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The effect of *CYP7A1* polymorphisms on lipid responses to fenofibrate

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Abstract

CYP7A1 encodes cholesterol 7α-hydroxylase an enzyme crucial to cholesterol homeostasis. Its transcriptional activity is down-regulated by fenofibrate. The goal of this study was to determine the effect of *CYP7A1* polymorphisms on lipid changes in response to fenofibrate. We examined associations of three tagging single nuclear polymorphisms (SNP) (i6782C>T, m204T>G, 3U12536A>C) at *CYP7A1* with triglyceride (TG) and HDL-C responses to a 3-week treatment with fenofibrate 160 mg/d in 864 US White participants from the Genetics of Lipid Lowering Drugs and Diet Network (GOLDN) study. The m204T>G variant significantly associated TG and HDL-C responses to fenofibrate. Individual homozygous for the common T allele of m204T>G SNP displayed both the greater reduction of TG (−32% for TT, −28% for GT, −25% for GG, $P = 0.004$) and an increase of HDL-C response compared to non-carriers (4.1% for TT, 3.4% for GT, 1.2% for GG, $P = 0.01$). Conversely, individuals homozygous for the minor allele of i6782C>T showed greater increase of HDL-C response compared to non-carriers (2.8% CC, 4.5% for CT, 5.8% for TT, P=0.02) albeit no significant effect on TG response. Our data suggest that common variants at *CYP7A1* locus modulate the TG lowering and HDL-C raising effects of fenofibrate, and contribute to the interindividual variation of the drug responses.

Keywords

CYP7A1; polymorphism; TG response; fenofibrate

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Introduction

Cholesterol 7α-hydroxylase, encoded by *CYP7A1* catalyzes the first and rate-limiting step in classical bile acid synthesis in the liver, and thus plays a key role in cholesterol homeostasis [1]. Because of the coordination between the regulation of bile acid on the synthesis and secretion of triglyceride (TG) and TG-rich lipoproteins [2, 3], CYP7A1 also regulates plasma TG levels[4]. A loss-of function mutation of this gene which causes the absence of cholesterol 7α-hydroxylase activity in humans has been linked to an atherogenic lipid profile including hypercholesterolemia and hypertriglyceridemia, and premature atherosclerosis [5]. In contrast to human, CYP7A1 deficiency in mice decreases VLDL particles and plasma triglycerides as a consequence of reduced bile acid biosynthesis [6]. CYP7A1 transgenic mice displays increase in hepatic production and secretion of triglyceride-rich apoB-containing lipoproteins although plasma levels of triglycerides and cholesterol are not significantly increased possibly due to concomitant up-regulated low density lipoprotein receptor [7]. The observed conflicting effects on plasma triglycerides could be due to sex, genetic background, diet or other environmental factors, more importantly, may reflect the complex role of CYP7A1 and bile acid biosynthesis in maintaining the homeostasis of the anabolic lipoprotein assembly/secretion pathway with the cholesterol catabolic pathway. The common variants at the *CYP7A1* locus, promoter variant m204T>G (rs3808607) in particular, have been studied extensively yielding descriptions of genetic impact on hydroxylase activity, fasting plasma LDL cholesterol and triglyceride [8-10] as well as modulation of lipid responses to statin treatment and dietary manipulations [11, 12].

Fenofibrate is a fibrate derivative commonly used in clinics for the management of hypertriglyceridemia [13]. As a potent ligand for peroxisome proliferator-activated receptor (PPARα), the metabolic effect of this drug is mediated through PPAR-dependent pathways which regulate transcription of target genes involved in lipid metabolism. Individual responses to fenofibrate vary considerably and are subject to genetic regulation. However, genetic determinants of fenofibrate response are largely unknown. It has been shown that fenofibrate down-regulates *CYP7A1* expression via activation of PPARα binding to the PPAR response element in the promoter region of the gene [14]. Hence, *CYP7A1* represents a candidate gene that may harbor genetic variants contributing to differential responses to fenofibrate among individuals. The goal of this investigation was to examine the effect of common variants at *CYP7A1* locus on lipid responses to a 3-week fenofibrate treatment in US Whites participating in the Genetics of Lipid Lowering Drugs and Diet Network (GOLDN) study.

