the participants of the MESA study, the Coordinating Center, MESA investigators, and study staff for their valuable contributions. A full list of participating MESA investigators and institutions can be found at http://www.mesa-nhlbi.org. MESA and the MESA SNP Health Association Resource (SHARe) project are conducted and supported by contracts N01-HC-95159 through N01-HC- 95169 and RR-024156 from the National Heart, Lung, and Blood Institute. Funding for MESA SHARe genotyping was provided by National Heart, Lung, and Blood Institute [Contract N02-HL-6-4278]. MESA Family is conducted and supported in collaboration with MESA investigators; support is provided by National Heart, Lung, and Blood Institute grants and contracts [grant numbers R01HL071051, R01HL071205, R01HL071250, R01HL071251, R01HL071252, R01HL071258, R01HL071259]. [representing authors: ACW, ATC, LS and SSR]	Bild DE, et al. Am. J. Epidemiol. 156 (9): 871-881.2002
Netherlands Epidemiology in Obesity (NEO) Study **The Netherlands**	The Netherlands Epidemiology of Obesity (NEO) study: The NEO was designed for extensive phenotyping to investigate pathways that lead to obesity-related diseases. The NEO study is a population-based, prospective cohort study that includes 6,671 individuals aged 45–65 years, with an oversampling of individuals with overweight or obesity. At baseline, information on demography, lifestyle, and medical history have been collected by questionnaires. In addition, samples of 24-h urine, fasting and postprandial blood plasma and serum, and DNA were collected. Genotyping was performed using the Illumina HumanCoreExome chip, which was subsequently imputed to the 1000 genome reference panel. Participants underwent an extensive physical examination, including anthropometry, electrocardiography, spirometry, and measurement of the carotid artery intima-media thickness by ultrasonography. In random subsamples of participants, magnetic resonance imaging of abdominal fat, pulse wave velocity of the aorta, heart, and brain, magnetic resonance spectroscopy of the liver, indirect calorimetry, dual energy X-ray absorptiometry, or accelerometry measurements were performed. The collection of data started in September 2008 and completed at the end of September 2012. Participants are currently being followed for the incidence of obesity-related diseases and mortality. The authors of the NEO study thank all individuals who participated in the Netherlands Epidemiology in Obesity study, all participating general practitioners for inviting eligible participants and all research nurses for collection of the data. We thank the NEO study group, Pat van Beelen, Petra Noordijk and Ingeborg de Jonge for the coordination, lab and data management of the NEO study. The genotyping in the NEO study was supported by the Centre National de Génotypage (Paris, France), headed by Jean-Francois Deleuze. The NEO study is supported by the participating Departments, the Division and the Board of Directors of the Leiden University Medical Center, and by the Leiden University, Research Profile Area Vascular and Regenerative Medicine. Dennis Mook-Kanamori is supported by Dutch Science Organization (ZonMW-VENI Grant 916.14.023).	de Mutsert R et al. The Netherlands Epidemiology of Obesity (NEO) study: study design and data collection. Eur J Epidemiol. 2013 Jun;28(6):513-23.

Cohort	Study Description and Acknowledgements	Relevant References
Western Australian Pregnancy Cohort (Raine) Study **Australia**	The Western Australian Pregnancy Cohort (Raine) Study is a prospective pregnancy cohort where 2900 were recruited from King Edward Memorial Hospital between 1989 and 1991. Data were collected throughout pregnancy and the children have been followed-up at ages 1, 2, 3, 5, 8, 10, 14, 17, 18, 20 and 22. Ethnics approval for this study was obtained from King Edward Memorial Hospital and Princess Margaret Hospital. Participants were consented to being involved in this study prior to each follow-up. The Raine Study acknowledges the National Health and Medical Research Council (NHMRC) for their long term contribution to funding the study over the last 29 years. Core Management of the Raine study has been funded by the University of Western Australia (UWA), Curtin University, the UWA Faculty of Medicine, Dentistry and Health Sciences, the Raine Medical Research Foundation, the Telethon Kids Institute, the Women's and Infants Research Foundation, Edith Cowan University, Murdoch University, and the University of Notre Dame. This study was supported by the National Health and Medical Research Council of Australia [grant numbers 572613, 403981 and 003209] and the Canadian Institutes of Health Research [grant number MOP-82893]. The authors gratefully acknowledge the assistance of the Western Australian DNA Bank (National Health and Medical Research Council of Australia National Enabling Facility). All analytic work was supported by resources provided by the Pawsey Supercomputing Centre with funding from the Australian Government and the Government of Western Australia.	Newnham JP, Evans SF, Michael CA, Stanley FJ, Landau LL. Effects of frequent ultrasound during pregnancy: a randomised controlled trial. *Lancet* 1993;342(8876):887-91.
Rotterdam Study I & II (RS1 & RS2) **The Netherlands**	The Rotterdam Study is a prospective population-based cohort study in Ommoord, a suburb of Rotterdam, designed to investigate the prevalence and incidence of and risk factors for chronic diseases in the elderly. The baseline exam of the first cohort (RS-I) was conducted between 1990 and 1993. A total of 7,983 adults, aged 55 years and over, participated in the study. In 2000, the study was extended with a second cohort (RS-II) of 3,011 participants who had moved into the area or who had become 55 years since the start of the study. For the current analysis, 5,784 adults were eligible as they had available data on DNA, dietary intake and outcome information, and consent to share genetic data.. The Rotterdam Study is supported by the Erasmus MC University Medical Center and Erasmus University Rotterdam; The Netherlands Organisation for Scientific Research (NWO); The Netherlands Organisation for Health Research and Development (ZonMw); the Research Institute for Diseases in the Elderly (RIDE); The Netherlands Genomics Initiative (NGI); the Ministry of Education, Culture and Science; the Ministry of Health, Welfare and Sports; the European Commission (DG XII); and the Municipality of Rotterdam. The contribution of inhabitants, general practitioners and pharmacists of the Ommoord district to the Rotterdam Study is gratefully acknowledged. ETML, JCK-dJ, TV and OHF work in ErasmusAGE, a center for aging research across the life course funded by Nestlé Nutrition (Nestec Ltd.), Metagenics Inc. and AXA. Nestlé Nutrition (Nestec Ltd.), Metagenics Inc. and AXA had no role in design and conduct of the study, collection, management, analysis, and interpretation of the data; and preparation, review or approval of the manuscript.	Hofman et al. Eur J Epidemiol. 2015;30(8):661-708
Women’s Genome Health Study (WGHS) **United States**	The Women’s Genome Health Study (WGHS) is a prospective cohort of initially healthy, female North American health care professionals at least 45 years old at baseline representing participants in the Women’s Health Study (WHS) who provided a blood sample at baseline and consent for blood-based analyses. The WHS was a 2x2 trial beginning in 1992-1994 of vitamin E and low dose aspirin in prevention of cancer and cardiovascular disease with about 10 years of follow-up. Since the end of the trial, follow-up has continued in observational mode. Additional information related to health and lifestyle were collected by questionnaire throughout the WHS trial and continuing observational follow-up. The WGHS is supported by the National Heart, Lung, and Blood Institute (HL043851 and HL080467) and the National Cancer Institute (CA047988 and UM1CA182913), the Donald W. Reynolds Foundation, and the Fondation Leducq, with collaborative scientific support and funding for genotyping provided by Amgen.	Ridker PM, Chasman DI, Zee RY, Parker A, Rose L, Cook NR, Buring JE; Women's Genome Health Study Working Group. Rationale, design, and methodology of the Women's Genome Health Study: a genome-wide association study of more than 25,000 initially healthy American women. Clin Chem. 2008 Feb;54(2):249-55. Epub 2007 Dec 10. PubMed PMID: 18070814.

Cohort	Study Description and Acknowledgements	Relevant References
Women's Health Initiative (WHI) **United States**	WHIMS randomized trials which examined the effects of postmenopausal hormone therapy on cognitive function in women agead 65-80 years. Recruitment began in 1995.. The WHI program is supported by contracts from the National Heart, Lung and Blood Institute, NIH. The authors thank the WHI investigators and staff for their dedication, and the study participants for making the program possible. A listing of WHI investigators can be found at http://www.whi.org/researchers/Documents%20%20Write%20a%20Paper/WHI%20Investigator %20Short%20List.pdf. The WHI program is funded by the National Heart, Lung, and Blood Institute, National Institutes of Health, U.S. Department of Health and Human Services through contracts HHSN268201600018C, HHSN268201600001C, HHSN268201600002C, HHSN268201600003C, and HHSN268201600004C.	Prentice R, Rossouw J, Furberg C, Johnson S, Henderson M, Cummings S, Manson J, Freedman L, Oberman A, Kuller L, Anderson G. Design of the WHI Clinical Trial and Observational Study. Control Clin Trials 1998;19:61-109.
Cardiovascular Risk in Young Finns Study (YFS) **Finland**	The Cardiovascular Risk in Young Finns (YFS) is a population-based 27 year follow up-study. The first cross-sectional survey was conducted in 1980, when 3,596 Caucasian subjects aged 3-18 years participated. In adulthood, the latest 27-year follow-up study was conducted in 2007 (ages 30-45 years) with 2,204 participants. The study cohort for the present analysis comprised subjects who had participated in the study in 2007 and had validated dietary data from FFQ, available genotype and other risk factor data (Raitakari OT et al. Cohort profile. Int. J Epidemiol. 2008;37:1220-6). The dietary intake of nutrients was assessed using a modified 131-item food frequency questionnaire developed by the Finnish National Institute for Health and Welfare (Paalanen et al. 2006). The study was approved by the local Ethical Committees and was performed according to Helsinki declaration.A total of 3,196 participants with available DNA and who provided complete dietary information were eligible for the current study. The Young Finns Study has been financially supported by the Academy of Finland: grants 286284, 134309 (Eye), 126925, 121584, 124282, 129378 (Salve), 117787 (Gendi), and 41071 (Skidi); the Social Insurance Institution of Finland; Competitive State Research Financing of the Expert Responsibility area of Kuopio, Tampere and Turku University Hospitals (grant X51001); Juho Vainio Foundation; Paavo Nurmi Foundation; Finnish Foundation for Cardiovascular Research ; Finnish Cultural Foundation; Tampere Tuberculosis Foundation; Emil Aaltonen Foundation; Yrjö Jahnsson Foundation; Signe and Ane Gyllenberg Foundation; and Diabetes Research Foundation of Finnish Diabetes Association.	Raitakari OT et al. Cohort profile. Int. J Epidemiol. 2008;37:1220-6.

Table 6.2. Dietary assessment methods of participating CHARGE cohorts.

Cohort	Dietary Assessment Method	Nutrient Database	Sugar-Sweetened Beverages	Fruit Intake	Vegetable Intake	Nuts/ Seeds Intake	Whole Grain Intake	Fish Intake	% Energy Saturated Fat	Relevant References
ARIC	66-item, interviewer-administered, modified Willett FFQ	Harvard	Regular soda/ fruit-flavored drink, quantified as servings/d	Apples or pears/ bananas/ peaches, apricots, plums/ oranges/ other fruit	Broccoli/ string beans, green beans/ cauliflower, cabbage, Brussels sprouts/ spinach, collards, other greens/ corn/ tomatoes (potatoes not included)	peanuts, almonds, peanut butter, cashews, sesame seeds, walnuts	Cooked cereals/ whole grain cold cereal/ dark or whole grain bread	Canned tuna fish/ dark meat fish/ other light meat fish	As a percent of total energy, where 1 g saturated fat has 9 kcal.	Willett WC, *et al. Am J Epidemiol.* 1985; 122(1):51–65. Stevens J, *et al. Nutrition Research* 1996;16:735–745.
CHS	99-item, self-administered, picture-sort version of National Cancer Institute FFQ	Harvard	Tang, Regular soft drinks	apples, applesauce, pears/bananas/peaches, apricots, nectarines (canned, frozen, whole year/in season), cantaloupe/watermelon/strawberries/oranges/grapefruit/any other fruit.	string beans, green beans/peas/corn/winter squash, baked squash/tomatoes, tomato juice/broccoli/cauliflower, brussel sprouts/spinach (raw/cooked)/mustard greens, turnip greens, collards/cole slaw, cabbage, sauerkraut/carrots, mixed vegetables containing carrot/green salad/sweet potatoes, yams/other veg including onions, summer squash	peanuts	dark bread (whole wheat, rye, pumpernickel)/ cooked cereals/high fiber, bran or granola cereals.	tuna fish, tuna salad, tuna casserole/other fish, broiled, baked	As a percent of total energy	Kumanyika S, et al. J Am Diet Assoc. 1996;96(2):137–144.

