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Title

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ORIGINAL ARTICLE

Sugar-Sweetened Beverage Consumption May Modify Associations Between Genetic Variants in the CHREBP (Carbohydrate Responsive Element Binding Protein) Locus and HDL-C (High-Density Lipoprotein Cholesterol) and Triglyceride Concentrations

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BACKGROUND: ChREBP (carbohydrate responsive element binding protein) is a transcription factor that responds to sugar consumption. Sugar-sweetened beverage (SSB) consumption and genetic variants in the *CHREBP* locus have separately been linked to HDL-C (high-density lipoprotein cholesterol) and triglyceride concentrations. We hypothesized that SSB consumption would modify the association between genetic variants in the *CHREBP* locus and dyslipidemia.

METHODS: Data from 11 cohorts from the Cohorts for Heart and Aging Research in Genomic Epidemiology consortium (N=63 599) and the UK Biobank (N=59 220) were used to quantify associations of SSB consumption, genetic variants, and their interaction on HDL-C and triglyceride concentrations using linear regression models. A total of 1606 single nucleotide polymorphisms within or near *CHREBP* were considered. SSB consumption was estimated from validated questionnaires, and participants were grouped by their estimated intake.

RESULTS: In a meta-analysis, rs71556729 was significantly associated with higher HDL-C concentrations only among the highest SSB consumers (β, 2.12 [95% CI, 1.16–3.07] mg/dL per allele; P<0.0001), but not significantly among the lowest SSB consumers (P=0.81; P_{Diff} <0.0001). Similar results were observed for 2 additional variants (rs35709627 and rs71556736). For triglyceride, rs55673514 was positively associated with triglyceride concentrations only among the

highest SSB consumers (β, 0.06 [95% CI, 0.02–0.09] ln-mg/dL per allele, P=0.001) but not the lowest SSB consumers (P=0.84; P_{Diff}=0.0005).

CONCLUSIONS: Our results identified genetic variants in the *CHREBP* locus that may protect against SSB-associated reductions in HDL-C and other variants that may exacerbate SSB-associated increases in triglyceride concentrations.

REGISTRATION: URL: https://www.clinicaltrials.gov; Unique identifier: NCT00005133, NCT00005121, NCT00005487, and NCT00000479.

Key Words: carbohydrates ■ dyslipidemia ■ epidemiology ■ genetics ■ nutrition ■ sugars ■ triglyceride

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Nonstandard Abbreviations and Acronyms

CHARGE	Cohorts for Heart and Aging Research in Genetic Epidemiology
ChREBP	Carbohydrate Responsive Element Binding Protein
GWAS	genome-wide association studies
HDL-C	high-density lipoprotein cholesterol
SNP	single nucleotide polymorphism
SSB	sugar-sweetened beverages
UKB	UK Biobank

Low circulating HDL-C (high-density lipoprotein cholesterol) and elevated fasting triglyceride concentrations are positively associated with risk of type 2 diabetes and cardiovascular disease.[1–5] Both genetic and environmental factors, including diet, are important determinants of HDL-C and triglyceride concentrations.[5–7] Genetic determinants of HDL-C and triglyceride concentrations have been identified in genome-wide association studies (GWAS),[8–12] but the extent to which genetic variants interact with environmental exposures is unknown. It is plausible that unrecognized genetic variants or genetic effects may be suppressed or exacerbated by environmental factors, such as diet.

ChREBP (carbohydrate responsive element binding protein) is a transcription factor that regulates glucose and lipid metabolism in response to sugar consumption, including sugar from sugar-sweetened beverages (SSB).[13,14] GWAS have consistently observed an association between single nucleotide polymorphisms (SNPs) in the *CHREBP* locus (also known as *MLXIPL*), and HDL-C and triglyceride concentrations.[8,9,15,16] In animal studies, hepatic ChREBP is robustly activated by dietary fructose, a major constituent of SSB, and potentiates hepatic lipogenesis and triglyceride secretion.[14,17–20] These findings are consistent with large population-based studies in which high SSB consumption has been associated with elevated fasting plasma triglyceride and reduced HDL-C concentrations,[21–24] and increased type 2 diabetes[25–27] and cardiovascular disease[21] risk. Thus, SNPs within the *CHREBP* locus present promising candidates for gene-SSB interactions on circulating HDL-C and triglyceride concentrations.