Materials and Methods

Subjects

The GOLDN study population consisted of 1120 individuals (539 men and 581 women) whose participants were re-recruited from the NHLBI Family Heart Study (FHS) [15] in two genetically homogeneous centers (Minneapolis, MN and Salt Lake City, UT) with predominantly White populations. The study was a single arm, uncontrolled, non-randomized intervention funded by the NHLBI with the purpose of identifying genetic variants associated with interindividual variability of triglyceride responses to a high-fat meal and fenofibrate. The design and methodology of the study have been described [16, 17]. Briefly, participants were given open-labeled fenofibrate 160 mg tablets (TriCor®, Abbott Laboratories, and Chicago, IL) and informed to take one tablet with a breakfast meal once daily for three weeks. A total of 859 participants with complete genotype data and full record for pre- and post-drug intervention lipid measurements were included in final analyses. The protocol for this study was approved by the Human Studies Committee of

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Institutional Review Board at University of Minnesota, University of Utah and Tufts University/New England Medical Center. Written informed consent was obtained from all participants.

Biochemical Measurements

Triglycerides were measured using a glycerol blanked enzymatic method (Trig/GB, Roche Diagnostics Corporation, Indianapolis, IN) on the Roche/Hitachi 911 Automatic Analyzer (Roche Diagnostics Corporation). Cholesterol was measured on the Hitachi 911 using a cholesterol esterase, cholesterol oxidase reaction (Chol R1, Roche Diagnostics Corporation). The same reaction was also used to measure HDL-C after precipitation of non-HDL-C with magnesium/dextran. LDL-C was measured by a homogeneous direct method (LDL Direct Liquid Select™ Cholesterol Reagent, Equal Diagnostics, Exton, PA) on the Hitachi 911. Lipoprotein particles concentration and size, including TG-rich lipoproteins, LDL and HDL, were measured by NMR. The quantification of the lipoprotein subclass was based on amplitudes of their spectroscopically distinct nuclear magnetic resonance signals of the lipid methyl group [18].

DNA isolation and genotyping

Genomic DNA was extracted from blood samples and purified using commercial Puregene® reagents (Gentra System, Inc.) following the manufacturer's instructions. Three common *CYP7A1* polymorphisms (i6782C>T (rs11786580), 3U12536 A>C (rs10957056), and m204T>G (rs3808607)) were selected for genotyping based on literature reports, the potential functionality or the representation of the common haplotypes at the *CYP7A1* locus which covers 10kb of gene region and 5kb upstream and 5kb downstream of the gene. Genotyping was performed using the 5′nuclease allelic discrimination Taqman assay with ABI 7900HT system (Applies Biosystems, Foster City, CA, USA). SNP names were designated by their positions relative to the transcription initiation site ("m" indicating the minus residing in the gene control or promoter region, "i" indicating the intronic region and "3u" indicating the 3′UTR region). The description of primer and probe sequences as well as ABI assay-on-demand IDs are presented in supplemental **Table 1**.

Statistical analyses

Statistical analyses were performed using SAS for Windows version 9.2 (SAS Institute, Cary, NC). A Chi-square test was used to determine if genotype frequency distribution followed Hardy-Weinberg equilibrium. A logarithmic transformation was applied to all lipid parameters to normalize the distribution of data. The lipid parameters in response to fenofibrate treatment were calculated as the percent change (the difference between pre and post-fenofibrate treatment values divided by the baseline value). We used the generalized estimating equation (GEE) approach with exchangeable correlation structure as implemented in the GENMOD procedure in SAS to adjust for correlated observations due to familial relationships. Potential confounding factors examined included age, gender, BMI, field center, smoking status, alcohol consumption, physical activity. Because drugs for lowering cholesterol, diabetes and hypertension, and hormones have been shown to affect lipid levels and therefore, may potentially change lipid responses, we also included those medi cation use in the final analysis. Pair-wise linkage disequilibrium (LD) between SNPs was estimated as a correlation coefficient in unrelated subjects using Haploview [19]. A two-tailed P-value of <0.05 was considered as statistically significant.