Cohort	Dietary Assessment Method	Nutrient Database	Sugar-Sweetened Beverages	Fruit Intake	Vegetable Intake	Nuts/ Seeds Intake	Whole Grain Intake	Fish Intake	% Energy Saturated Fat	Relevant References
Fenland	160 -item, self-administered, modified EPIC-Norfolk FFQ	FETA	Fizzy soft drinks, eg. Coca cola, lemonade (glass) + Fruit squash or cordial (glass)	apples/pears/oranges satsumas mandarins/grapefruits/bananas/grapes/melon/peaches plums apricots/strawberries raspberries kiwi fruit/tinned fruit/dried fruit eg raisins prunes	carrots/spinach/broccoli spring greens kale/brussels sprouts/cabbage/peas/green beans broad beans runner beans/marrow courgettes/cauliflower/parsnips turnips swedes/leeks/onions/garlic/mushrooms/sweet peppers/beansprouts/green salad lettuce cucumber celery/watercress/tomatoes/sweetcorn/beetroot/coleslaw/avocado/baked beans/driedLnetils/tofu	peanuts/ peanut butter	Brown bread and rolls/wholemeal bread and rolls/brown rice/wholemeal pasta/porridge/breakfast creals	fried fish in batter as in fish and chips/fish fingers fish cakes/other white fish fresh or rozen eg. Cod haddock plaice sole halibut/oily fish fresh or canned eg mackerel kippers tuna salmon sardines herring/shellfish eg crab prawns mussels/fish roe taramasalata	As a percent of total energy, where 1 g saturated fat has 9 kcal.	Bingham et al. Br J Nutr. 1994;72(4):619–43 Bingham et al. Int J Epidemiol. 1997;26(suppl_1):S137-151.
FHS	126-item, semi-quantitative FFQ	USDA	Caffeinated colas, caffeine-free colas, carbonated noncola soft drinks, and noncarbonated sugar-sweetened beverages (fruit punches, lemonades, or other fruit drinks).	Raisins, prunes, bananas, cantaloupe, watermelon, apples, pears, oranges, grapefruit, fruit juices, strawberries, blueberries, peaches	Tomatoes, tomato juice, red chili sauce, tofu, soybeans, string beans, broccoli, cabbage, coleslaw, cauliflower, Brussels sprouts, carrots, corn, peas, lima beans, mixed vegetables, beans/lentils, squashes, potatoes, spinach, kale/mustard/chard, lettuce, celery, beets, alfalfa, garlic	nuts, peanut butter	Quantified as g/day (ready to eat breakfast cereal, cooked oatmeal, dark bread, brown rice, other grains (i.e., bulgur, kasha, couscous), popcorn, bran, wheat germ, other (fill-in) whole grain foods)	Canned tuna fish; dark meat fish; other fish; shrimp, lobster, scallops as main dish	As a percent of total energy, where 1 g saturated fat has 9 kcal.	Rimm et al. Am J Epidemiol 1992;135:1114–26, 1127–36.

Cohort	Dietary Assessment Method	Nutrient Database	Sugar-Sweetened Beverages	Fruit Intake	Vegetable Intake	Nuts/ Seeds Intake	Whole Grain Intake	Fish Intake	% Energy Saturated Fat	Relevant References
MESA	120-item, self-administered, modified-Block FFQ	NDSI	Coke, Pepsi, 7-up or other carbonated (not diet) + other juice (not fruit) quantified as servings / d	Peaches, apricots, nectarines, plums / cantaloupe mango, papaya / strawberries, blueberries, other berries / apples, applesauce, pears / bananas, plantains / oranges, grapefruit, tangerines, kiwi / any other fruit	Tomato / cruciferous veg / yellow veg / green leafy veg / other veg	serv/day	Whole grain breakfast cereal; oatmeal; dark bread; bran muffins; brown or wild rice	Weighted amounts defined from Tuna, salmon, sardines; including sashimi or sushi / Other broiled, steamed, baked or raw fish– trout, sole, halibut, poke, grouper /tuna / boiled fish	As a percent of total energy, where 1 g saturated fat has 9 kcal	Nettleton JA, et al. Am J Clin Nutr 2006;83:1369–79. Norris J et al Am J Public Health 1997;87:740–6. Mayer-Davis EJ, et al Ann Epidemiol 1999;9:314–24.
NEO	semi-quantitative food frequency questionnaire, originally validated in the Dutch general population	Dutch food composition table (NEVO) (version 2011)	regular soda/ fruit-flavored drink, quantified as servings/d	apples or pears/ bananas/ peaches, apricots, plums/ oranges/ other fruit	Cooked, baked and raw vegetables	peanuts, almonds, peanut butter, cashews, sesame seeds, walnuts	N/A	canned tuna fish/ dark meat fish/ other light meat fish	As a percent of total energy, where 1 g saturated fat has 9 kcal	(Verkleij-Hagoort AC, *et al. Eur J Clin Nutr* 2007;61:610-5.)
Raine	self-adminitered, 74-item FFQ designed to measure dietary intake over the past 12 months	Australian NUTTAB 1995 food composition database (Cancer Council Victoria Australia)	Soft drink, regular energy drinks	oranges, apples, pears, bananas, melon, pineapples, strawberries, apricots, peaches, mangoes, avocado	tomatoes, pumpkin, zucchini, cucumber, capsicum, lettuce, celery, beetroot, carrots, cabbage, cauliflower, broccoli, spinach, peas, green beans, bean sprouts, onion, garlic	mixed nut, peanut butter	Ryebread, multigrain bread, All bran, bran flakes, porridge, muesli.	grilled fish, fried fish, tinned fish	As a percent of total energy, where 1 g saturated fat has 9 kcal	

Cohort	Dietary Assessment Method	Nutrient Database	Sugar-Sweetened Beverages	Fruit Intake	Vegetable Intake	Nuts/ Seeds Intake	Whole Grain Intake	Fish Intake	% Energy Saturated Fat	Relevant References
RS1	170-item semi-quantitative food frequency questionnaire	Dutch Food Composition Table 1993 (NEVO 1993).	Soft drink	Apple without skin / Strawberries / Banana / Grapes white/black with skin / Grapefruit / Tangerines / Pear without skin / Plums with skin / Orange / Apple sauce canned / Fruit cocktail in syrup canned / Kiwi fruit	Endive boiled / Cauliflower boiled / Kale curly boiled / Mushrooms boiled / Cabbage green boiled / Cucumber raw / Cucumber boiled / Leek boiled / Rhubarb raw / Cabbage red boiled / Cabbage Savoy boiled / Lettuce raw / Spinach boiled / Cabbage oxhair boiled / Brussel sprouts boiled / Tomatoes raw / Onions boiled / Chicory raw / Chicory boiled / Cabbage white cooked / Carrots raw / Carrots boiled / Cabbage sauerkraut cooked / Vegetables mixture raw / Gherkins sweet pickled / Sweet pepper red boiled without salt / Broccoli boiled / Beetroot boiled	serv/day	Wheat germ / Bread brown wheat / Rye bread dark / Rye bread light / Bread brown wholemeal / Porridge oatmeal / Wheat bran / Muesli without sugar / Rice brown boiled	Herring salted / Mackerel raw / Sardines/pilchards in oil canned / Salmon canned / Eel smoked / Plaice raw / Fish fingers uncooked / Cod raw / Herring marinated	As a percent of total energy, where 1 g saturated fat has 9 kcal	Klipstein-Grobusch et al. Eur J Clin Nutr. 1998; 52(8):588-96
RS2	389-item semi-quantitative food frequency questionnaire	Dutch Food Composition Table 2011 (NEVO 2011).	Soft drinks	Apple / Strawberries / Banana / Grapes / Grapefruit / Tangerines / Pear / Plums / Orange / Kiwi fruit / Other fruit	Cauliflower / Kale / Cabbage / Cucumber / Leek / Lettuce / Spinach / Brussel sprouts / Tomatoes / Onions / Broccoli / Peppers / Carrots / Other vegetables	serv/day	Whole-wheat crackers / Wheat germ / Wheat bread / Rye bread / Whole-wheat bread / Oatmeal / Whole-wheat cereal / Muesli without sugar / Whole-grain rice	Herring / Mackerel / Sardines/pilchards in oil canned / Salmon / Eel / Trout / Flounder / Fish fingers / Other fish	As a percent of total energy, where 1 g saturated fat has 9 kcal.	Goldbohm RA, et al. Eur J Clin Nutr 1994; 48(4):253-65 Feunekes GI, et al. Am J Clin Nutr 1993; 58(4):489-96

Cohort	Dietary Assessment Method	Nutrient Database	Sugar-Sweetened Beverages	Fruit Intake	Vegetable Intake	Nuts/ Seeds Intake	Whole Grain Intake	Fish Intake	% Energy Saturated Fat	Relevant References
WGHS	126-item, semi-quantitative FFQ	Harvard	Caffeinated colas, caffeine-free colas, carbonated noncola soft drinks, and noncarbonated sugar-sweetened beverages (fruit punches, lemonades, or other fruit drinks).	apples, apple juice, avocados, bananas, blueberries, cantaloupes, grapefruit, grapefruit juice, oranges, orange juice, peaches, prunes, raisins, strawbetties, other juice	beans, beets, broccoli, brussels sprouts, cabbage, cooked & raw carrots, cauliflower, celery, red chili sauce, corn, eggplant, green pepper, iceberg lettuce, kale, mixed vegetables, cooked & raw onions, dark orange squash, peas, romaine lettuce, cooked & raw spinach, string beans, tofu, tomatoes, tomato juice, tomato sauce, yams	peanuts, peanut butter, other nuts	whole grain cold cereal, dark bread, popcorn, oatmeal, wheat germ, brown rice, oat bran, other grain	dark meat fish, canned tuna fish, other fish, shrimp, lobster, scallops as main dish	As a percent of total energy, where 1 g saturated fat has 9 kcal.	Rimm et al. Am J Epidemiol 1992;135:1114–26, 1127–36.
WHI			Tang/Kool-Aid/Hi-C/other fruit drinks,Regular soft drinks (not diet)	serv/day	serv/day	serv/day	serv/day	serv/day	As a percent of total energy, where 1 g saturated fat has 9 kcal.	
YFS	131-item FFQ	Finnish food composition database Fineli, maintained by the Nutrition Unit, National Institute of Health and Welfare, Finland.	Sugar-sweetened soda/ sugar-sweetened cola drinks/ sugar-sweetened fruit or berry drinks	Grams per day (approximate conversions used for presentation in Table 1)	Grams per day (approximate conversions used for presentation in Table 1)	Grams per day (approximate conversions used for presentation in Table 1)	Servings per day	Grams per day (approximate conversions used for presentation in Table 1)	As a percent of total energy, where 1 g saturated fat has 9 kcal	Paalanen L, et al. J Clin Epid. 2006;59(9):994 –1001.

Table 6.3. Genotyping information of participating CHARGE cohorts.

Cohort	Genotyping Array & Calling Algorithm	Sample QC	SNP Inclusion Criteria	Phasing & Imputation	Correction for Ancestry/ Relatedness
ARIC	Affymetrix 6.0	>95% call rate	≥ 90% call rate; Hardy-Weinberg equilibrium $<10^{-6}$	IMPUTE2; HRC r1.1 2016 on the Michigan imupation server	Principal Components
CHS	Illumina CNV370 merged with ITMAT-Broad-CARe Illumina iSELECT chip; Illumina BeadStudio	>95% call rate; Individuals additionally excluded for: Presence at study baseline of coronary heart disease, congestive heart failure, peripheral vascular disease, valvular heart disease, stroke or transient ischemic attack; lack of available DNA; genotype discordant with known sex or prior genotyping. SNPs additionally excluded for >2 duplicate errors or Mendelian inconsistencies, or heterozygote frequency = 0.	≥ 97% call rate; Hardy-Weinberg equilibrium $<10^{-5}$	SHAPE-IT; HRC r1.1 2016 on the Michigan imputation server	None
Fenland	Affymetrix Axiom UKBiobank	≥95% call rate; >0.19 and <0.21 calculated on SNPs with MAF > 1%, >0.004 and <0.0125 calculated on SNPs with MAF < 1%; Channel contrast (DishQC <0.82), sex discrepancy, unusually high number of singleton genotypes, impossible IBD values	>0% MAF; ≥ 95% call rate; Hardy-Weinberg equilibrium $<10^{-6}$		
	Illumina Metabochip	≥95% call rate (for including individuals) and sex mismatch	>0% MAF; ≥ 90% call rate; Hardy-Weinberg equilibrium $<10^{-6}$	HRC + UK10K + 1000G phase 3 (IMPUTE, http://mathgen.stats.ox.ac.uk/impute/impute.html)	Kinship matrix
	Infinium Core Exome 24 v1	≥98% call rate; Sex call based on X heterozygosity consistent with self-reported sex; ethnically aware rare variant heterozogosity (SNPs with MAF <=1%) Heterozygosity > 0.005; Ethnically aware common variant heterozogosity (SNPs with MAF >1%) Heterozygosit >0.363 or <0.336; Ethnically aware rare allele count outliers: > 60 singleton calls, >60 doubleton calls or > 60 tripleton calls; Ethnic outliers with inconsistent heterozygosity patterns; Duplicates: removed sample with lower call rate where both passed	≥ 95% call rate; Hardy-Weinberg equilibrium $<10^{-6}$;		
FHS	Affymetrix 500K (250K Nsp and 250K Sty) and MIPS 50K	Sample call rate >95%; Number of Mendelian errors greater or equal to 1000	≥0.01% MAF; ≥ 90% call rate; Hardy-Weinberg equilibrium $<10^{-6}$	ShapeIT v2.r837; HRC r1.1 2016 on the Michigan imputation server	Kinship Matrix
MESA	Affymetrix Genome-Wide Human 6.0 array	Sample call rate >95%;	>97% call rate; Hardy-Weinberg equilibrium $<10^{-6}$	HRC r1.1 2016: Michigan Imputation server	Principal Components
NEO	Illumina HumanCoreExome-24v1; Illumina GenomeStudio	1) call rate <98%; 2) PCA outliers; 3) sex mismatch; 4) heterozygosity +/-3SD from mean of ancestry distribution; 5) first degree relatedness;	>0% MAF; ≥95% call rate; Hardy-Weinberg equilibrium $<10^{-6}$	Impute version 2.2 (ftp://ftp.1000genomes.ebi.ac.uk/vol1/ftp/release/20110521/)	Principal Components