These pieces of biological, epidemiological, and genetic evidence suggest that SSB consumption may modify how genetic variants within the *CHREBP* locus influence plasma lipid concentrations in some individuals. Although reduction of SSB consumption is increasingly being encouraged globally,[28] public health efforts to reduce SSB consumption have achieved limited success and SSB consumption remains a modifiable dietary exposure that contributes substantially to the burden of type 2 diabetes and cardiovascular disease worldwide.[29,30] A better understanding of the mechanisms underlying the SSB-ChREBP-lipid relationship may reveal novel mechanisms that contribute to the pathogenesis of type 2 diabetes and cardiovascular disease risk. Understanding these mechanisms may provide alternative strategies and approaches to reduce metabolic disease that may complement or facilitate dietary interventions.

The present study aimed to examine whether SSB consumption may modify the association of genetic variants within the *CHREBP* locus on HDL-C and triglyceride concentrations in aggregated data from cohorts who are part of the Cohorts for Heart and Aging Research in Genetic Epidemiology (CHARGE) consortium.[31] Descriptions of the CHARGE cohorts are included in the Table I in the Data Supplement. We further used data from the UK Biobank (UKB) to assess the reproducibility of these findings in an independent cohort.[32]

METHODS

Methods are available in the Data Supplement. The data that support the findings of this study are available from the corresponding author upon reasonable request. All study participants provided written informed consent, and approval for all study protocols was granted by local institutional review boards and oversight committees.

RESULTS

General characteristics and mean dietary intakes for the 11 CHARGE cohorts are shown in Table 1. Replication of previous findings on associations of SSB consumption and SNPs with lipid traits in the CHARGE cohorts are presented in the Results in the Data Supplement.

Table 1.   General Characteristics of Participating CHARGE Consortium Cohorts*

	Raine Study	ARIC	FHS	NEO	Fenland	YFS	WGHS	WHI	MESA	CHS	RS
Characteristics											
Country	Australia	United States	United States	the Netherlands	United Kingdom	Finland	United States	United States	United States	United States	the Netherlands
n	617	10924	6382	5694	10022	1782	16284	1109	1805	3196	5784
Age, y	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
Body mass index, kg/m²	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/mo, %	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/mo, %	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/wk, %	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/wk, %	29.2	25.7	21.3	11.7	15.2	21.0	15.1	15.3	8.6	18.7	7.5
>1 serving/d, %	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	1857 (850)	1644 (599)	1956 (645)	2291 (763)	1935 (578)	2381 (762)	1732 (524)	1698 (670)	1708 (734)	2024 (654)	2046 (1409)
Saturated fat intake, % 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)	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)

ARIC indicates Atherosclerosis Risk in Communities Study; CHARGE, Cohorts for Heart and Aging Research in Genomic Epidemiology; CHS, Cardiovascular Health Study; FHS, Framingham Heart Study; HDL-C, high-density lipoprotein cholesterol; MESA, Multi-Ethnic Study of Atherosclerosis; n, total sample size; NEO, Netherlands Epidemiology in Obesity Study; RS, the Rotterdam Study; SSB, sugar-sweetened beverages; TG, triglyceride; WGHS, Women's Genome Health Study; WHI, Women's Health Initiative; and YFS, Young Finns Study.

*Means (SD) or percentage for maximum observations available for analysis. Sample sizes may vary depending on availability of genotype and covariate information. Cohorts are ordered by estimate of sugar-sweetened beverage intake.

Difference Test Interactions Between SSB Consumption and SNPs on HDL-C and Triglyceride in CHARGE Cohorts

We identified 55 SNPs that displayed a significant (P_{Diff} <0.0001) or suggestive (P_{Diff} <0.005) difference in estimated effect by category of SSB consumption on HDL-C concentrations in either of the 2 covariate models in the meta-analysis of the CHARGE cohorts. Among these 55 top SNPs, 4 distinct signals for HDL-C concentrations were observed when applying the different test interaction. Two distinct SNPs in moderate LD (linkage disequilibrium) with one another (rs35709627 and rs71556729; R^2=0.55 [Figure II in the Data Supplement]) and in low LD with the top SNP identified in the overall analysis for HDL-C concentrations (R^2<0.3)