Results

The demographic, biochemical and genotypic characteristics of subjects at baseline (mean age ± SD, 49±16y, 52% female) are presented in Table 1. The genotype frequency for the

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3U12536 A>C deviated from Hardy-Weinberg equilibrium (P<0.05), and thus was excluded from the analysis. The minor allele frequencies for the i6782C>T and m204T>G variants were 0.20 and 0.43, respectively. There was weak linkage disequilibrium between those two SNPs (r^2=0.145). Table 2 describes the effect of the m204T>G on lipid response to a 3-week fenofibrate intervention. Because no significant gene-gender interactions were observed, we analyzed men and women together. There were no significant cross-sectional associations between this SNP and lipid measurements both before and after the intervention. However, there were significant genotypic differences of the TG lowering and HDL raising effects of fenofibrate. Major T allele homozygotes for m204T>G displayed the greatest reduction of TG after treatment, whereas heterozygotes and minor allele homozygotes displayed an intermediate and the smallest reduction of TG, respectively (−33% for TT, −28% for TG, −25% for GG, P = 0.004). Major T allele carriers also had higher increases in HDL response than non-carriers (4.1% for TT, 3.4% for GT, 1.2% for GG, P = 0.01). Moreover, there were significant differences of TG-rich lipoprotein responses after fenofibrate treatment across the genotype groups (Table 3). Major TT carriers showed the greatest reduction of VLDL-TG, large and medium VLDL compared with G allele carriers (VLDL-TG: −43% for TT, −38% for TG, −33% for GG, P =0.007; Large VLDL: −36% for TT, −31% for TG, −23% for GG, P=0.035; medium VLDL: −56% for TT, −49% for TG, −41% for GG, P=0.003). As for SNP i6782C>T, there was no significant genotypic effect on TG responses to fenofibrate (−29% for CC, −29% for CT, −37% for TT, P=0.19). However, major C allele homozygous individuals displayed less induction of HDL in response to fenofibrate than those with minor T allele carriers (2.8% CC, 4.5% for CT, 5.8% for TT, P=0.02) (Table 4). We further examined the associations with changes of particle size and HDL subclass in response to fenofibrate and observed no significant genotypic effect for both SNPs. (Supplemental Table 2-4).

Discussion

In this study we observed differential responses of TG and HDL-C levels by CYP7A1 genotype to a 3-week fenofibrate intervention. Specifically, individuals homozygous for the major T allele of the promoter SNP m204T>G displayed a greater reduction of total plasma TG and TG-rich VLDL than did those carrying GT and GG genotypes. The common T allele carriers also had greater increases in HDL-C compared to non-carriers. Conversely, individuals homozygous for the minor T allele of i6782C>T showed greater increase of the HDL response compared to non-carriers although there was no significant effect of this SNP on TG response.

Fenofibrate improves the dyslipidemic profile through the reduction of plasma TG, VLDL-C and the increase of HDL-C [20]. As a target of fenofiibrates, CYP7A1 mediates the effect of this drug on lipid and lipoprotein metabolism. In the present study, a common genetic variant in the regulatory region of *CYP7A1*, namely, m204T>G significantly modulates fenofibrate efficacy with the major allele associated with a more favorable response relative to the minor allele. Our findings agree with previous studies reporting that the major T allele was associated with high responsiveness with regard to atorvastatin-induced total cholesterol and LDL-C reduction [21, 22]. Furthermore, patients with coronary atherosclerosis carrying the T allele showed better responses to a 2-year pravastatin treatment with a greater reduced progression of atherosclerosis compared to non-carrier patients [23]. However, in response to a high intake of dietary cholesterol, the minor G allele was associated with a higher response of HDL-C and total cholesterol compared to non-carriers [12]. Likewise, individuals carrying the minor G allele experienced a greater reduction of plasma TG levels than did those non-carriers after a 6- to 8-week low-fat diet in a group of dyslipidemic males [11]. Despite the inconsistent directionality of the allele effect, current studies provide

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evidence that the m204T>G SNP significantly determines the effect of lipid-lowering drug and thus, may predict the future clinical CVD event.

