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ARTICLE

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Genetic variation in the hepatic lipase gene and the risk of coronary heart disease among US diabetic men: potential interaction with obesity

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Abstract *Aims/hypothesis:* The −514 C to T polymorphism of the hepatic lipase gene (*LIPC*) has been associated with lowered LIPC activity and elevated HDL-cholesterol concentrations. Previous findings on the association of this polymorphism with the risk of CHD are inconsistent. Moreover, data on this association among diabetic patients are limited. We investigated the association of the *LIPC* polymorphism with CHD risk among US diabetic men and evaluated whether this association was modified by adiposity status. *Subjects and methods:* The case group consisted of 220 diabetic men who were recruited from the Health Professionals Follow-up Study (years 1986–2000) and were free of cardiovascular disease at baseline, but subsequently developed CHD. A total of 641 diabetic men from the same study but without cardiovascular disease constituted the control group. *Results:* No overall association between the *LIPC* polymorphism and CHD risk was observed. However, we did observe a significant interaction between this polymorphism and BMI in association with CHD risk. Among obese men, after adjustment for age, duration of diabetes and major lifestyle factors, the CT or TT genotype was associated with an increased CHD risk compared with the CC genotype (odds ratio [OR] 2.52, 95% CI 1.08–5.90); the corresponding ORs (95% CI) were 0.99 (0.58, 1.69) for overweight men (25≤BMI <30 kg/m^2) and 0.37 (0.17, 0.79) for lean men (BMI <25 kg/m^2) (p for interaction 0.001). Stratified analyses by waist circumference (tertiles) showed a similar pattern of interaction (adjusted p for interaction 0.023). *Conclusion/interpretation:* These data suggest that obesity may modify the association between the *LIPC* C(−514) T polymorphism and CHD risk among diabetic men.

Keywords Coronary heart disease · Diabetes · Gene · Hepatic lipase · Interaction · Obesity

Abbreviations CABG: coronary artery bypass grafting · HPFS: Health Professionals Follow-up Study · IDL: intermediate density lipoprotein · LIPC: hepatic lipase · *LIPC*: hepatic lipase gene · OR: odds ratio

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Introduction

A decreased plasma HDL-cholesterol (HDL-C) concentration is an established risk factor for CHD among diabetic patients [1]. Allelic variations at the gene encoding hepatic lipase (LIPC) account for up to 25% of the variability in plasma HDL-C concentrations [2]. LIPC is a lipolytic enzyme that is primarily synthesised in the parenchymal liver cells and secreted and bound extracellularly to the endothelial cells in the hepatic sinusoids. It plays a crucial role in remodelling remnant lipoprotein, LDL and HDL [3–5] as both a catalyst and a ligand. Specifically, it catalyses the conversion of large HDL2 particles to small, dense HDL and acts on large LDL and intermediate density lipoprotein (IDL) particles to form small, dense LDL (sdLDL) particles. In addition, it can facilitate the hepatic uptake of lipoproteins, including HDL, by acting as a ligand mediating the binding and uptake of lipoproteins through proteoglycans and/or receptor pathways [6, 7].

LIPC activity has been reported to be determined by sex [8, 9], body adiposity [10], dietary fat intake [11, 12],

alcohol consumption [13, 14], physical activity [15–17] and, most importantly, by genotype. The human hepatic lipase gene (*LIPC*) maps to chromosome 15q21 [18]. Four polymorphisms in the 5′-flanking region of this gene (G→A at position −250, C→T at −514, T→C at −710 and A→G at −763) are in complete linkage disequilibrium [19]. They were designated as the −514 C and T alleles together [4, 19, 20]. The C→T substitution disrupts the upstream stimulatory factor 1 binding site present in the proximal promoter region of the hepatic lipase gene and has been shown to decrease transcriptional activity of the *LIPC* promoter in vitro [21, 22]. Furthermore, it accounted for up to 38% of the variation in LIPC activity [23] and was associated with increased concentrations of plasma HDL and HDL2 and more buoyant LDL particles [6, 20, 24, 25].