Raine	Illumina Human660W Quad BeadChip; GenomeStudio	1) call rate >97%; 2) duplicates and plate controls; 3) gender discrepency when compared to core data; 4) cryptic relatedness (IBD), pi > 0.1875, 4) heterozygosity, h>0.32	≥ 95% if MAF ≥ 5%, ≥ 99% if MAF < 5%; Hardy-Weinberg equilibrium $<10^{-6}$	ShapeIT v2.r790; 1000G Phase 3 v5, HRC r1.1 2016: Michigan Imputation server	Principal Components
RS	Array type Illumina 550K	≥ 97.5% call rate	≥ 95% call rate; Hardy-Weinberg equilibrium $<10^{-6}$	SHAPEIT2; HRC r1.1 2016 on the Michigan imputation server	None
WGHS	Illumina HumanHap300 Duo & HumanHap300 Duo and iSelect chips with the Infinium II protocol; Illumina BeadStudio	Sample call rate >95%	≥ 1% MAF; ≥ 90% call rate; Hardy-Weinberg equilibrium $<10^{-6}$	HRC r1.1 2016 on the Michigan imputation server	Principal Components
WHI	1000 genomes; Illumina, Affymetrix, HumanOmni	Sample call rate >95%	>0.5% MAF; ≥ 90% call rate; Hardy-Weinberg equilibrium $<10^{-4}$	Beagle/MACH	None
YFS	Illumina 670K; Illuminus clustering algorithm	1) call rate ≥95%; Sanger genotyping pipeline QC: heterozygosity, call rate, duplicate/related and Sequenom fingerprint discrepancy. Sex status match genotyping sex. Cryptic relatedness: pi-hat<0.2	≥ 1% MAF; ≥ 95% call rate; Hardy-Weinberg equilibrium $<10^{-6}$	SHAPEIT v1; IMPUTE v2.2.2	Principal Components

Table 6.4. Assessment of additional characteristics of participating CHARGE cohorts.

Cohort	Education	Smoking Status	Physical Activity	Alcohol Intake	Relevant References
ARIC	Categorized into 6 groups: grade school or none, some high school, high school graduate, vocational school, college, graduate/ professional school	Classified as current, former, never smoker or missing/ unknown; current smokers given in descriptive	Assessed as both sport and leisure time using the Baecke questionnaire. A sports activity score and a leisure activity score ranged from low to high (five quintile categories).	grams/day	
CHS	Classified as having completed high school or GED based on self-report	Classified as current, former, never smoker by self report	Assessed as kcal expended in physical activities excluding household chores based on self-report of specific activities, used as a continuous variable.	drinks/day converted to grams/day by multiplying by 14.	
Fenland	Participants were asked to confirm 12 types of British educational certificates. If they had a school leaving certificate or a GCE AS level, AS level or higher, they were classified as having an educatino level of high school or above.	Classified as current, former, and never smoker, according to questions about current smoking and smoking history	Physical activity was objectively assessed over 6 days using a combined heart rate and movement sensor (Actiheart, CamNTech, Cambridge, UK), with individual calibration of heart rate performed using a treadmill test. Data from free-living was pre-processed and modelled using a branched equation framework to estimate intensity time-series, which were summarised over time as daily Physical Activity Energy Expendi- ture (PAEE) (kJ/kg/d). Then, kj/d was computed by PAEE times a body weight for each individual.	grams/day	Brage et al. PLoS One 2015;10(9), e0137206.
FHS	Categorized into 4 groups: less than high school, graduated high school, some college, or graduated college	Regular cigarette smoking in the last year: classified as yes (current) or no (never)	Physical activity score taking into account typical number of hours sleeping, sitting, slight activity, moderate activity, and heavy activity	grams/day	Kannel WB, Sorlie P Arch Intern Med. 1979;139:857–61.
MESA	Measured as 'highest level achieved' for 9 categories: no school, grades 1-8, grades 9-11, completed high school / GED, some college but no degree, technical school certificate, associate's degree, Bachelor's degree, graduate school	Classified as current, former, never smoker or missing/ unknown	Sport and leisure time as METs / week	grams/day	
NEO	Classified as having completed high school based on self-report	Classified as current, former, never smoker or missing/ unknown	Assessed using the SQUASH questionnaire. For the current analyses, MET-hours were calculated for sports/leisure activities		

Cohort	Education	Smoking Status	Physical Activity	Alcohol Intake	Relevant References
Raine	Classified as having completed high school based on self-report	Classified as current, never smoker or missing/ unknown	Self-reported using 'The International Physical Activity Questionnaire'. METMINperWeek calculated for activities of different intensities - Vigorous, moderate, walking.	Expressed as precentage of total daily energy intake	
RS	2 Categories: 1) primary education, lower vocational training or lower general education, 2) intermediate vocational training, higher general education, higher vocational training, college, or university.	Classified as current, former, never smoker	NA	grams/day	
WGHS		three category smoking status variable (never/past/current)	Total energy expended from recreational physical activity (MET-hours/week)	grams/day	
WHI	Classified as having completed high school based on self-report	three category smoking status variable (never/past/current)	Total energy expended from recreational physical activity (MET-hours/week)	alcohol from beer, wine, and liquor, as well as from foods (some foods contain alcohol due to minute amounts of alcohol in vanilla extract, almond extract etc used in baking)	
YFS	Continuous, years of total education	Classified as current, former, never smoker	Continuous: MET-exercise index, which refers to energy consumption of 1 kcal per hour, per 1kg of person weight.	grams/day	

Table 6.5. General characteristics of participating CHARGE cohorts.[a]

	Raine	ARIC	FHS	NEO	Fenland	YFS	WGHS	WHI	MESA	CHS	RS
Characteristics											
n	617	10,924	6,382	5,694	10,022	1,782	16,284	1,109	1,805	3,196	5,784
Age (years)	20 (1)	55 (6)	49 (14)	56 (6)	49 (7)	38 (5)	55 (7)	65 (7)	70 (10)	72 (5)	66 (8)
Sex (% women)	52.4	52.7	54.3	52.0	53.3	55.9	100	100	51.2	61.0	57.8
BMI (kg/m^2)	24.5 (5.2)	27.0 (4.8)	27.4 (5.5)	30.0 (4.8)	26.9 (4.8)	25.9 (4.6)	25.9 (4.9)	28.6 (5.7)	28.0 (5.3)	26.3 (4.4)	26.5 (3.7)
Current Smoker (%)	13.5	24.2	13.4	16.0	12.0	27.6	11.7	10.1	7.0	11.4	23.4
Completed High School (%)	81.5	84.9	98.0	93.0	81.8	75.4	100	94.7	96.5	75.1	60.8
Fasting HDL-C (mg/dl)	51 (13)	51 (17)	54 (17)	55 (16)	59 (16)	52 (13)	54 (15)	58 (15)	57 (18)	55 (16)	53 (14)
Fasting TG (mg/dl)	85 (2)	137 (90)	117 (87)	130 (85)	106 (81)	122 (82)	119 (89)	156 (92)	107 (59)	140 (76)	137 (71.0)
Dietary Intakes											
SSB intake (servings/d)	0.7 (1.0)	0.5 (0.9)	0.4 (0.8)	0.4 (0.8)	0.3 (0.6)	0.3 (0.5)	0.3 (0.6)	0.2 (0.6)	0.1 (0.5)	0.1 (0.3)	0.1 (0.2)
<1 serving/month (%)	13.6	35.7	33.9	49.4	35.8	23.6	44.8	58.0	70.0	63.4	71.9
1-4 serving/month (%)	14.4	16.3	24.3	13.8	24.6	31.9	22.0	19.3	12.4	16.9	13.5
1-2 serving/week (%)	23.8	12.1	9.76	14.1	14.0	17.1	13.1	3.5	2.2	0.06	6.4
3-7 serving/week (%)	29.2	25.7	21.3	11.7	15.2	21.0	15.1	15.3	8.6	18.7	7.5
>1 serving/day (%)	19.0	10.3	10.8	11.0	10.4	6.3	5.0	3.9	2.3	0.9	0.8
Energy Intake (kcal/d)	1,857 (850)	1,644 (599)	1,956 (645)	2,291 (763)	1,935 (578)	2,381 (762)	1,732 (524)	1,698 (670)	1708 (734)	2,024 (654)	2,046 (1,409)
SFA (% total energy)	16.1 (3.1)	12.2 (3.1)	11.1 (2.9)	12.4 (2.9)	12.5 (3.0)	11.8 (2.4)	10.2 (2.5)	11.6 (3.3)	11.3 (3.3)	10.4 (2.2)	14.4 (3.1)
Fruit intake (servings/d)	1.9 (1.3)	1.5 (1.3)	1.1 (1.0)	1.1 (0.9)	2.7 (2.2)	3.4 (3.1)	1.9 (1.2)	1.8 (1.2)	2.1 (1.7)	2.7 (1.5)	1.2 (1.0)
Vegetable Intake (servings/d)	1.7 (0.9)	1.7 (1.2)	2.0 (1.1)	2.8 (1.5)	5.0 (2.5)	1.4 (1.8)	3.9 (2.3)	2.2 (1.3)	2.4 (1.5)	2.8 (1.5)	2.8 (2.1)
Whole Grain Intake (servings/d)	0.8 (1.0)	1.1 (1.1)	1.2 (1.2)	NA	1.8 (1.4)	3.2 (1.9)	1.5 (1.2)	1.2 (0.8)	1.0 (0.8)	1.0 (0.7)	3.4 (2.9)
Fish Intake (servings/d)	0.4 (0.6)	0.3 (0.3)	0.4 (0.4)	0.2 (0.2)	0.4 (0.3)	1.3 (0.9)	0.3 (0.2)	0.2 (0.2)	0.3 (0.3)	0.3 (0.3)	0.1 (0.2)
Nuts/Seeds Intake (servings/d)	0.1 (0.2)	0.4 (0.6)	0.6 (0.9)	0.8 (1.0)	0.2 (0.3)	0.1 (0.1)	0.3 (0.4)	0.2 (0.3)	0.5 (0.6)	0.2 (0.3)	0.2 (2.1)
Alcohol Intake (g/d)	7.8 (8.9)[b]	6.7 (13.5)	10.5 (14.8)	15.5 (17.4)	9.5 (12.7)	8.6 (13.4)	4.3 (8.5)	5.0 (10.2)	8.8 (15.5)	5.5 (12.9)	11.1 (15.5)

[a] Data are means (standard deviation) or percentage for maximum observations available for SSB intake and lipid outcomes. Sample sizes may vary depending on availability of genotype and covariate information. Cohorts are ordered by estimate of sugar-sweetened beverage intake. Cohort study name (study acronym) (country): Western Australian Birth Cohort Study (Raine) (Australia), Atherosclerosis Risk in Communities Study (ARIC) (USA), Framingham Heart Study (FHS) (USA), Netherlands Epidemiology in Obesity Study (NEO) (The Netherlands), The Fenland Study (Fenland) (United Kingdom), Young Finns Study (YFS) (Finland), Women's Genome Health Study (WGHS) (USA), Women's Health Initiative (WHI) (USA), Multi-Ethnic Study of Atherosclerosis (MESA) (USA), Cardiovascular Health Study (CHS), and the Rotterdam Study (RS) (The Netherlands).

[b] Expressed as % total energy intake

Abbreviations: BMI, body mass index; CHARGE, Cohorts for Heart and Aging Research in Genomic Epidemiology; HDL-C, high-density lipoprotein cholesterol concentrations; *n*, total sample size; SFA, saturated fatty acids; SSB, sugar-sweetened beverages; TG, triglyceride concentrations.

Table 6.6. Meta-analysis of the associations between SSB intake and lipid traits.[a]

		<1 serv/mo	1-4 serv/mo	1-2 serv/wk	3-7 serv/wk	>1 serv/day		
	n		*β2* (SE)	*β3* (SE)	*β4* (SE)	*β5* (SE)	p_{trend}	I^2
HDL-C (mg/dl)								
Model 1[b]	63,527	Ref.	-0.9 (0.2)	-2.1 (0.3)	-2.7 (0.2)	-4.3 (0.5)	<0.0001	49%
Model 2[c]	61,168	Ref.	-0.5 (0.2)	-1.7 (0.3)	-2.0 (0.2)	-3.1 (0.4)	<0.0001	31%
Model 3[d]	60,991	Ref.	-0.9 (0.1)	-1.8 (0.3)	-2.1 (0.3)	-3.0 (0.5)	<0.0001	47%
Model 4[e]	60,908	Ref.	-0.8 (0.1)	-1.6 (0.2)	-1.8 (0.2)	-2.1 (0.4)	<0.0001	40%
TG (ln-mg/dl)								
Model 1[b]	61,879	Ref.	0.01 (0.01)	0.04 (0.01)	0.06 (0.01)	0.11 (0.02)	<0.0001	41%
Model 2[c]	60,716	Ref.	0.01 (0.01)	0.03 (0.01)	0.05 (0.01)	0.10 (0.02)	<0.0001	45%
Model 3[d]	60,539	Ref.	0.02 (0.01)	0.04 (0.01)	0.06 (0.01)	0.09 (0.02)	<0.0001	48%
Model 4[e]	60,455	Ref.	0.02 (0.01)	0.03 (0.01)	0.05 (0.01)	0.06 (0.02)	<0.0001	38%

[a] Regression coefficients are shown as *βs* (SEs). *β2, β3, β4,* and *β5* represent beta-coefficients for 1-4 servings SSB/month (n=12,581), 1-2 servings SSB/week (n=7,185), 3-7 servings SSB/week (n=10,725), and >1 servings SSB/day (n=4,685) compared to <1 serving SSB/month (reference; n=28,356), respectively. I^2 represents heterogeneity in multivariate meta-analysis of categorical SSB intake on lipid traits, and p_{trend} represents statistical significance in univariate meta-analysis when SSB intake was assessed using an additive model (per increase in category).