Table 2. Top SNPs in Meta-Analysis of Difference Test (P_{Diff} <0.005) and Cross-Product ($P_{interact}$ <0.005) Interactions Between SSB Consumption and SNPs on HDL-C and TG Concentrations in CHARGE Consortium Cohorts*

SNP	Location (Hg19)	Alleles (E/A)†	MAF	Model‡	SSB intake category	n	Effect size (SE)§	P value	Direction‖	I^2	P value¶
HDL-C, mg/dL											
Difference test											P_{Diff}
rs35709627#	72999171	A/G	0.05	Model 1	<1 serving/mo	24389	−0.01 (0.04)	0.86	+-++--+++??	23%	1.98×10⁻⁵**
					>1 serving/d	4033	3.23 (0.77)	2	+?+?+?+?+??	0%	
				Model 2	<1 serving/mo	23801	0.006 (0.04)	0.86	+-++--+++??	30%	0.0001
					>1 serving/d	3955	2.72 (0.72)	0.0002	+?+?+?+?+??	0%	
rs71556729#	72989516	T/C	0.05	Model 1	<1 serving/mo	23974	0.02 (0.06)	0.77	+?++-+++-??	0%	4.78×10⁻⁵**
					>1 serving/d	3359	4.47 (1.10)	5	??+?+?+?+??	0%	
				Model 2	<1 serving/mo	22835	0.01 (0.05)	0.83	+?+++-?-??	0%	0.0001
					>1 serving/d	3299	3.89 (1.04)	0.0002	??+?+?+?+??	0%	
rs71556736	73034929	T/C	0.13	Model 1	<1 serving/mo	24389	−0.0005 (0.02)	0.98	+-+++-++?-	60%	0.0003
					>1 serving/d	4033	1.65 (0.47)	0.0004	+?+?+?+?+??	0%	
				Model 2	<1 serving/mo	23801	0.007 (0.02)	0.69	+-++++-++??	67%	0.002
					>1 serving/d	3955	1.34 (0.43)	0.002	+?+?+?+?+??	0%	
rs73137017	72974413	G/A	0.04	Model 1	<1 serving/mo	24020	−0.05 (0.06)	0.46	-+--+-++-??	0%	0.002
					>1 serving/d	3933	−3.13 (0.99)	0.002	-?-?-?-?-??	0%	
				Model 2	<1 serving/mo	23437	−0.008 (0.05)	0.88	++--+-++-??	0%	0.003
					>1 serving/d	3855	−2.64 (0.91)	0.004	-?-?-?-?-??	0%	
Cross-product interaction test											$P_{interact}$
rs71556729	72989516	T/C	0.03	Model 1	…	55418	0.66 (0.21)	…	+++++?+-+--	0%	0.001
				Model 2	…	53394	0.68 (0.20)	…	++-++?+++?-	26%	0.0007
rs79578725	73002455	A/G	0.05	Model 1	…	53662	−0.51 (0.18)	…	+?-+-?-----	0%	0.005
				Model 2	…	52328	−0.18 (0.17)	…	+?++-?-----	0%	0.28
TG, ln-mg/dL											
Difference test											P_{Diff}
rs799157	73020301	T/C	0.05	Model 1	<1 serving/mo	23974	0.02 (0.01)	0.11	+?++++-++??	59%	0.005
					>1 serving/d	4033	0.11 (0.03)	0.002	+?+?+?+?+??	0%	
				Model 2	<1 serving/mo	23403	0.02 (0.01)	0.17	+?++--?+?	68%	0.008
					>1 serving/d	3955	0.09 (0.03)	0.004	+?+?++?+?	0%	
Cross-product interaction test											$P_{interact}$
rs55673514	73021456	G/A	0.04	Model 1	…	57977	0.02 (0.01)	…	-+++++++?++	17%	0.04
				Model 2	…	56578	0.02 (0.01)	…	-+++++++++?	0%	0.005

CHARGE indicates Cohorts for Heart and Aging Research in Genetic Epidemiology; CHS, Cardiovascular Health Study; FHS, Framingham Heart Study; HDL-C, high-density lipoprotein cholesterol; MAF, minor allele frequency; MESA, Multi-Ethnic Study of Atherosclerosis; NEO, Netherlands Epidemiology in Obesity Study; RS, the Rotterdam Study; SNP, single nucleotide polymorphism; SSB, sugar-sweetened beverages; TG, triglyceride; WGHS, Women's Genome Health Study; and YFS, Young Finns Study.