The relationship of the *LIPC* polymorphism to CHD risk has not been clearly established. Findings from several studies suggested a pro-atherogenic role of this polymorphism [24, 26–31]. The *LIPC* C(−514)T polymorphism was associated with reduced coronary flow reserve (an early marker of atherosclerosis) [28], coronary artery calcification [26] and increased risk of CHD [27, 29–31]. However, other studies reported no association or an inverse association with CHD risk [20, 32–34]. We speculated that some of the inconsistencies may be, at least in part, due to different study population characteristics, such as underlying environmental factors that can affect lipid metabolism. Indeed, in our previous study, we observed that body adiposity significantly modified the association between the *LIPC* polymorphism and plasma HDL concentrations [35]; only among lean diabetic men was the *LIPC* polymorphism associated with higher HDL-C concentrations, whereas obesity abolished the beneficial effect of the polymorphism on HDL-C concentrations. In the present study we investigated the relationship between the *LIPC* −514 polymorphism and the risk of CHD and evaluated whether this association was modified by adiposity status among diabetic patients.

Subjects and methods

Study background

The Health Professionals Follow-up Study (HPFS) is a prospective cohort study of 51,529 American male health professionals who were aged 40–75 years at study initiation in 1986. This cohort has been and continues to be followed through biennial mailed questionnaires focusing on various lifestyle factors and health outcomes. In addition, between 1993 and 1999 (more than 95% of them between 1993 and 1995), 18,159 study participants provided blood samples by overnight courier. Among them, 999 (>97% were people of European extraction) had confirmed type 2 diabetes (at baseline or during follow up, 1986–2000). From this group of diabetic men, we excluded those with reported CHD or stroke at baseline, those who developed stroke or other non-coronary cardiovascular disease during follow-up, and those who developed CHD before diabetes was diagnosed (total exclusions, 138). Among the remaining 861 diabetic men, 220 who developed CHD (i.e. non-fatal myocardial infarction, *n*=66; fatal CHD, *n*=18; coronary artery bypass grafting [CABG], *n*=136) constituted the case group and 641 diabetic men who remained free of CHD and stroke constituted the control group. The 201 cases and 604 control subjects for whom complete data on both *LIPC* genotype and BMI existed constituted the analytical population of this study. The participants provided written informed consent to be included in the study and all investigations were approved by the institutional review board ethics committee.

Diagnosis of type 2 diabetes

A diagnosis of diabetes was established when at least one of the following criteria was reported on a supplementary questionnaire sent to all men who reported a diagnosis of diabetes in any biennial follow-up questionnaire: (1) one or more classic symptoms (excessive thirst, polyuria, weight loss, hunger or coma) plus a fasting plasma glucose concentration ≥7.8 mmol/l or a random plasma glucose concentration ≥11.1 mmol/l; (2) at least two elevated plasma glucose concentrations on different occasions (fasting ≥7.8 mmol/l and/or random ≥11.1 mmol/l and/or ≥11.1 mmol/l after 2 h or more on oral glucose tolerance testing) in the absence of symptoms; or (3) treatment with hypoglycaemic medication (insulin or oral hypoglycaemic agents). We used the National Diabetes Data Group criteria [36] to define diabetes because the majority of our cases were diagnosed before the release of the American Diabetes Association criteria [37]. Men with type 1 diabetes were excluded. A validation study in a subsample of the HPFS demonstrated that our supplementary questionnaire is highly reliable in confirming diabetes diagnosis [38].

Diagnosis of cardiovascular end-points

The cardiovascular end-points for this analysis comprised fatal CHD, non-fatal myocardial infarction, CABG and percutaneous transluminal coronary angioplasty occurring between the return of the 1986 questionnaire and 31 January 2000. The end-points did not include angina pectoris. Non-fatal myocardial infarction was confirmed by a review of medical records based on World Health Organization criteria that included characteristic symptoms with either typical electrocardiographic changes or elevations of cardiac enzymes [39]. Probable cases of myocardial infarction (no available records but confirmed by hospitalisation and information from telephone interview/letter) were also included in the analysis. Confirmation of CABG or percutaneous transluminal coronary angioplasty was based on self-report only; hospital records obtained for a sample of 102 men in the HPFS confirmed the procedure for 96% [40]. Deaths were reported by next of kin, the postal system, and through records of the National Death

Index. Using all sources combined, it was estimated that follow-up for deaths was >98% complete [41]. Fatal CHD was confirmed by reviewing medical records or autopsy reports with the permission of the next of kin. Physicians who reviewed the records had no knowledge of the self-reported risk factor status. Sudden deaths (deaths within 1 h of symptom onset in men without known disease that could explain death) were included in the fatal CHD category.