[b] Model 1: adjusted for age, sex, total energy intake and study site for multi-centered cohorts (CHS, FHS, YFS, Fenland, RS, MESA).

[c] Model 2: adjusted for Model 1 covariates and smoking status, education status, physical activity, and alcohol intake.

[d] Model 3: adjusted for Model 2 covariates and BMI.

[e] Model 4: adjusted for Model 3 covariates and fruit intake, vegetable intake, whole grains intake (except in YFS), fish intake, and saturated fatty acids (% of total energy).

Supplemental Table 6.7. Meta-analysis of associations between SSB intake (per category) and lipid traits stratified by potential moderators*

Stratification criteria		HDL-C (mg/dl)				TG (ln-mg/dl)			
		β (95% CI)	$P_{interaction}$	Direction	I^2	β (95% CI)	$P_{interaction}$	Direction	I^2
Region	Australia	-0.41 (-1.18, 0.37)	**<0.0001**	-	0%	0.001 (-0.03, 0.03)	0.30	+	0%
	USA	-0.77 (-0.89, -0.65)		------	0%	0.02 (0.01, 0.03)		++++++	65%
	Europe	-0.25 (-0.41, -0.09)		----	0%	0.01 (0.002, 0.02)		++++	0%
Age	< 40 yrs	-0.28 (-0.83, -0.26)	0.50	--	0 %	0.01 (-0.01, 0.02)	**0.004**	++	0 %
	40-60 yrs	-0.64 (-0.91, -0.37)		-----	88 %	0.02 (0.02, 0.03)		+++++	55 %
	> 60 yrs	-0.52 (-0.88, -0.15)		----	0 %	0.01 (0.001, 0.01)		++++	0%
BMI	< 27 kg/m^2	-0.46 (-0.72, -0.21)	0.25	------	80%	0.01 (0.01,0.02)	0.10	++++++	61 %
	≥ 27 kg/m^2	-0.71 (-1.04, -0.38)		-----	0 %	0.02 (0.01, 0.03)		+++++	44 %
SSB Intake	< 0.4 serv/d	-0.49 (-0.74, -0.24)	0.36	-------	76 %	0.01 (0.01, 0.02)	0.12	+++++++	65 %
	≥ 0.4 serv/d	-0.70 (-1.06, -0.33)		----	0 %	0.02 (0.01, 0.03)		++++	52 %
Study Date	≤ 2005	-0.65 (-0.88, -0.42)	0.24	------	29 %	0.01 (0.01, 0.02)	0.51	++++++	67 %
	> 2005	-0.43 (-0.71, -0.15)		-----	77 %	0.02 (0.01, 0.03)		+++++	62 %
Smoking	< 20 %	-0.60 (-0.86, -0.34)	0.54	--------	76 %	0.02 (0.01, 0.02)	0.51	++++++++	64 %
	≥ 20 %	-0.46 (-0.83, -0.09)		---	51 %	0.01 (0.002, 0.02)		+++	48 %
Sample Size	≤ 3,500	-0.46 (-0.81, -0.12)	0.52	-----	0 %	0.01 (-0.003, 0.02)	0.05	+++++	0 %
	> 3,500	-0.60 (-0.85, -0.35)		------	85 %	0.02 (0.01, 0.02)		++++++	55 %

* Regression coefficients represent beta-coefficients for each increase in category of SSB intake [<1 serving SSB/month (reference; n=28,356), 1-4 servings SSB/month (n=12,581), 1-2 servings SSB/week (n=7,185), 3-7 servings SSB/week (n=10,725), and >1 servings SSB/day (n=4,685) in meta-analysis. Regression models were adjusted for age, sex, total energy intake, study site for multi-centered cohorts, smoking status, education status, physical activity, alcohol intake, BMI, fruit intake, vegetable intake, whole grains intake (except in YFS), fish intake, and saturated fatty acids (% of total energy). Stratification criteria are based on mean values in cohorts, and $P_{interaction}$ represents statistical significance of the stratification criteria as a moderator in meta-regression

Table 6.8. Results from meta-analysis and conditional and joint association analysis of *CHREBP* SNPs on lipid traits.[a]

SNP	Basepair (hg19; chr7)	Nearest gene	Nearest Reported SNP	r^2 with GWAS SNP[b]	Alleles (E/A)[c]	Minor Allele Frequency	Effect Size (SE)[d]	*P*	Direction[e]	I^2	Joint Effect Size (SE)[f]	*P*
HDL-C (mg/dl)												
rs71556736	73034929	*CHREBP*	rs3812316[g]	0.94	T/C	0.13	0.92 (0.13)	4.62E-12	+++++??+++++	0 %	0.92 (0.13)	4.73E-12
TG (ln-mg/dl)												
rs71556736	73034929	*CHREBP*	rs3812316[g]	0.94	T/C	0.13	-0.08 (0.005)	9.47E-63	-----??-----	0.0	-0.06 (0.007)	2.36E-16
rs42124	72832340	*FZD9*	rs799160[g]	0.02	A/G	0.04	0.05 (0.009)	1.50E-07	++++++++-+++	20 %	0.05 (0.009)	1.23E-07
rs13225660	73006388	*CHREBP*	rs1051921[h]	1.00	T/C	0.19	-0.06 (0.004)	1.25E-58	-----??-----	17 %	-0.03 (0.006)	1.92E-07
rs10245965	73063515	*VPS37D*	rs799160[g]	0.45	C/T	0.36	-0.01 (0.004)	6.69E-02	-----??----+	74 %	0.02 (0.004)	6.94E-07

[a] Regression coefficients are shown as β (SE). β represents the direction and magnitude of change in the outcome trait with each additional effect allele.
[b] Population genotype data from European ancestry groups in Phase 3 (Version 5) of the 1000 Genomes Project.
[c] Alleles presented as effect (E)/alternative (A) alleles
[d] Regression coefficient in linear regression models used an additive genetic effect and adjusted for age (years), sex (male/female) and cohort/field center (CHS, FHS, YFS, Fenland, RS, MESA); where applicable) while accounting for family, or population structure (where applicable: CHS, FHS, YFS, Fenland, RS, MESA, WGHS).
[e] Order of cohorts for regression coefficient directions: FHS, YFS, Fenland, CHS, NEO, RS1, RS2, MESA, WHI, ARIC, Raine, WGHS (+, positive effect size; -, negative effect size; ?, SNP not available in cohort).
[f] Approximate regression coefficients in joint model of all top signals on lipid outcome
[g] Kooner et al. Nat Genet. 2008 Feb;40(2):149–51.
[h] Talmud et al. Am J Hum Genet. 2009 Nov 13;85(5):628–42.

Table 6.9. Top signals in meta-analysis of difference test interactions between SSB consumption and SNPs on lipid traits[a]

SNP	Location (Hg19)	Nearest gene	Alleles (E/A)[b]	Minor Allele Frequency	P_{Diff}/ Model[c]	SSB Intake Category	*n*	Effect Size (SE)	*P*-value	Direction[d]	I^2
HDL-C (mg/dl)											
rs71556729	72989516	*TBL2*	T/C	0.05	**0.0001/ Model 1	<1 serving/month	20,975	0.48 (0.48)	0.31	+?++-+?-??	0 %
						>1 serving/day	3,359	4.47 (1.10)	5.02E-05	??+?++?+??	0 %
					0.0003/ Model 2	<1 serving/month	20,479	0.37 (0.44)	0.41	+?++--?-??	0 %
						>1 serving/day	3,299	3.89 (1.04)	0.0002	??+?++?+??	0 %
rs73137017	72974413	*BCL7B*	G/A	0.04	0.0002/ Model 1	<1 serving/month	21,050	-0.40 (0.49)	0.90	-+--++?-??	0 %
						>1 serving/day	3,933	3.12 (0.99)	0.002	+?+?++?+??	0 %
					0.0009/ Model 2	<1 serving/month	20,537	-0.23 (0.45)	0.60	--++--?+??	0 %
						>1 serving/day	3,855	2.64 (0.91)	0.004	+?+?++?+??	0 %
rs941294	73065500	*VPS37D*	A/G	0.13	0.0002/ Model 2	<1 serving/month	20,877	0.64 (0.23)	0.006	--++++?-??	0 %
						>1 serving/day	3,955	-1.00 (0.46)	0.03	-?-?--?-??	6 %
rs35709627[e]	72999171	*TBL2*	A/G	0.05	0.0004/ Model 1	<1 serving/month	21,390	0.62 (0.36)	0.09	+-++-+?+??	16 %
						>1 serving/day	4,033	3.23 (0.77)	2.94E-05	+?+?++?+??	0 %
					0.002/ Model 2	<1 serving/month	20,877	0.62 (0.34)	0.06	+-++-+?+??	19 %
						>1 serving/day	3,955	2.72 (0.72)	0.0002	+?+?++?+??	0 %
rs4377835	73069245	*VPS37D*	T/C	0.38	0.0005/ Model 2	<1 serving/month	20,961	0.08 (0.15)	0.59	-+-+-+?--?	0 %
						>1 serving/day	4,181	-0.94 (0.31)	0.002	---?--?--?	0 %
rs73133094	72833516	FZD9	A/G	0.07	0.002/ Model 1	<1 serving/month	21,050	-0.33 (0.42)	0.43	--+---?+??	5 %
						>1 serving/day	3,933	2.19 (0.88)	0.01	+?+?++?+??	0 %
					0.002/ Model 2	<1 serving/month	20,537	-0.21 (0.39)	0.58	--+---?+??	11 %
						>1 serving/day	3,855	2.09 (0.80)	0.009	+?+?++?+??	0 %
rs11768232	73066538	*VPS37D*	A/G	0.43	0.002/ Model 2	<1 serving/month	20,961	-0.14 (0.15)	0.35	+-+---?++?	0 %
						>1 serving/day	4,181	0.74 (0.30)	0.01	+++?++?+-?	0 %

TG (ln-mg/dl)											
rs799157	73020301	*CHREBP*	T/C	0.05	0.002/ Model 1	<1 serving/month	20,975	0.01 (0.01)	0.45	+?+++-?+??	66 %
						>1 serving/day	4,033	0.11 (0.03)	0.002	+?+?++?+??	0 %
					0.003/ Model 2	<1 serving/month	20,479	0.01 (0.01)	0.59	+?++--?+??	74%
						>1 serving/day	3,955	0.09 (0.03)	0.004	+?+?++?+?	0 %

[a] Top hits represent suggestive P_{Diff}[c] <0.005
[b] Alleles presented as effect (E)/alternative (A) alleles
[c] P_{Diff} represents the difference test for the highest and lowest category of SSB intake (<1 serving/month vs. >1 serving/day). Linear regression models used an additive genetic effect, and adjusted for following: Model 1 adjusted for age (years), sex (male/female), total energy intake (kcal/day) field center (CHS, FHS, YFS, Fenland, RS, MESA), and accounted for family or population structure where applicable (FHS, YFS, Fenland, NEO, MESA, WGHS, Raine, MESA); Model 2 adjusted for Model 1 covariates plus cohort-specific definition of education, smoking, physical activity, alcohol intake, and body mass index (kg/m^2).
[d] Order of cohorts for regression coefficient directions: FHS, YFS, Fenland, CHS, NEO, WGHS, WHI, ARIC, Raine, MESA (+, positive effect size; -, negative effect size; ?, SNP not available in cohort).
[e] Linkage Disequilibrium with rs13240662 (Table 4) = 0.98 in European ancestry groups of Phase 3 (Version 5) of the 1000 Genomes Project

[**] Indicates a statistically significant interaction based on Bonferonni-corrected P_{Diff} <0.0001

Table 6.10. Top signals in meta-analysis of multiplicative interactions between SSB consumption and SNPs on lipid traits.[a]

SNP	Location (Hg19)	Nearest gene(s)	Alleles[b]	Minor Allele Frequency	Sample Size	Effect Size (SE)[c]	$P_{interaction}$/ Model[d]	P_{Diff}[e]	Direction[f]	I^2
HDL (mg/dl)										
rs71556729	72989516	*TBL2*	T/C	0.03	52,563	0.74 (0.22)	0.001/ Model 1	0.0001	++++++?+-	9 %
					35,015	0.66 (0.25)	0.008/ Model 2	0.0003	++-++??+?	51 %
rs79578725	73002455	*CHREBP*	A/G	0.05	50,807	-0.51 (0.18)	0.006/ Model 1	0.05	+?-+--?--	23 %
rs13240662[g]	73032823	*CHREBP*	A/G	0.06	52,563	0.42 (0.16)	0.008/ Model 1	0.0003	+++-++?--	68 %
rs17145717	72929608	*BAZ1B*	A/G	0.07	35,632	0.37 (0.15)	0.01/ Model 2	0.65	+++++??++	0 %
TG (ln-mg/dl)										
rs72649028	73022647	*CHREBP*	T/C	0.01	51,926	0.03 (0.01)	0.008/ Model 1	NA[h]	++++++?+?	0 %