Although there were no direct measurements of gene expression levels or protein enzyme activity of *CYP7A1* associated with m204T>G in the study, several line of evidence may help predict the potential functionality of this variant. The m204T>G is located in a region of conserved sequence containing several transcription factor binding motifs for PPARs and hepatocyte nuclear factor-4 (HNF4) [24] and represents the haplotype block covering a substantial part of the promoter and the first exon of this gene [25]. *In vitro* studies have indicated that PPARα and fibrate reduce the availability of HNF-4 for binding to the response sequence or competing with HNF-4 for the same binding site and therefore attenuate the transactivation of CYP7A1 by HNF-4 [24, 26]. We speculate that minor allele substitution associated with poor responsiveness to fenofibrate may decrease the binding affinity or even abolish the binding site for those transcription factors, and thus impair the transrepression of the *CYP7A1* gene in response to drug-dependent PPAR activation. In contrast, normal or high drug responsiveness associated with the major allele could be due to its ability to maintain the integrity of the transcription factor binding motifs. In fact, a recent study showed that the major T allele was associated with higher enzymatic activity of CYP7A1 compared with the minor G allele in patients with ileal resection, a condition associated with up-regulated *CYP7A1* expression due to bile salt malabsorption, whereas in healthy individuals there was no genetic effect of this SNP on CYP7A1 activity [27]. Those observations together with ours suggest that the m204T>G polymorphism could be functional and important in response to different types of stimuli, such as pathophysiological conditions, exposure to a pharmacological agent or dietary modifications which either up- or down-regulates *CYP7A1* expression.

Emerging evidence suggests a role for CYP7A1 in the regulation of triglyceride metabolism. In our study individuals carrying the major T allele of the m204 T>G presumably associated with greater reduction of CYP7A1 after fenofibrate had greater reduction of TG, mainly due to significantly decreased total VLDL and large and medium VLDL subfractions, compared with those non-carriers. The underlying mechanisms may include the regulation of *CYP7A1* on sterol response element-binding protein, the main regulator of hepatic fatty acid and TG biosynthesis [28], and its targeted genes which encode enzymes required for VLDL production and assembly and secretion [7, 29]. Bile acids may additionally mediate CYP7A1 impact through bile acid-activated nuclear hormone receptor farnesoid X receptor (FXR or NR1H4) which regulates a number of genes involved in lipogenesis and fatty acids metabolism [6, 30]. Our data also demonstrated that the m204T>G polymorphism significantly determined HDL-C responses with the T allele associated with the greater responsiveness of fenofibrate-induced HDL-C increases. Interestingly, genetic loci affecting *CYP7A1* expression have been linked also to diet-induced HDL-C changes in mice [31], suggesting coordinate regulation between CYP7A1 and HDL-C levels. In our study, the genetic effect of this SNP on HDL-C responses was not specific to any types of HDL subfractions. Nonetheless, the observed effect of the m204T>G SNP on HDL-C responses could reflect its impact on TG responses through the coordinated regulation of TG and HDL metabolism with its known seesaw effect. Adjusting for the change of TG response attenuated, but did not remove the statistical significance of our findings, suggesting additional mechanisms may be involved. One such mechanism may be the bile acid-activated FXR pathway which also has profound effects on HDL-C metabolism and remodeling [32].

In contrast to the m204T>G, there was no significant association between the intronic i6782C>T polymorphism and TG responses after fenofibrate intervention. However, the major allele of this variant displayed less responsiveness to fenofibrate-induce HDL–C

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increases compared with the minor allele. The differential modulation of these two variants on fenofibrate responses may stem from independent mechanisms which the two variants mediate as both variants are identified as tagging SNPs [25] with limited correlation and each representing an independent haplotype in a White population.