Measurements of body weight and waist circumference and ascertainment of characteristics of participants

At baseline, participants were asked to report their height (in inches; 1 in=2.54 cm) and current body weight (in pounds; 1 lb=0.45 kg); weight was then updated during the biennial follow-up. In 1987, HPFS participants were asked in a supplementary questionnaire to measure their waist circumference with a paper tape and were given detailed measuring directions. BMI was calculated as weight in kilograms divided by height squared in metres. Waist circumference was used as an indicator of central obesity. Self-reports of body weight, waist circumference and height have been shown to be highly correlated with technician-measured weight, waist circumference and height (r=0.97, 0.95 and 0.94, respectively) in HPFS participants [42].

Participants also provided information biennially on their cigarette smoking, aspirin use and physical activity. Physical activity (metabolic equivalent task [MET] hours/week) was calculated from the reported time spent on various activities, weighting each activity by its intensity level. History of high blood pressure was determined from self-reports preceding the blood collection. Family history of CHD was reported in 1986. Alcohol intake was estimated with a dietary questionnaire in 1986.

Laboratory analysis methods

Details about the blood collection and processing procedure have been reported previously [43]. Briefly, approximately 95% of participants in the present study provided blood samples in 1993 and 1994. Blood samples were collected in three 10-ml liquid EDTA blood tubes, placed on ice packs stored in Styrofoam containers and returned to our laboratory by overnight courier; over 95% of the samples arrived within 24 h. After receipt, the chilled blood was centrifuged, divided into plasma, erythrocytes and buffy coat, and stored in continuously monitored nitrogen freezers at temperatures not higher than −130°C. We requested information on the date and time of drawing the blood sample and the time elapsed since the preceding meal to identify non-fasting (<8 h) subjects. Measurements of lipid profiles were done among diabetic men who developed CHD after their blood samples were collected in 1993–1994 (n=109) and their controls (n=604). All lipid profiles were assayed in the laboratory of N. Rifai (The Children's Hospital, Boston, MA, USA), which was certified by the NHLBI/CDC (National Heart, Lung and Blood Institute/Centers for Disease Control) Lipid Standardization Program. All assays except the ELISA and RIA employed a Hitachi 911 analyser (Roche Diagnostics, Indianapolis, IN, USA). Concentrations of total cholesterol, triacylglycerols and HDL-C were analysed simultaneously with enzymatic assays with CVs of 1.7, 1.8 and 2.5%, respectively. LDL-cholesterol (LDL-C) was determined by a homogeneous direct method (Genzyme, Cambridge, MA, USA), with CV <3.1%. Measurement of HbA_{1c} was based on turbidimetric immunoinhibition using haemolysed packed red cells. The intra-assay CVs at HbA_{1c} values of 5.5 and 9.1 were 1.9 and 3.0%, respectively.

DNA was extracted from the buffy coat fraction of centrifuged blood using the QIAmp Blood Kit (Qiagen, Chatsworth, CA, USA). The genotyping technique was Taqman SNP allelic discrimination by means of an ABI 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA, USA). Primers and probes are available on request.

Statistical analysis

Frequency distributions of characteristics of study subjects were examined according to case–control status. Student's t tests and χ^2 tests were used for comparisons of means and proportions. Because of the small number of subjects homozygous for the less common allele (genotype TT; nine cases, 25 controls) and similar magnitude of associations with CHD risk for individuals with this genotype and heterozygotes (genotype CT), individuals with genotypes CT and TT were grouped together.

Unconditional logistic regression was used to calculate odds ratios (OR) and 95% CIs for the association between the *LIPC* genotype and CHD risk adjusted for risk factors for CHD. Adjustment for baseline variables (age, smoking status [never, past, current smoker], physical activity [quartile], alcohol consumption [non-drinker, <5.0, <10.0, >10.0 g/day], total energy intake), and duration of diabetes changed the OR slightly, but we kept these variables in the final model because of their recognition as risk factors for CHD and interpretable variables that may account for the heterogeneity of study participants. Adjustment for other covariates, such as family history of myocardial infarction, aspirin use, history of hypertension and hypercholesterolaemia at baseline, did not show a significant effect on the ORs, and because they might be intermediates for the association they were not included in the final models.

Stratified analyses were conducted to examine whether the association between the *LIPC* polymorphism and CHD risk was modified by overall body adiposity status (lean, BMI <25; overweight, BMI 25.0–29.9; obese, BMI ≥30 kg/m^2) defined using the World Health Organization criteria [44], and central obesity status (waist circumference: tertile [high, medium, low]) at baseline. Interactions between *LIPC* genotypes and BMI and waist circumference were

assessed using a cross-product term between genotypes and these factors. Tests for interactions were performed using likelihood ratio tests by comparing two nested multivariate models with and without the interaction term. All reported p values are two-tailed and statistical significance was defined at the α=0.05 level. All analyses were performed with SAS version 8.12 software (SAS Institute, Cary, NC, USA).