*Top signals represent suggestive interactions P_{Diff} <0.005 or $P_{interact}$ <0.005.

†Alleles presented as effect (E)/alternative (A) alleles.

‡Model 1 adjusted for age (y), sex (male/female), total energy intake (kcal/d) field center (CHS, FHS, YFS, Fenland, RS, MESA), and accounted for family or population structure where applicable (FHS, YFS, Fenland, NEO, MESA, WGHS, Raine Study, MESA); model 2 adjusted for model 1 covariates plus cohort-specific definition of education, smoking, physical activity, alcohol intake, and body mass index (kg/m²).

§For the difference test, β (SE) represents the direction and magnitude of the difference in the outcome trait with each additional effect allele among categories of SSB consumption. For the cross-product interaction test, β (SE) represents the direction and magnitude of the difference in the outcome trait with each additional effect allele, per each increase in category of SSB intake (<1 serving/mo, 1−4 servings/mo, 1−2 servings/wk, 3−7 servings/wk, >1 serving/d).

‖Order of cohorts for regression coefficient directions: FHS, YFS, Fenland Study, CHS, NEO, RS, WGHS, WHI, ARIC, Raine Study, MESA (+, positive effect size; −, negative effect size; and ?, SNP not available in cohort).

¶P represents P_{Diff} for the difference test for the highest and lowest category of SSB intake (<1 serving/mo vs >1 serving/d). P represents $P_{interact}$ for the cross-product interaction regression coefficient of additive SSBxSNP categories.

#Linkage disequilibrium (R^2) between rs13240662 and rs71556729=0.55 in European ancestry groups of phase 3 (version 5) of the 1000 genomes project.

**Statistically significant interaction based on Bonferonni-corrected P_{Diff} or $P_{interact}$ <0.0001.

displayed a statistically significant difference in effect by category of SSB intake on HDL-C concentrations in fully adjusted models (model 2; P_{Diff} <0.0001; Table 2 and Figures III and IV in the Data Supplement). In model 2, each additional minor allele at rs35709627 (β [SE], 2.72 [0.72], P=0.0002) and rs71556729 (β [SE], 3.89 [1.04], P=0.0002) was associated with higher mean concentrations of HDL-C concentrations among the highest SSB consumers (>1 serving/d), but was not associated with mean HDL-C concentrations among the lowest SSB

consumers (<1 serving/mo; P>0.05). The effect sizes of these SNPs among the highest SSB consumers were consistent across all the cohorts. There was no heterogeneity (I^2=0%) observed for the top 4 distinct signals (statistically significant and suggestive) among the highest SSB consumers (>1 serving/d), which could be due to low power to detect heterogeneity given the smaller sample size available among the highest SSB consumers (maximum, n=4033).

No statistically significant differences in effect by category of SSB intake on triglyceride concentrations were observed when applying the difference test (P_{Diff} >0.0001 for all SNPs). One SNP (rs799157) in moderate LD with a top SNP identified in the overall analysis for triglyceride concentrations (Table X in the Data Supplement; R^2 with rs42124=0.44) displayed a suggestive difference in effect by category of SSB intake on triglyceride concentrations in minimally adjusted models (model 1; P_{Diff}=0.005; Table 2). Each additional minor allele at rs799157 was associated with higher mean triglyceride concentrations among the highest SSB consumers (>1 serving/d; 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=0.11; Figure V in the Data Supplement). The direction of the effect size of this SNP among the highest SSB consumers was consistent across all the cohorts in which these SNPs were available, and heterogeneity was low among the highest SSB consumers (I^2=0%).

Cross-Product Interactions Between SSB Consumption and SNPs on HDL-C and Triglyceride in CHARGE Cohorts

No statistically significant cross-product interactions between SNPs and SSB consumption on HDL-C or triglyceride concentrations were observed ($P_{interaction}$ >0.0001), while some tests were suggestive ($P_{interaction}$ <0.005; Table 2). Three SNPs displayed a suggestive interaction with SSB consumption on HDL-C concentrations in either covariate model, and the clumping identified 2 distinct signals (rs71556729 and rs79578725). One SNP (rs55673514) displayed a suggestive interaction with SSB on triglyceride concentrations in model 2. Forest plots for top distinct signals in SSBxSNP interaction analyses on lipid traits are presented in Figures VI and VII in the Data Supplement.