Our findings could be clinically relevant for several reasons. First, clinical evidence regarding the efficacy of fibrates on the reduction of CVD events has been inconsistent [33, 34] despite the well-established role of fibrates in normalizing lipid profiles [35]. Our data provide insight into the heterogeneity of the treatment response, suggesting that genetic difference could define individuals' response to fenofibrate, and thus, potentially may affect the disease outcome. Second, if an increase in HDL-C level translates into the reduction of CVD [36, 37], those carrying the major allele of the m204T>G or /and the minor allele of the i6782C>T variant which predicted the greater HDL-C responses may benefit the most from fenofibrate and therefore, have better clinical outcomes than the non-carriers who may require different lifestyle modification or therapeutic regime. However, because we were unable to detect significant effect of *CYP7A1* variants on lipid response in subjects with hypertriglyceridemia (triglycerides over 200 mg/dl) possibly due to low power given only 151 subjects in this subgroup (data not shown), our finding should be interpreted with caution in terms of clinical utility. Furthermore, the results from the current study need to be replicated in independent intervention studies particular in hyperlipidemia patients to validate association signals.

Conclusions

Our data suggest that common variants at the *CYP7A1* locus modulate the TG lowering and HDL-C rising effects of fenofibrate, and contribute to the interindividual variation of the response to fenofibrate. These findings have clinical implications as to the identification of individuals who may benefit the most from fenofibrate and ultimately provide more personalized treatment based on genetic differences.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Table1

Baseline characteristics of the study population

Characteristics	N= (1120)
Female	579 (52%)
Age (years)	48.6±16.4
BMI (kg/m^2)	28.3±5.6
Insulin (uU/ml)	13.7±8.1
Glucose (mg/dl)	101.5±18.8
Triglycerides (mg/dl)	138.7±116
Cholesterol (mg/dl)	190.6±39
HDL-C (mg/dl)	47.1±13
LDL-C(mg/dl)	121.5±31.3
Current drinker	559 (50%)
Current smoker	87 (7.7%)
Physical activity score	34±6.3
CYP7A1_m204	
TT	364
TG	547
GG	207
CYP7A1_i6782	
CC	711
CT	359
TT	47

Values are presented as number (%), or mean ± standard deviation

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Table 2

Lipid responses to fenofibrate according to *CYP7A1* m204T>G genotypes.

	Genotype	Before drug: Geometric mean (95%CI)	Before drug: P*	After drug: Geometric mean (95%CI)	After drug: P*	Response: % change (95%CI)	Response: P*
Triglycerides (mg/dl)	TT	138.68(123.34-155.92)	0.79	94.36(84.70-105.12)	0.13	−31.9(−36.7-−26.7)	0.004
	TG	139.22(124.68-155.45)		98.67(88.67-109.77)		−28.3(−32.9-−23.3)	
	GG	136.14(118.47-156.49)		101.58(89.76-114.95)		−24.6(−30.9-−17.9)	
Total cholesterol (mg/dl)	TT	181.26(173.54-189.37)	0.38	161.85(155.78-168.11)	0.16	−10.9(−13.2-−8.4)	0.4
	TG	181.26(174.10-188.71)		162.93(157.00-169.08)		−10.0(−12.4-−7.6)	
	GG	184.63(176.20-193.46)		166.04(158.96-173.42)		−10.2(−12.9-−7.5)	
HDL-C (mg/dl)	TT	45.49(42.87-48.27)	0.23	47.34(44.53-50.32)	0.02	4.1(1.6-6.6)	0.01
	TG	45.14(42.69-47.74)		46.60(43.81-49.56)		3.4(0.8-5.9)	
	GG	43.98(41.33-46.82)		44.34(41.40-47.49)		1.2(−1.6-4.0)	
LDL-C (mg/dl)	TT	110.18(103.80-116.95)	0.19	96.34(90.97-102.05)	0.05	−12.8(−16.1-−9.4)	0.24
	TG	110.31(104.52-116.39)		96.85(91.47-102.52)		−12.3(−15.8-−8.6)	
	GG	114.13(107.15-121.53)		101.98(95.54-108.84)		−10.9(−14.5-−7.2)	

Values were adjusted for age, gender, BMI, field center, smoking status, alcohol intake, physical activity, drugs for lowering cholesterol, diabetes, hypertension, and hormone treatment in women.

* Additive model.