[a] Top hits represent suggestive $P_{interact}$[c] <0.01
[b] Alleles presented as effect (E)/alternative (A) alleles
[c] Interaction coefficients are shown as β (SE). β represents the direction and magnitude of change in the outcome trait with each additional effect allele, per each increase in category of SSB intake (<1 serving/month, 1-4 servings/month, 1-2 servings/week, 3-7 servings/week, >1 serving/day).
[d] $P_{interact}$ represents the p-value for the multiplicative interaction regression coefficient of SNPxSSB (additive categories). Linear regression models used an additive genetic effect, and adjusted for following: Model 1 adjusted for age (years), sex (male/female), total energy intake (kcal/day) field center (CHS, FHS, YFS, Fenland, RS, MESA), and accounted for family or population structure where applicable (FHS, YFS, Fenland, NEO, MESA, WGHS, Raine, MESA); Model 2 adjusted for Model 1 covariates plus cohort-specific definition of education, smoking, physical activity, alcohol intake, and body mass index (kg/m^2).
[e] P_{Diff} represents the difference test for the highest and lowest category of SSB intake (<1 serving/month vs. >1 serving/day)
[f] Order of cohorts for regression coefficient directions: FHS, YFS, Fenland, CHS, NEO, WGHS, WHI, ARIC, Raine (+, positive effect size; -, negative effect size; ?, SNP not available in cohort).
[g] Linkage Disequilibirum with rs35709627 (Table 3) = 0.98 in European ancestry groups of Phase 3 (Version 5) of the 1000 Genomes Project
[h] Meta-analysis data not available among participants consuming >1 serving SSB/month

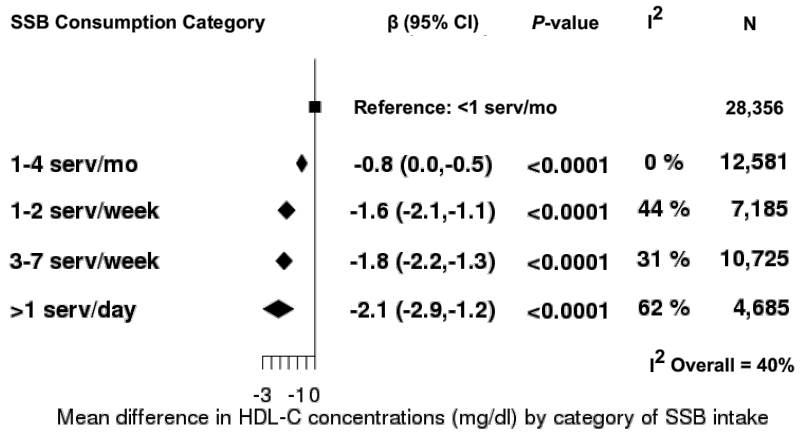

Figure 6.1. Forest plot of multivariate meta-analysis results for main association between category of SSB intake and HDL-C concentrations

Compared to the lowest SSB consumers (<1 serving/month), participants consuming 1-4 servings SSB/month [β (95% CI):-0.8 (0.00, -0.5)], 1-2 servings SSB/week [β (95% CI):-1.6 (-2.1, -1.1)], 3-7 servings SSB/week [β (95% CI): -1.8 (-2.2, -1.3)], and >1 serving SSB/day [β (95% CI): -2.1 (-2.9, -1.2)] displayed lower mean HDL-C concentrations (mg/dl) in a dose-response manner. All regression models were adjusted for age, cohort (where applicable), sex (where applicable), total energy, education, current smoking status, physical activity, alcohol intake, body mass index, percent energy from saturated fat, and servings per day of vegetables, total fruits, whole grains (except YFS), nuts/seeds, and seafood. I^2 represents heterogeneity for individual regression coefficients and I^2 overall represents heterogeneity in multivariate meta-analysis.

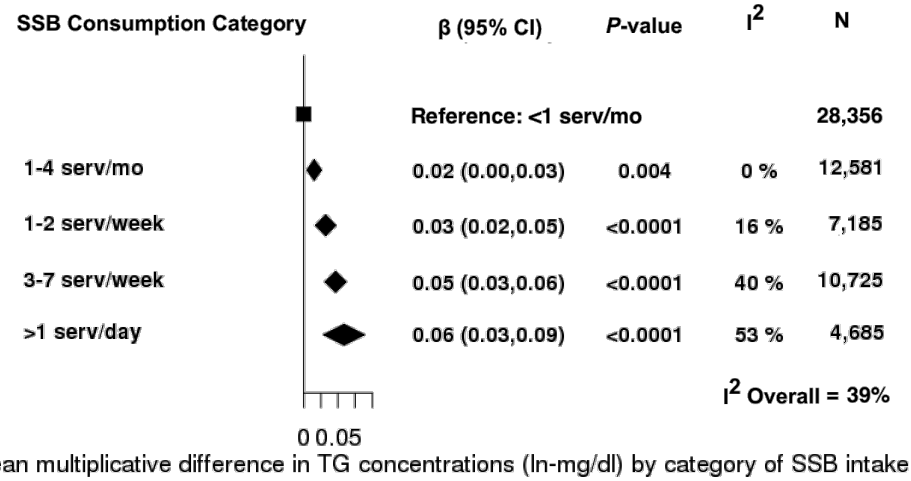

Figure 6.2. Forest plot of multivariate meta-analysis results for main association between category of SSB intake and TG concentrations

Compared to the lowest SSB consumers (<1 serving/month), participants consuming 1-4 servings SSB/month [β (95% CI): 0.02 (0.00, 0.03)], 1-2 servings SSB/week [β (95% CI): 0.03 (0.02, 0.05)], 3-7 servings SSB/week [β (95% CI): 0.05 (0.03, 0.06)], and >1 serving SSB/day [β (95% CI): 0.06 (0.03, 0.09)] displayed higher mean TG concentrations (ln-mg/dl) in a dose-response manner. All regression models were adjusted for age, cohort (where applicable), sex (where applicable), total energy, education, current smoking status, physical activity, alcohol intake, body mass index, percent energy from saturated fat, and servings per day of vegetables, total fruits, whole grains (except YFS), nuts/seeds, and seafood. I^2 represents heterogeneity for individual regression coefficients and I^2 overall represents heterogeneity in multivariate meta-analysis.

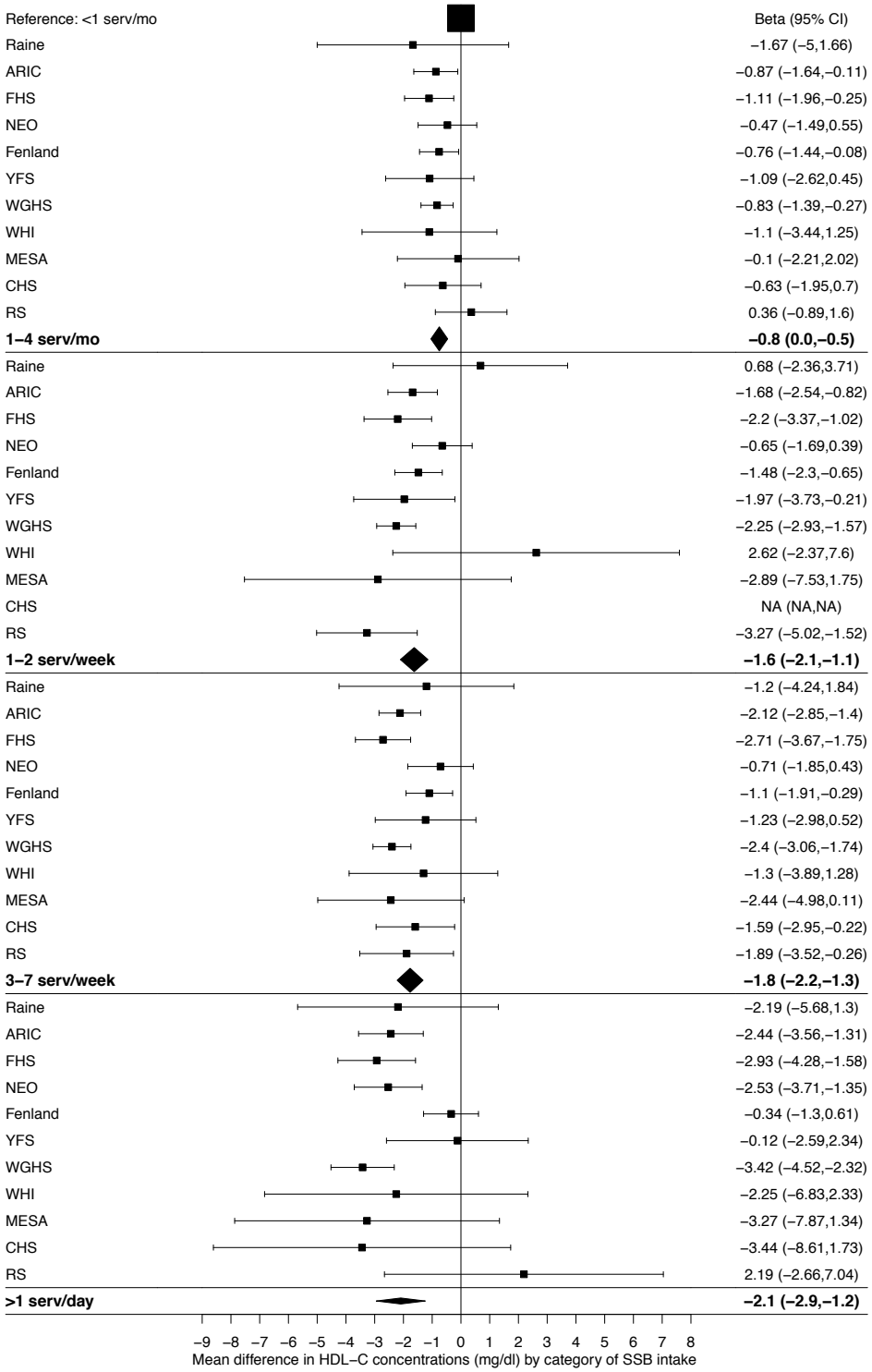

Figure 6.3. Forest plot of main association between SSB intake and HDL-C concentrations

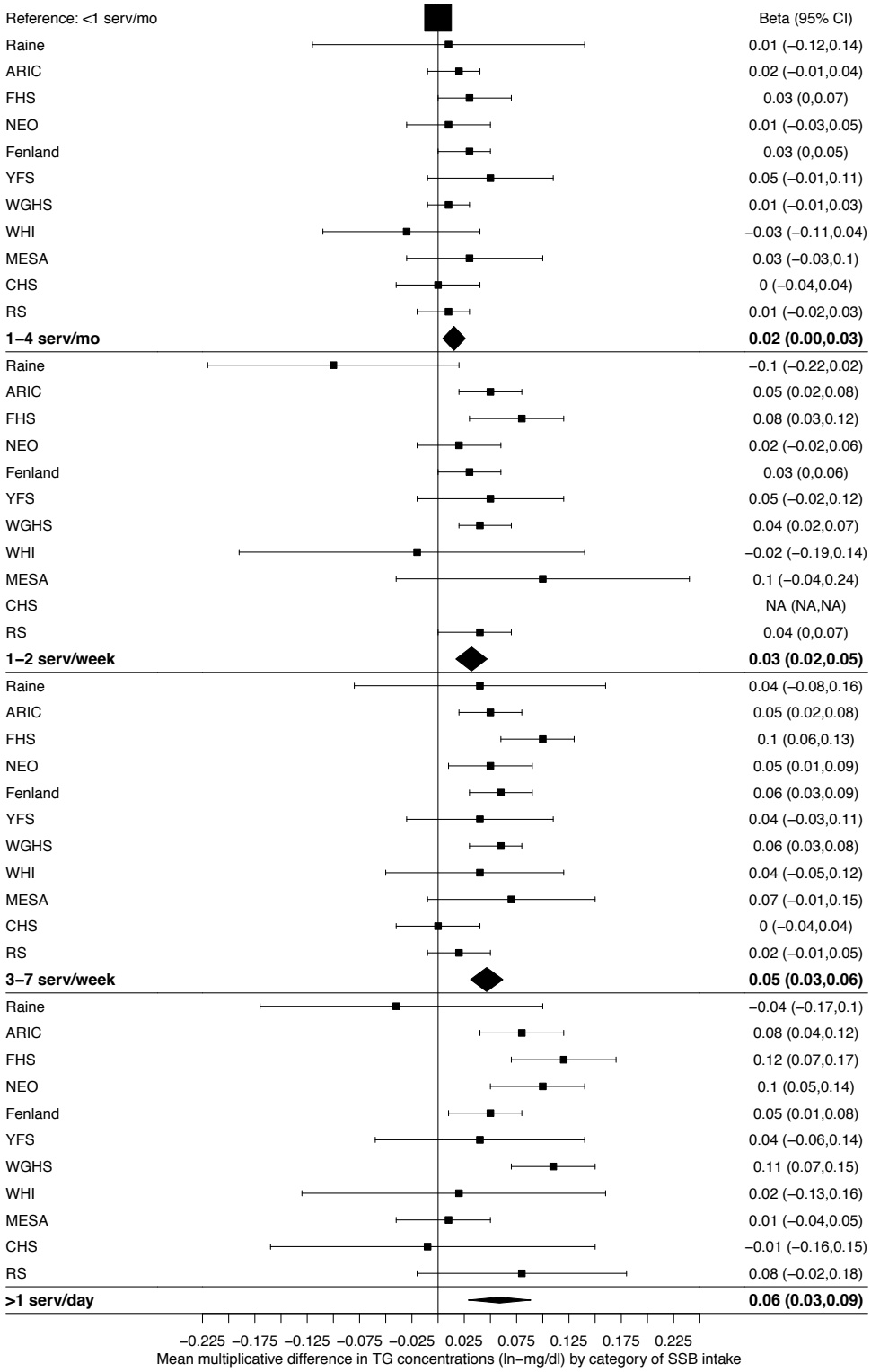

Figure 6.4. Forest plot of main association between SSB intake and TG concentrations