Interactions Between SSB Consumption and SNPs on Lipid Traits in the UKB and Meta-Analysis With CHARGE Cohort Results

General characteristics and mean dietary intakes for the 59 220 UKB participants are shown in Table VI in the Data Supplement. Two out of 5 top signals for HDL-C

(rs35709627 and rs71556729) and one out of 2 top signals for triglyceride in the CHARGE consortium were replicated among the UKB participants (Table VII in the Data Supplement). In a meta-analysis of the top results from the CHARGE consortium and data from the UKB, 3 out of the 5 top SNPs for HDL-C and one out of the 2 top SNPs for triglyceride concentrations displayed statistically significant interactions (Table 3). The top SNP for HDL-C concentrations was located at rs71556729 (Figure 1A). In fully adjusted models, the association between the minor allele at rs71556729 with HDL-C concentrations was observed only among the highest SSB consumers (β, 2.12 [95% CI, 1.16–3.07] mg/dL, P<0.0001) and not the lowest SSB consumers (P=0.81; P_{Diff} <0.0001). Similarly, 2 SNPs in low to moderate LD with rs71556729 (TBL2-rs35709627: R^2 with rs71556729=0.55; rs71556736: R^2 with rs71556729=0.19) displayed similar statistically significant differences in effect by category of SSB intake (P_{Diff} <0.0001). The SNP at rs55673514 displayed a suggestive interaction with triglyceride concentrations in the CHARGE meta-analysis and was statistically significant after including data from the UKB (Figure 1B, P_{Diff} <0.0005). The association of the minor allele at rs55673514 with triglyceride concentrations was observed only among the highest SSB consumers (β, 0.06 [95% CI, 0.02–0.09] ln-mg/dL, P=0.001) and not the lowest SSB consumers (P=0.84). The SNP at rs55673514 is not in appreciable LD with any of the top SNPs in the overall analysis for triglyceride concentrations (R^2<0.1). A heatmap of LD among top SNPs in overall and interaction analyses is provided in Figure II in the Data Supplement. Sensitivity analyses examining the influence of adjustment for other dietary factors and fasting hours among UKB participants yielded similar results for the top SNPs identified in the meta-analysis (Results in the Data Supplement).

DISCUSSION

In this study, including up to 86 241 participants for whom genetic and SSB consumption data were available, we identified novel interactions between genetic variants at the CHREBP locus and SSB consumption on HDL-C and triglyceride concentrations. Our data suggest that the magnitude of the inverse association between SSB consumption and HDL-C concentrations is lower among individuals harboring genetic variants at rs71556729, rs35709627, and rs71556736, and the positive association between SSB consumption and triglyceride concentrations is exacerbated among individuals harboring genetic variants at rs55673514. In the CHARGE cohorts, we also observed a consistent inverse association between SSB consumption on fasting HDL-C and positive association on triglyceride concentrations. We also replicated previously observed main associations between SNPs in the CHREBP locus and HDL-C and triglyceride concentrations.

Table 3. Fixed-Effect Meta-Analysis of Top Candidate SNPs for Difference Test Interactions Between SSB Consumption and SNPs on HDL-C and TG Concentrations in CHARGE Consortium Cohorts and UKB*

SNP	Location (Hg19)	Alleles (E/A)†	MAF	SSB intake category	n	Effect size (SE)	P value	Direction‡	I^2	P_{Diff}
HDL-C, mg/dL										
rs71556729§	72989516	T/C	0.05	Low	68701	0.01 (0.05)	0.81	++	0%	1.5×10^{-6}‖
				High	15227	2.06 (0.44)	3.48×10^{-6}	++	74%	
rs35709627§	72999171	A/G	0.05	Low	69667	0.01 (0.04)	0.74	++	0%	1.0×10^{-5}‖
				High	15883	1.37 (0.32)	2.15×10^{-5}	++	87%	
rs71556736	73034929	T/C	0.13	Low	69667	0.02 (0.02)	0.33	++	93%	2.5×10^{-5}‖
				High	15882	0.84 (0.20)	3.27×10^{-5}	++	42%	
rs73137017	72974413	G/A	0.04	Low	69303	0.01 (0.05)	0.82	++	0%	0.04
				High	15783	0.73 (0.37)	0.05	++	81%	
rs79578725	73002455	A/G	0.05	Low	68929	−0.02 (0.04)	0.64	− −	21%	0.55
				High	15783	−0.22 (0.36)	0.53	− −	0%	
TG, ln-mg/dL										
rs55673514	73021456	G/A	0.04	Low	69096	−0.002 (0.01)	0.84	+−	29%	0.0005‖
				High	15395	−0.06 (0.02)	0.001	− −	0%	
rs799157	73020301	T/C	0.05	Low	70235	0.03 (0.01)	2.55×10^{-7}	++	59%	0.05
				High	16006	0.06 (0.02)	0.0002	++	19%	