Sample size: N for TT=290, N for TG=415, N for GG=140

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Table 3

TG-rich lipoprotein responses to fenofibrate according to *CYP* 7A1 m204T>G genotypes

	Before drug			After drug		Response	
	Genotype	**Geometric Mean(95%CI)**	**P***	**Geometric Mean(95%CI)**	**P***	**% change (95%CI)**	**P***
VLDL-TG (mg/dl)	TT	99.9(86.4-115.5)	0.67	55.3(47.2-64.8)	0.16	−42.6(−48.9-−35.7)	0.007
	TG	99.0(86.3-113.7)		58.2(49.7-68.0)		−38.4(−44.5-−31.5)	
	GG	96.7(81.0-115.6)		61.4(50.8-74.2)		−33.3(−41.3-−24.2)	
Large VLDL (mg/dl)	TT	23.2(17.9-30.2)	0.45	14.4(11.4-18.2)	0.63	−36.3(−46.5-−24.1)	0.035
	TG	23.3(18.1-29.9)		15.2(12.2-18.9)		−31.4(−41.9-−18.9)	
	GG	21.1(15.0-29.7)		15.0(11.5-19.6)		−23.3(−37.1-−6.5)	
Medium VLD(mg/dl)	TT	47.9(39.2-58.5)	0.37	19.7(15.5-25.0)	0.06	−56.3(−64.7-−46.0)	0.003
	TG	45.1(37.0-55.0)		21.4(16.7-27.5)		−49.2(−58.5-−38.0)	
	GG	43.5(34.34-55.2)		24.4(18.9-31.6)		−40.9(−53.1-−25.6)	
Small VLDL (mg/dl)	TT	10.3(8.5-12.5)	0.61	7.63(6.36-9.16)	0.49	−25.3(−36.9-−11.5)	0.78
	TG	10.0(8.1-12.4)		7.67(6.28-9.37)		−21.8(−34.3-−6.9)	
	GG	10.9(8.6-13.9)		8.14(6.59-10.06)		−24.5(−39.3-−6.1)	

Values were adjusted for age, gender, BMI, field center, smoking status, alcohol intake, physical activity, drugs for lowering cholesterol, diabetes, hypertension, and hormone treatment in women.

*Additive model.

Sample size: N for TT=290, N for TG=415, N for GG=140

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Table 4

Lipid responses to fenofibrate according to *CYP 7A1* i6782C>T genotypes

		Before drug		After drug		Response	
	Genotype	Geometric Mean (95%CI)	P*	Geometric Mean (95%CI)	P*	% change (95%CI)	P*
Triglycerides (mg/dl)	CC	138.4(123.9-154.5)	0.38	97.1(87.8-107.4)	0.84	−29.1(−33.6-−24.3)	0.19
	CT	138.3(123.2-155.4)		98.0(87.3-109.9)		−29.0(−34.1-−23.6)	
	TT	156.2(127.5-191.3)		97.1(81.9-115.2)		−37.4(−43.7-−30.2)	
Total cholesterol(mg/dl)	CC	182.0(174.8-189.6)	0.71	162.6(156.7-168.8)	0.77	−10.6(−12.8-−8.3)	0.46
	CT	180.8(173.4-188.6)		163.2(157.1-169.5)		−9.9(−12.4-−7.2)	
	TT	181.7(169.8-194.4)		163.5(154.8-172.7)		−10.3(−14.0-−6.4)	
HDL-C (mg/dl)	CC	45.5(43.1-47.9)	0.43	46.6(44.0-49.3)	0.74	2.8(0.4-5.1)	0.02
	CT	44.4(41.8-47.2)		46.3(43.3-49.6)		4.5(1.7-7.3)	
	TT	45.6(41.4-50.2)		48.3(44.2-52.7)		5.8(1.7-10.1)	
LDL-C (mg/dl)	CC	111.0(105.0-117.3)	0.62	96.9(91.5-102.6)	0.74	−12.8(−16.0-−9.5)	0.36
	CT	110.2(104.1-116.6)		97.7(92.3-103.6)		−11.5(−15.3-−7.6)	
	TT	109.4(99.7-120.1)		97.3(89.0-106.5)		−11.4(−18.2-−4.0)	

Values were adjusted for age, gender, BMI, field center, smoking status, alcohol intake, physical activity, drugs for lowering cholesterol, diabetes, hypertension, and hormone treatment in women.

* Additive model.

Sample size: CC=526, CT=274, GG=41