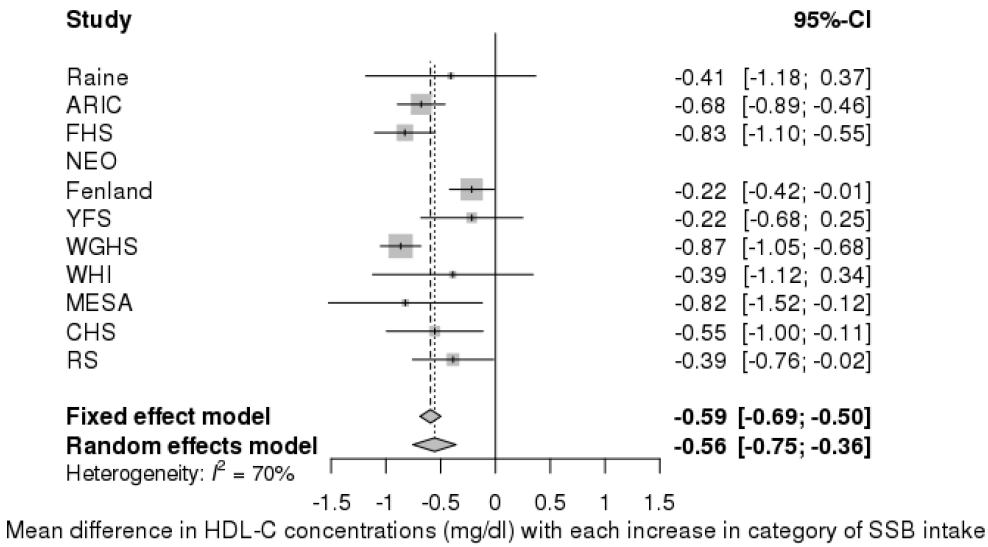

Figure 6.5. Forest plot of univariate meta-analysis results for mean difference in HDL-C concentrations (mg/dl) per increase in category of SSB intake

In random-effects meta-analysis, each increase in SSB intake category was associated with lower mean HDL-C concentrations [β (95% CI): -0.56 (-0.75, -0.36) mg/dl; p_{trend} = 0.002]. All regression models were adjusted for age, cohort (where applicable), sex (where applicable), total energy, education, current smoking status, physical activity, alcohol intake, body mass index, percent energy from saturated fat, and servings per day of vegetables, total fruits, whole grains (except YFS), nuts/seeds, and seafood.

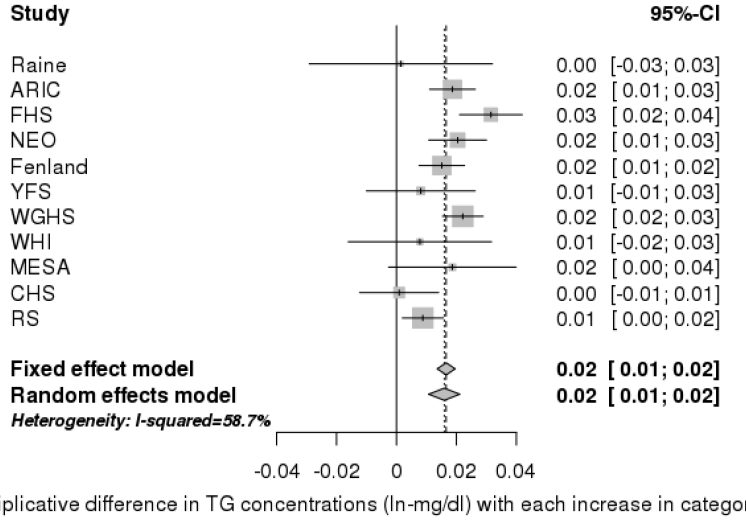

Figure 6.6. Forest plot of univariate meta-analysis results for mean multiplicative difference in TG concentrations (ln-mg/dl) per increase in category of SSB intake

In random-effects meta-analysis, each increase in SSB intake category was associated with higher mean TG concentrations [β (95% CI): 0.02 (0.01, 0.02) ln-mg/dl; p_{trend} < 0.0001]. All regression models were adjusted for age, cohort (where applicable), sex (where applicable), total energy, education, current smoking status, physical activity, alcohol intake, body mass index, percent energy from saturated fat, and servings per day of vegetables, total fruits, whole grains (except YFS), nuts/seeds, and seafood.

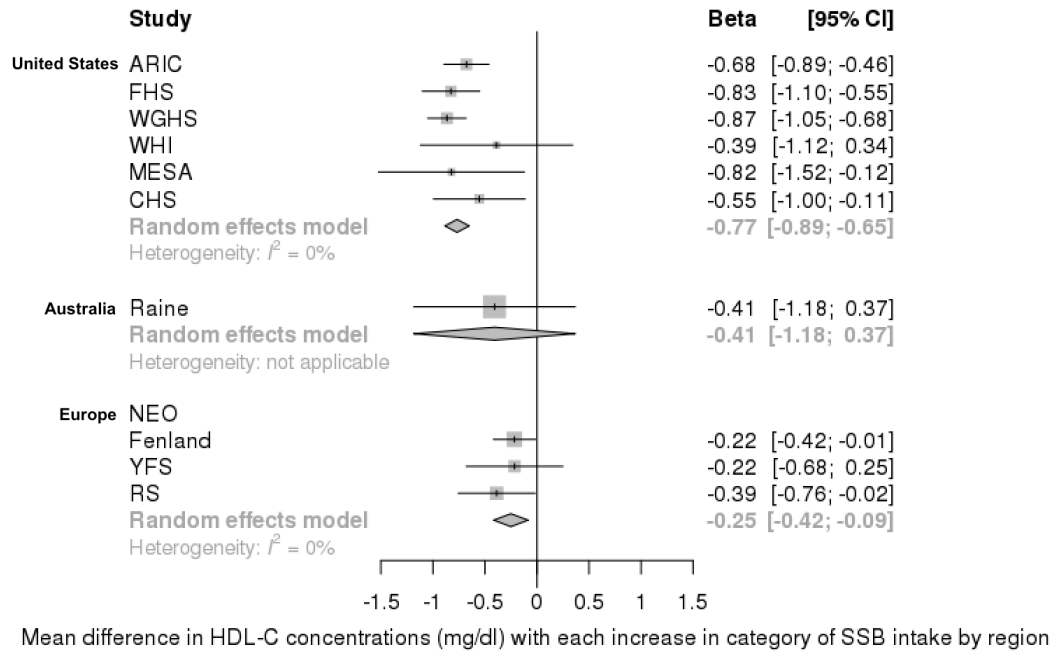

Figure 6.7. Forest plot of main association between SSB intake and HDL-C concentrations stratified by study region

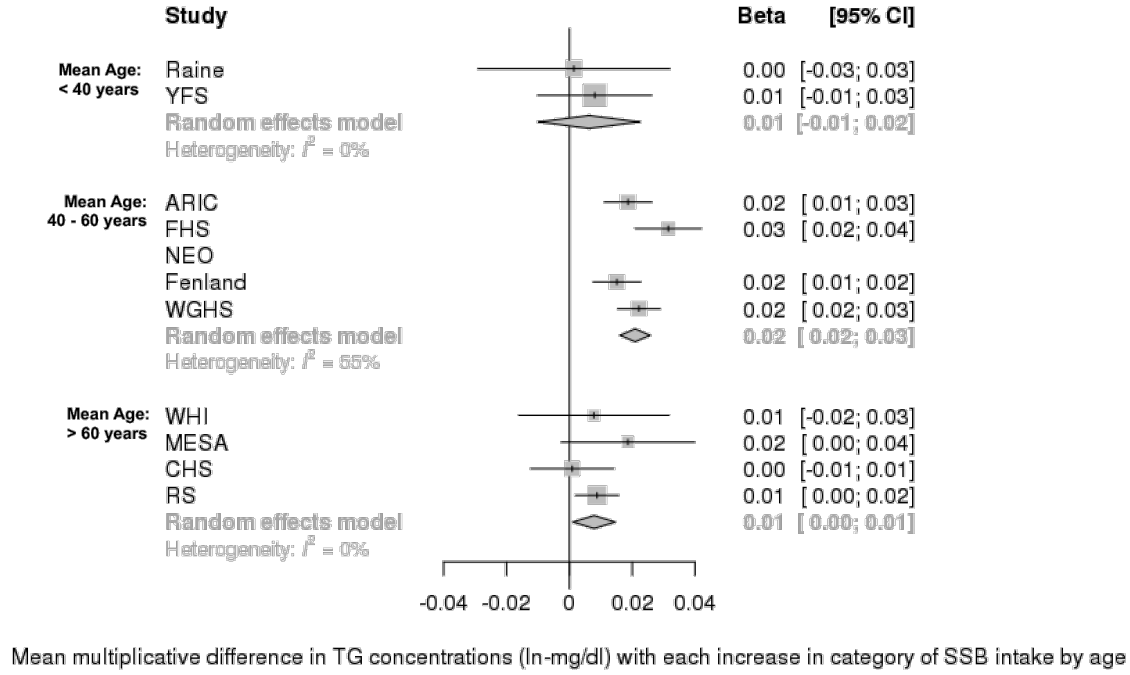

Figure 6.8. Forest plot of main association between SSB intake and TG concentrations stratified by mean study age

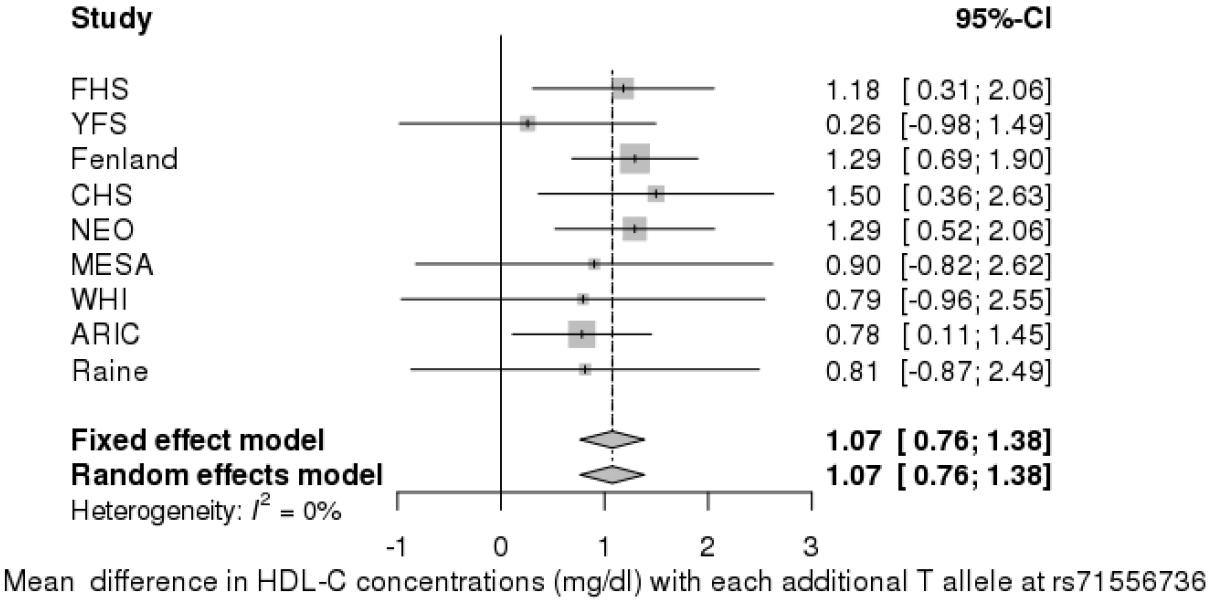

Figure 6.9. Forest plot of association between rs71556736 and HDL-C concentrations