CHARGE indicates Cohorts for Heart and Aging Research in Genetic Epidemiology; HDL-C, high-density lipoprotein cholesterol; MAF, minor allele frequency; SNP, single nucleotide polymorphism; SSB, sugar-sweetened beverages; TG, triglyceride; and UKB, UK Biobank.

*Top candidates represent statistically significant or suggestive interactions (P_{Diff} <0.005 or $P_{interact}$ <0.005) in CHARGE cohort meta-analysis. Models adjusted for age, sex, total energy intake, field center, and accounted for family or population structure where applicable plus education, smoking, physical activity, alcohol intake, and body mass index (kg/m²). For the difference test, interaction coefficients are shown as β (SE), where β represents the direction and magnitude of change in the outcome trait with each additional effect allele among participants with low (CHARGE <1 serving/mo; UKB: nonconsumers) or high (CHARGE >1 serving/d; UKB: consumers) SSB consumption.

†Alleles presented as effect (E)/alternative (A) alleles.

‡Order of cohorts for regression coefficient directions: CHARGE cohorts, UKB (+, positive effect size; −, negative effect size).

§Linkage disequilibrium (R^2) between rs13240662 and rs71556729=0.55 in European ancestry groups of phase 3 (version 5) of the 1000 genomes project.

‖Indicates a statistically significant interaction based on Bonferroni-corrected P_{Diff} <0.01 (0.05/5 top signals) for HDL-C and P_{Diff} <0.025 (0.05/2 top signals) for TG concentrations.

Our study provides evidence that SSB consumption may modify the association of genetic variants in the *CHREBP* locus with HDL-C and triglyceride concentrations. Participants with the minor allele at rs71556729, rs35709627, and rs71556736 and high SSB consumption had higher mean HDL-C concentrations than those with the major allele who also had high SSB consumption. This suggests that participants with the minor allele at rs71556729 (MAF [minor allele frequency]=0.05), rs35709627 (MAF=0.05), and rs71556736 (MAF=0.13) may be protected against SSB-induced reductions in HDL-C concentrations. The region containing these SNPs is enriched for enhancer histone marks and these SNPs lie within putative regulatory motifs for transcription factors that could potentially regulate ChREBP expression and function in an SSB-dependent manner.[33] Similarly, rs55673514, which associates with triglyceride only among the highest SSB consumers, lies within a region enriched for enhancer histone marks in several tissues, including liver.[33] Given the strong inverse relationship between HDL-C and triglyceride concentrations, additional investigation into how these SNPs may independently influence HDL-C or triglyceride concentrations could provide new insights into the distinct mechanisms contributing to plasma HDL-C and triglyceride concentrations. Additional discussion of main associations between SNPs and SSB on triglyceride and HDL-C in the CHARGE cohorts is provided in the Discussion in the Data Supplement.

The rs71556729 interaction was a top signal when testing for interactions using the difference test and the cross-product interaction test on HDL-C concentrations in the CHARGE cohorts. However, when applying the cross-product interaction test, the interaction appeared less significant than the result from the difference test. This may be due to heterogeneity in the association between rs71556729 and HDL-C concentrations resulting from increased misclassification of SSB consumption among those reporting low (1−4 servings/mo) to moderate (1−2 and 3−7 servings/wk) SSB consumption (Figure IV in the Data Supplement). These results 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 nonlinear interaction effects. However, we do not comprehensively compare the difference test to the cross-product interaction test. Future methodological studies comparing the usefulness of these 2 methods with varying degrees of
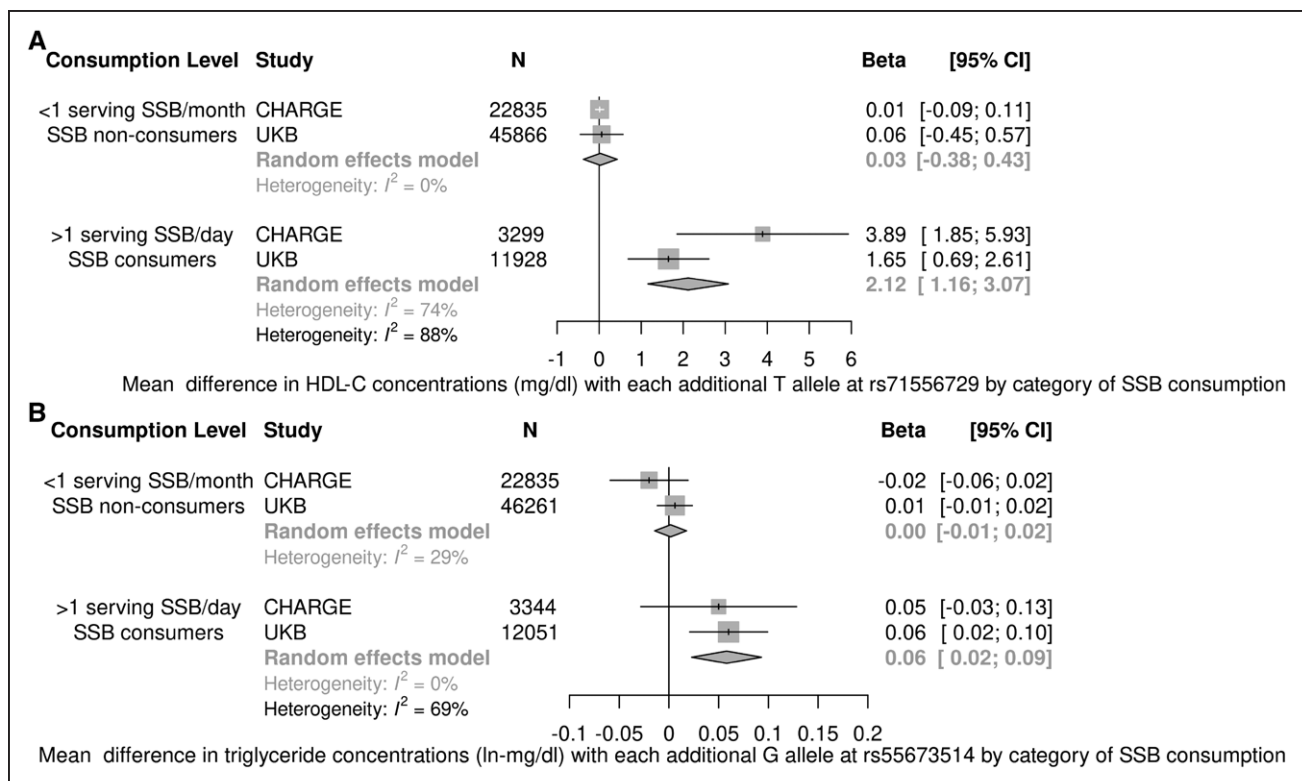
Figure. Associations between top candidate single nucleotide polymorphisms (SNPs) and HDL-C (high-density lipoprotein cholesterol) and triglyceride (TG) concentrations stratified by category of sugar-sweetened beverages (SSB) intake in a random effects meta-analysis of the Cohorts for Heart and Aging Research in Genetic Epidemiology (CHARGE) cohorts and the UK Biobank (UKB).
A, In a random effects meta-analysis of the CHARGE cohorts and the UKB, the association of the minor allele at rs71556729 with HDL-C concentrations was observed only among the highest SSB consumers (β, 2.12 [95% CI, 1.16–3.07] mg/dL, $P<0.0001$) and not the lowest SSB consumers ($P=0.81$; $P_{Diff}<0.0001$). **B**, In a random effects meta-analysis of the CHARGE cohorts and the UKB, the association of the minor allele at rs55673514 with TG concentrations was observed only among the highest SSB consumers (β, 0.06 [95% CI, 0.02–0.09] ln-mg/dL, $P=0.001$), and not the lowest SSB consumers ($P=0.84$; $P_{Diff}<0.0005$); linear regression models represent associations between each additional effect allele and HDL-C (mg/dL) or TG (ln-mg/dL) concentrations among SSB consumption categories accounting for family, population structure, and field center (where applicable) and adjusting for age, sex, total energy intake, education, smoking, physical activity, alcohol intake, and body mass index. Intake categories are different for the highest SSB consumers (CHARGE: >1 serving/d; UKB: SSB consumers) and lowest SSB consumers (CHARGE: <1 serving/mo; UKB: SSB nonconsumers) in the 2 samples.
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misclassification and types of exposures may be useful to inform future gene-diet interaction studies.