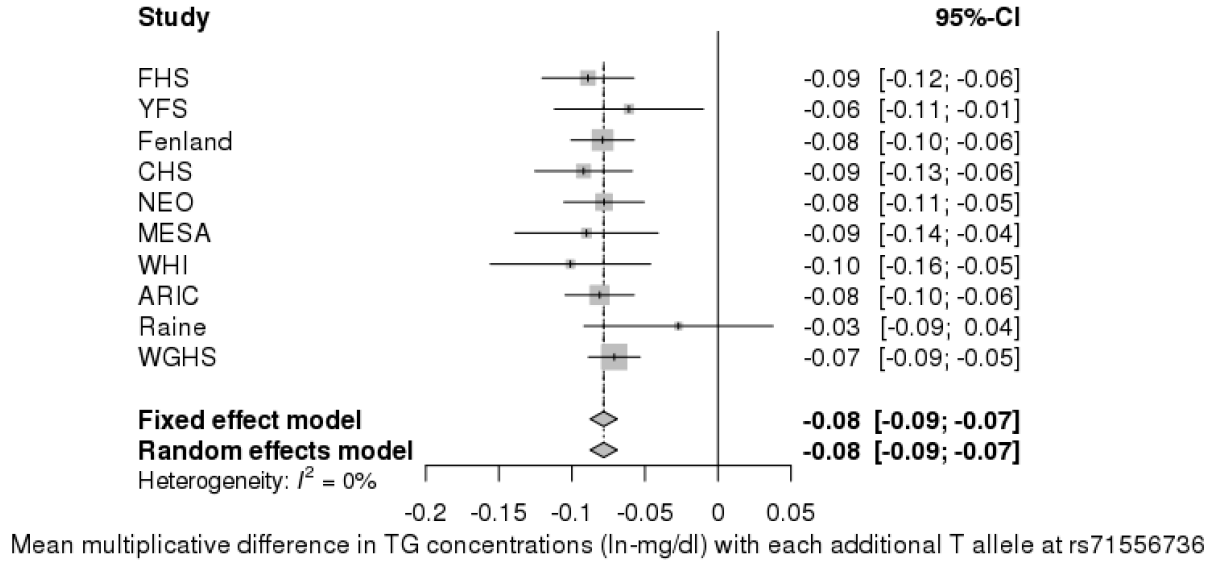

Figure 6.10. Forest plot of association between rs71556736 and TG concentrations

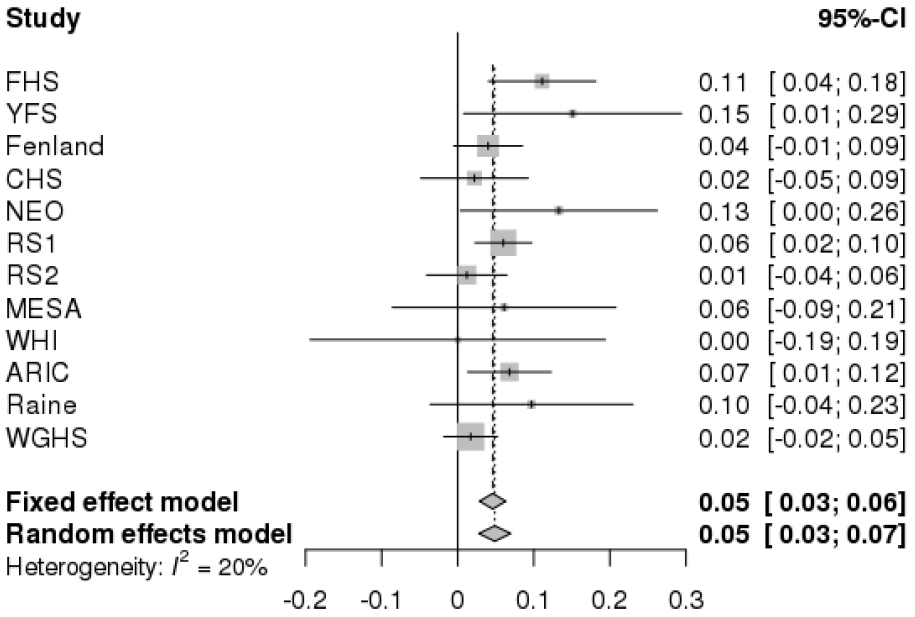

Figure 6.11. Forest plot of association between rs42124 and TG concentrations

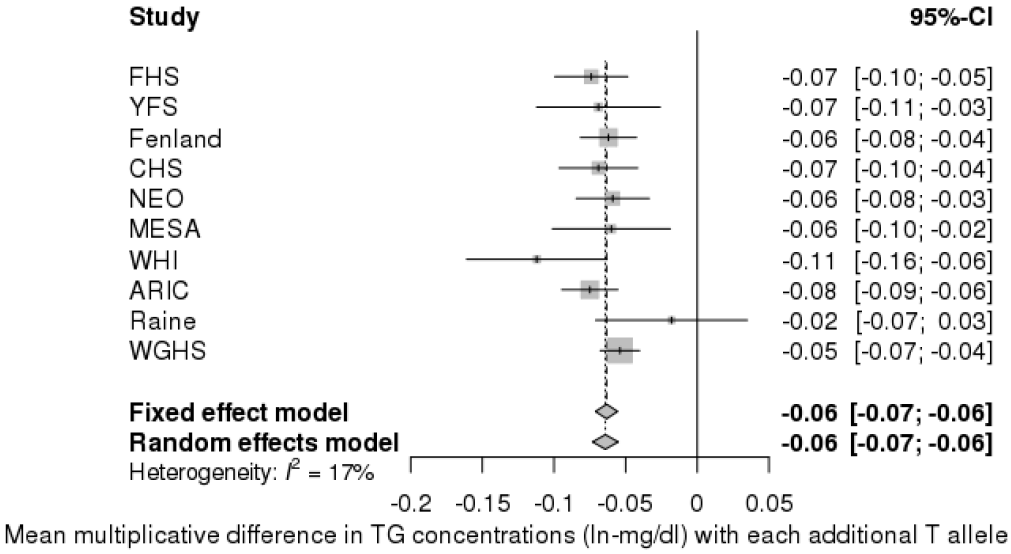

Figure 6.12. Forest plot of association between rs13225660 and TG concentrations

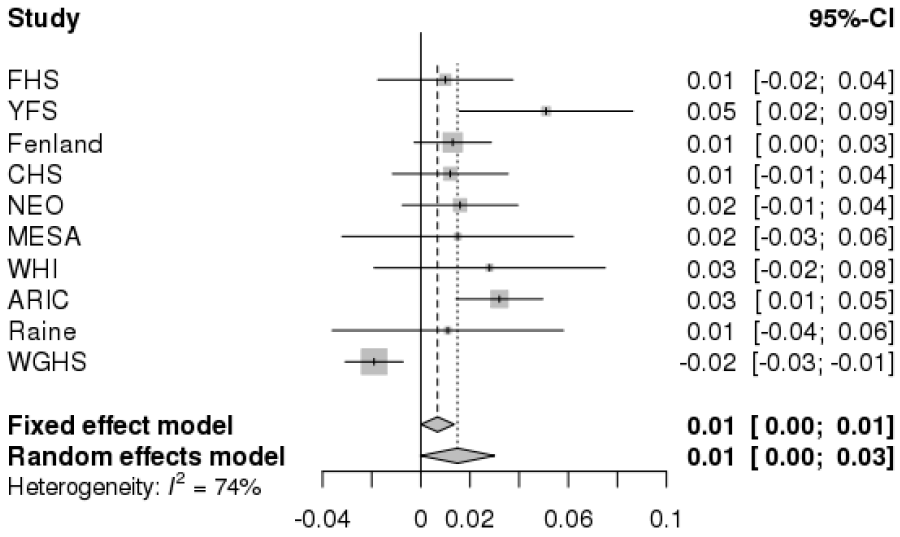

Figure 6.13. Forest plot of association between rs10245965 and TG concentrations

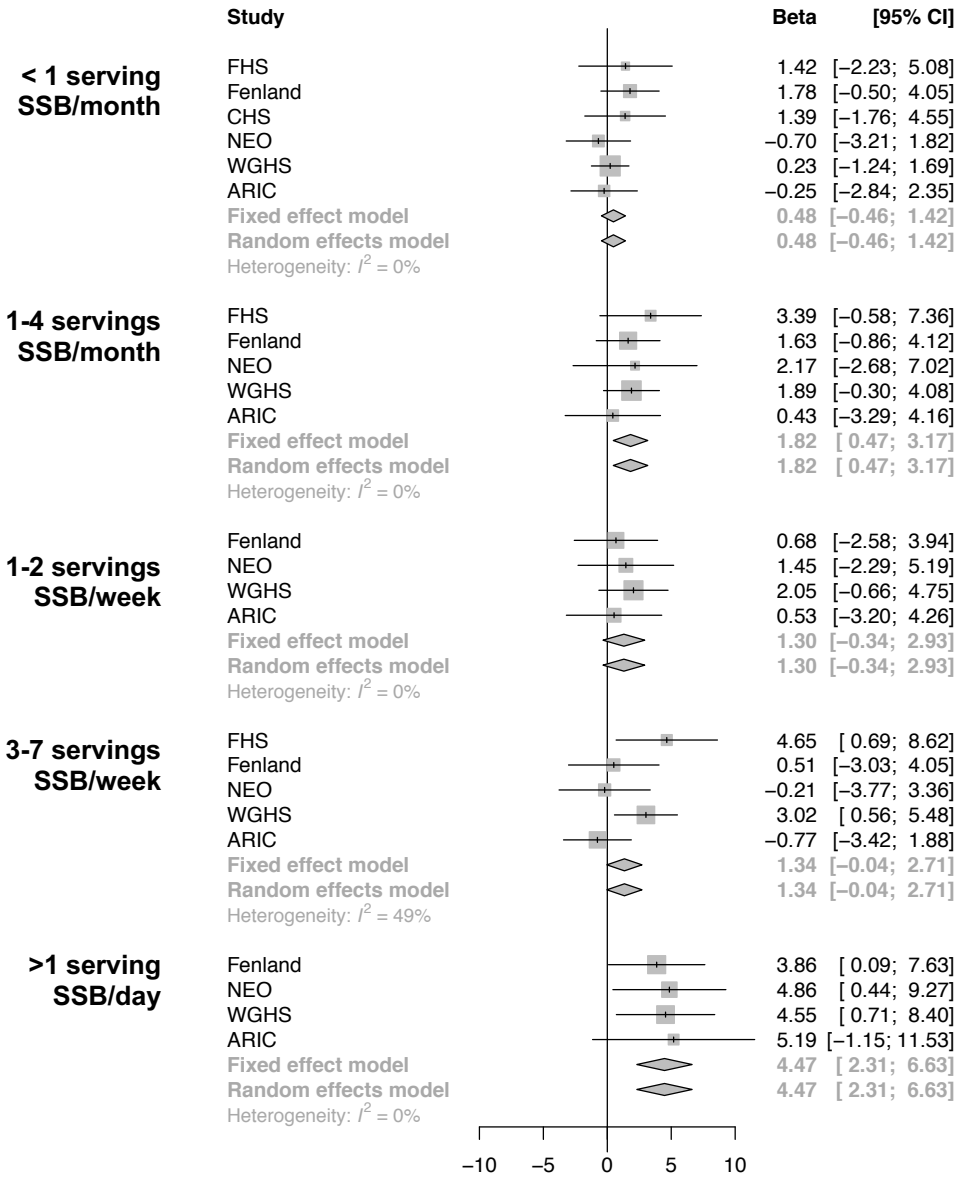

Figure 6.14. Forest plot of association between rs71556729 and HDL-C concentrations stratified by category of SSB intake (Model 1)

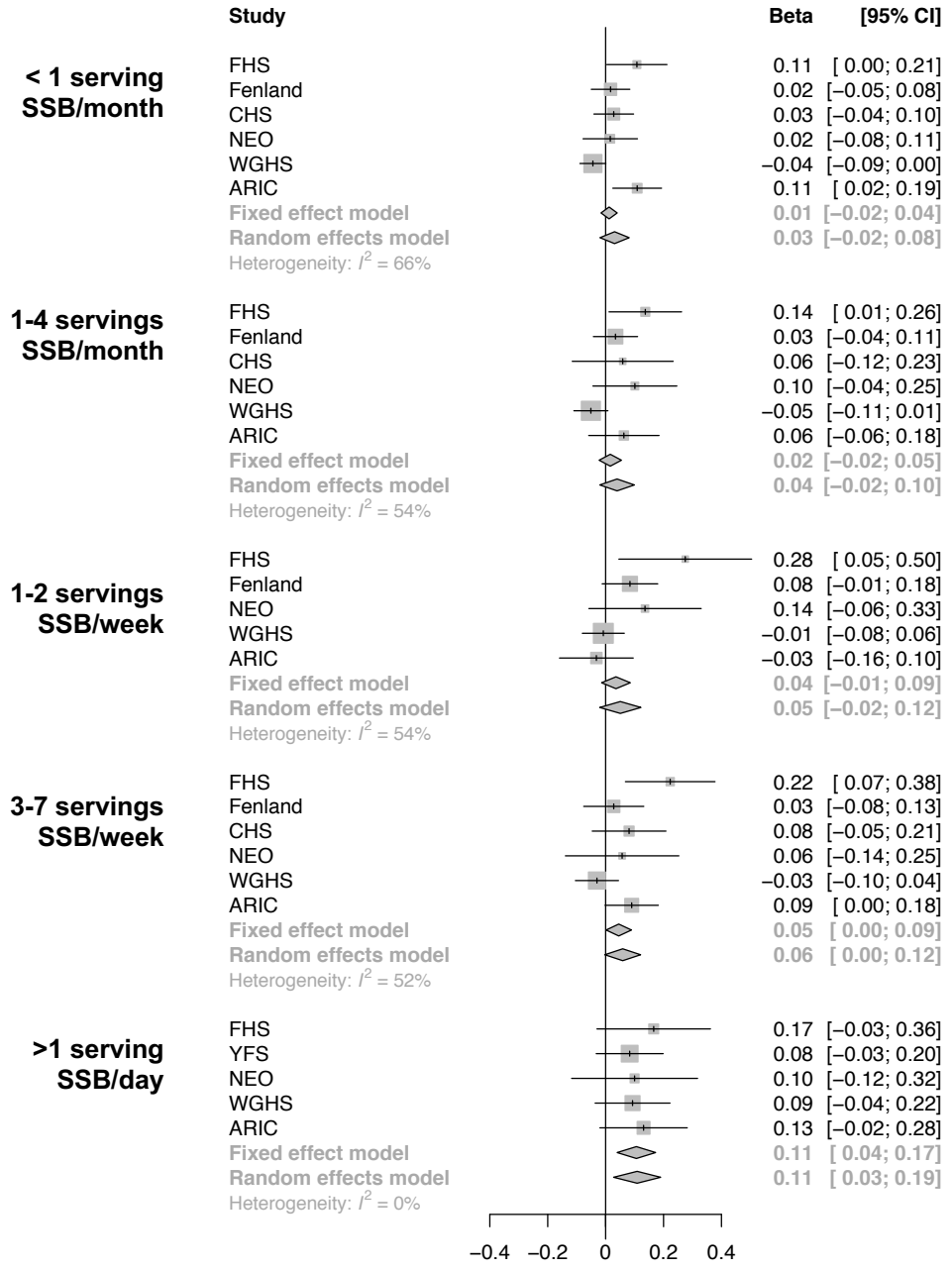

Figure 6.15. Forest plot of association between rs799157 and TG concentrations stratified by category of SSB intake (Model 1)