There is a limited body of evidence describing how genes implicated in various diseases may interact with SSB consumption to modify cardiometabolic health and noncommunicable disease risk.[34] 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.[35] Although genetic risk scores can be useful for translation, as previously shown for the interaction between SSB consumption and genetic risk for obesity,[36] 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, interaction effects of SNPs may be mitigated by the null interaction 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 cohorts, but we did not identify a significant cross-product interaction that was consistent among the discovery and replication phases of that study.[37] In the current study, we applied a 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 indicate that there may be additional SNPs not identified in GWAS contributing to the heritability of HDL-C and triglyceride concentrations, but their contribution is influenced by SSB consumption. Similar to previous GWAS for body mass index that have identified new loci when adjusting for environmental factors,[38,39] we provide an additional example of how missing genetic heritability may be revealed when accounting for environmental factors, such as SSB consumption in the current study.

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, the use of an external cohort to validate findings, and the use of multiple methods to screen for potential interactions between SSB consumption and over 1606 SNPs in the CHREBP region on HDL-C and triglyceride concentrations. The analytic approach revealed novel SNPs that may contribute to unexplained heritability of HDL-C and triglyceride concentrations. Limitations of this study include its observational design that constrain our ability to infer causality, the sample of European-descent adults that limits generalizability, the use of self-reported dietary data from food frequency questionnaires and 24-hour recall that may lead to misclassification of food and nutrient intakes, and the possibility of residual confounding, even after controlling for potential dietary and lifestyle factors that co-vary with SSB intake. Our focus on the comparison of the highest SSB consumers to the lowest SSB consumers helps minimize this potential misclassification by focusing on extreme consumption patterns. Misclassification in the UKB is likely given that a snapshot of intake on a single day cannot provide a reliable estimate of usual SSB consumption. However, this misclassification is likely nondifferential by genotype, which would only result in attenuation of our results. Additionally, while our definition of SSB did consider a range of SSB, it was not comprehensive. For example, it did not include commonly consumed beverages, such as sweetened tea or coffee, and we included several types of SSB in the same exposure definition (colas and fruit drinks). The blood collection among UKB participants was conducted after less than the recommended 8 hours of fasting before measurement of lipids. We adjusted for fasting hours to help account for this variability and conducted a sensitivity analysis to examine the top interactions observed by fasting hours. The LD-based method used to estimate the number of independent tests in the region may be overly conservative, which could potentially lead to inflation of type II error rate. Thus, we additionally present suggestive results that did not reach statistical significance. Given these weaknesses, results from this study should be used to inform future studies with larger samples sizes or detailed experimental studies. Minority populations are disproportionality burdened by dyslipidemia and have higher SSB intake,[40,41]

and thus more studies in these populations may help reduce health inequality and disparity.

In conclusion, our findings suggest that the minor alleles of 3 SNPs in the CHREBP region (rs71556729, rs35709627, and rs71556736) may be protective against SSB-induced low HDL-C concentrations and the minor allele at rs55673514 may exacerbate positive associations between SSB consumption and triglyceride concentrations. Several of the top SNPs identified in the interaction analyses were not top SNPs identified in the overall analyses, providing evidence that some genetic associations may be revealed only when conditioned on environmental factors, such as the range of SSB consumption in the current study. As larger data sets with genetics, diet, and lipids data become available, additional suggestive interactions between SSB consumption and SNPs within the CHREBP region on HDL-C and triglyceride concentrations observed here may warrant further investigation.

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Supplemental Materials

Supplemental Methods
Supplemental Results
Supplemental Discussion
Supplemental Tables I–XI
Supplemental Figures I–XXII
Appendix I

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