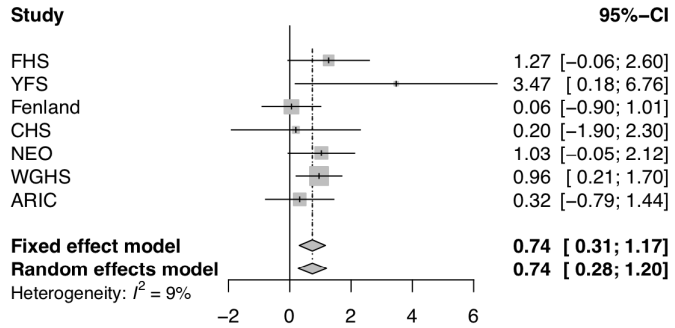

Figure 6.16. Forest plot of interaction between SSB intake and rs71556729 on HDL-C concentrations (Model 1)

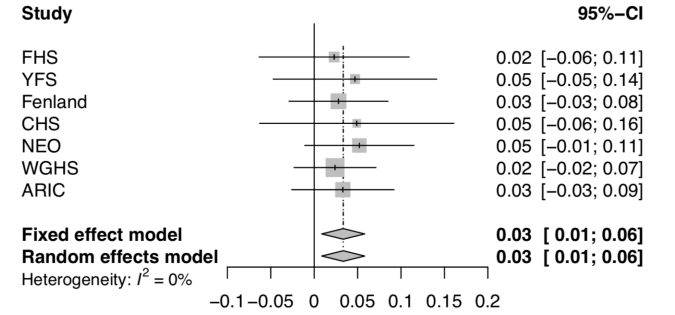

Figure 6.17. Forest plot of interaction between SSB intake and rs72649028 on TG concentrations (Model 1)

CHAPTER 7: SUMMARY AND FUTURE DIRECTIONS

7.1 Summary

The overall objective of this project was to contribute to the understanding of how genes and dietary sugar interact to increase cardiometabolic risk and inform new preventative and therapeutic strategies to prevent or treat dyslipidemia. The *central hypothesis* was that SSB consumption and genetic variants within *CHREBP* were associated with an unfavorable lipoprotein profile, and a genetic predisposition to dyslipidemia would be exacerbated through high SSB consumption (>1 serving/day), thereby predisposing susceptible individuals to greater risk of CVD. We tested our hypothesis through analysis of population-level data in the Framingham Heart Study [Offspring (FOS) and Third Generation (GEN3)] and Women's Genome Health Study (WGHS), as well as nine additional cohorts participating in the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) consortium's Nutrition Working Group.

First, we observed that SSB consumption was associated with concentrations of plasma cholesterol, triglyceride and apolipoproteins, and lipoprotein particle size concentrations. Some of these assoications have been linked to adverse cardiometabolic outcomes among FOS and WGHS participants. We identified novel associations between SSB intake and higher apo B:apo A1 ratio and concentrations of non-HDL-C, apo B, RLP-TG and RLP-C, lower concentrations of HDL-C and apo A1, and smaller LDL-P and HDL-P size. We also replicated previously observed associations of SSB consumption with TC: HDL-C ratio and HDL-C, LDL-C, and TG concentrations.

Next, we observed that greater consumption of SSB was associated with prospective adverse changes in lipid concentrations and development of dyslipidemia in Framingham Heart Study participants. Consumption of SSB was adversely associated with mean 4-year changes in HDL-C and TG concentrations, along with increased incidence of low HDL-C and high TG concentrations. Mixed results were observed for LCSB consumption. LCSB consumption was not associated with significant changes in lipid concentrations, with the exception of increased incidence of high non-HDL-C concentration. Given this inconsistent evidence, it is likely that this significant finding has a high risk of reverse causality. FJ consumption was not significantly associated with lipid concentrations or the development of dyslipidemia.

Finally, we observed a significant relationship between SSB consumption and dyslipidemia, characterized by an inverse association of SSB and fasting HDL-C and positive association with TG concentrations in a meta-analysis of data from 11 participating CHARGE consortium cohorts. We confirmed that one distinct SNP in the CHREBP region is associated with HDL-C concentrations. We identified two novel SNPs near the *CHREBP* locus that were significantly associated with TG concentrations, and also replicated two previously observed associations in this region. We also found suggestive evidence that SSB consumption may modify the association between SNPs near the CHREBP locus on HDL-C and TG concentrations.

Taken together, these observations support our hypothesis that SSB consumption and genetic variants within *CHREBP* associate with an unfavorable lipoprotein profile. We also present data that suggest *CHREBP* SNPs significantly associated with lipoprotein concentrations in GWAS do not exacerbate the adverse association of daily SSB consumption on lipoprotein concentrations, which is contrary to our hypothesis. However, our data suggests that SSB

consumption may modify the association between SNPs in the *CHREBP* region on lipoprotein concentrations, thus revealing additional SNPs in the *CHREBP* region that may influence lipoprotein concentrations dependent on SSB consumption level.

7.2 Public Health Importance

Given that CVD is the leading cause of death globally, representing 31% of all global deaths (1), strategies to reduce the burden of CVD are a global health priority (2). Dyslipidemia is a risk factor for CVD (3) present in approximately 30-40% of adults in the US (1,4). Thus, managing patients' blood lipid concentrations is a major focus for health professionals as a CVD risk reduction strategy and to prevent and treat dyslipidemia (5)(1,6).

Prospective cohort studies have found an association between added sugar intake and CVD (7–9) and CVD-mortality (7,10), particularly in the form of SSB (7,10–13). Consumption of SSB is likely to contribute to the development of CVD through weight gain, but data also through independent metabolic effects, including insulin resistance, hyperinsulinemia, hyperuricemia, increases in inflammatory biomarkers, and/or dyslipidemia (14). The Scientific Report of the 2015 Dietary Guidelines Advisory Committee (DGAC) indicates that evidence for associations between added sugars and dyslipidemia are not consistent, and the available observational studies are mostly cross-sectional, and thus potentially confounded by dietary and lifestyle factors (15). We present data that helps address this research gap by providing high-quality prospective evidence that consumption of added sugar in the form of SSB is associated with the development of dyslipidemias related to high TG and low HDL-C concentrations. These

prospective data also suggest that FJ consumption is not adversely associated with the development of dyslipidemia, supporting current recommendations that FJ consumption up to ½ cup/day can contribute to a healthy dietary pattern when consumed within recommended limits (16). Another data gap identified by the 2015 DGAC was a lack of prospective studies investigating the association between low-calorie sweeteners and cardiometabolic risk factors (15). Our data adds to this gap in knowledge suggesting that LCSB consumption does not associate with changes in lipoprotein concentrations but may influence risk for dyslipidemia. These conflicting findings suggest that additional high-quality prospective studies are necessary to inform recommendations related to LCSB consumption and development of dyslipidemia.

In addition to this prospective data, we also present data that can assist in the development of new hypotheses to investigate potential underlying mechanisms by which SSB consumption may influence CVD risk. Our data suggest that differences in lipoprotein metabolism are a potential pathway by which SSB intake may increase risk for cardiometabolic diseases. Furthermore, we present evidence that SSB consumption is associated with the characteristic features of two emerging dyslipidemias known as diabetic dyslipidemia and atherogenic dyslipidemia. Diabetic dyslipidemia is characterized by the high concentrations of TG and small dense LDL, and low concentrations of HDL-C, frequently found in diabetics (17,18). Although there is currently no widely accepted definition for atherogenic dyslipidemia, it has been characterized by high TG, small low-density lipoprotein particles (LDL-P) and remnant-like particle (RLP) concentrations, and low HDL-C concentrations (19,20). Although the contribution of these two dyslipidemias to the development of CVD is uncertain, the results of this study may be informative for the design of future studies.

Evidence for an interaction between genetics and SSB consumption on CVD is limited (21), and a better understanding of how genes and nutrients may interact to increase cardiometabolic risk may inform new strategies for prevention and treatment of CVD. We present a novel gene-diet interaction approach designed to illuminate SNPs within the *CHREBP* region that may influence lipoprotein concentrations dependent on SSB consumption. Utilizing this approach, we found suggestive SNPs that may contribute to the heritability of HDL-C and TG concentrations in a manner that is dependent on SSB consumption. Our data suggest that some SNPs in the *CHREBP* region may help protect against the potential adverse consequences of SSB consumption on HDL-C and TG concentrations, thus these SNPs could be potential targets for future therapies aimed to reduce dyslipidemia. Additional SNPs within the CHREBP region exacerbated the negative health effects of high SSB consumption, thus indicating that some individuals may be genetically predisposed for an adverse association between SSB intake and lipoprotein concentrations.

7.3 Future Research

Beverage consumption

In this study, presweetened teas and coffees are not included in the estimates of SSB consumption due to limitations in the dietary assessment methods utilized in the included cohorts. However, presweetened coffees and teas have more recently been captured in estimates of SSB consumption (22,23). The amount of sugar added to these presweetened coffees and teas

is similar to that of sodas and fruit drinks and the inability to capture their intake may lead to an underestimation of added sugar from beverages in observational studies. Given that coffee and tea contain phytochemicals and other potentially bioactive compounds, they may have different physiological health effects compared to traditional SSB. Moving forward, studies exploring beverage consumption patterns on cardiometabolic risk could be improved if estimates of beverage exposure included a separate category including presweetened coffees and teas and examined the independent associations of each beverage consumption category with health outcomes.

Validation of Gene-SSB Interactions

Replication of findings in genetic studies is of utmost importance to ensure the observation represents a credible association and prevent reports of spurious associations (24). Our study included 11 independent population-based cohorts, thus providing an internal replication of all analyses. However, our observation that high SSB consumption may modify the association between genetic variants within or near the *CHREBP* locus on HDL-C and TG concentrations was of nominal statistical significance, suggesting that an external validation study is warranted to further confirm these findings.

The UK Biobank is a prospective cohort study of over 500,000 individuals in the UK with information available on genetics, dietary consumption, and lipoprotein concentrations (https://www.ukbiobank.ac.uk). A validation study in the UK Biobank would allow us to increase the sample size of our study and provide an additional independent cohort to validate our findings, thus increasing the likelihood that we identified a credible association. This would also allow us to investigate additional covariate models that could reveal sources of attenuation

in interaction results with the addition of lifestyle factors and body mass index, thus enhancing the value of our findings.

Exploration of Further Gene-SSB Interactions

In addition to their association with TG and HDL-C concentrations, genetic variants within *CHREBP* have been associated with distinct lipoprotein particle size profiles in GWAS, which may further indicate that *CHREBP* could play a role in the modification of lipid metabolism. Given that our data suggest an association between SSB consumption and distinct lipoprotein size profiles, the examination of whether SSB consumption interacts with *CHREBP* SNPs to influence lipoprotein profiles may provide further insight into how *CHREBP* and SSB consumption may together influence lipoprotein metabolism.

Despite the strengths of our candidate gene approach, focusing our efforts on discovering gene-SSB interactions in a single region limit the scope of our findings. Examination of gene-SSB interactions at the genome-wide level could reveal novel pathways by which SSB consumption may influence health outcomes. Additional availability of -omics data in large populations, such as proteomic, transcriptomic, epigenomic, and metabolomic data, may also provide a means to enhance and validate findings through multi-omics study designs. As the availability of big data in area of genetic and nutritional epidemiology grows, exploration of these novel approaches could lead to important discoveries that could enhance our understanding of disease pathogenesis and lead to advancements in the field of personalized nutrition and medicine.

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