Results

Compared with those without CHD, diabetic men who developed CHD tended to be older and were more likely to have a family history of myocardial infarction and to have hypertension and hypercholesterolaemia at baseline (Table 1). They were also more likely to use aspirin at baseline, probably because of their elevated cardiovascular risk factors. Plasma concentrations of LDL were higher and HDL-C lower among cases than controls.

Genotype frequencies did not deviate from Hardy–Weinberg equilibrium either among cases or among controls (p=0.97 and p=0.92, respectively). Overall, genotype distributions of the *LIPC* C(−514)T polymorphism were not statistically significantly different between cases and controls (p=0.87, χ^2 test) (Table 2). The frequency of the −514 T allele was 21% for cases and 20% for controls. Thirty-three percent of both cases and controls were heterozygous for the T allele and 4% of both cases and controls were homozygous for the T allele. Because of the small number of subjects homozygous for the less common allele, individuals with CT and TT genotypes were grouped together in the following analyses. There was no evidence of a statistically significant association between the *LIPC* polymorphism (CT/TT genotype) and CHD risk before and after adjustment for age, BMI, smoking status, alcohol consumption, physical activity, duration of diabetes, and total energy intake (OR [95% CI]: age-adjusted, 1.07 [0.76, 1.50]; multivariate-adjusted, 0.96 [0.67, 1.38]). Additional adjustment for family history of myocardial infarction, history of hypertension and hypercholesterolaemia, and use of aspirin at baseline did not change the results materially (adjusted OR [95% CI]: 0.94 [0.66, 1.35]). In addition, results did not change appreciably after we excluded subjects taking cholesterol-lowering medications at baseline (n=14). We therefore included these subjects in subsequent analyses.

The association between the *LIPC* −514 polymorphism and risk of CHD varied significantly according to BMI status (Table 3). After adjustment for smoking status, alcohol consumption, physical activity, duration of diabetes and total energy intake, among lean men (BMI <25 kg/m^2), those with the CT or TT genotype had a 64% lower risk of CHD (age-adjusted OR=0.36, 95% CI 0.17, 0.79) than those with the CC genotype. No significant association was observed among overweight men (25≤BMI <30 kg/m^2). The CT or TT genotype, however, was associated with more than two-fold greater risk of CHD among obese men (multivariate-adjusted OR=2.52, 95% CI 1.08, 5.90). The interaction between the *LIPC* C(−514)T polymorphism and BMI in relation to CHD risk (likelihood, χ^2=12.31, df=1, p for interaction=0.001) was statistically significant. The results did not change after excluding those who reported taking

Table 1 Comparison of cardiovascular risk factors[a] between CHD cases and controls at baseline (1986) among US diabetic men

Characteristic	CHD cases n=201	Controls n=604	p value
Age (years)	59.2±7.4	55.0±8.6	<0.001
BMI (kg/m^2)	28.0±4.6	28.0±4.6	0.32
Waist circumference (cm)	102.4±13.2	101.3±11.4	0.35
Family history of MI (%)	20.9	12.1	0.002
History of hypertension (%)	48.3	31.5	<0.001
History of hypercholesterolaemia (%)	25.9	12.1	<0.001
Physical activity (MET-hours/week)	14.4±19.3	14.2±17.8	0.86
Total energy intake (kcal/day)	2,024±618	2,020±635	0.94
Alcohol consumption (g/day)	8.9±15.6	11.0±16.5	0.10
Never smokers (%)	35.8	39.4	0.70
Aspirin users (%)	45.3	29.5	<0.001
Plasma lipoprotein[a] (mmol/l)			
Triglyceride[b]	2.2±1.1	2.0±1.1	0.12
Cholesterol	5.6±1.0	5.5±1.1	0.07
HDL-C	1.0±0.2	1.1±0.3	0.002
LDL-C	3.4±0.9	3.2±0.9	0.02

All data are mean±SD unless otherwise indicated
MET metabolic equivalent task, *MI* myocardial infarction
[a]Only for subjects whose blood sample was available before CHD was identified (109 CHD cases, 604 controls)
[b]Only for subjects whose blood sample was available before CHD was identified and who fasted before blood was drawn (60 CHD cases, 335 controls)

Table 2 Association between *LIPC* genotypes and the risk of CHD among US diabetic men

LIPC genotype	Cases (201)	Controls (604)	Age-adjusted[a] OR (95% CI)	Multivariate-adjusted[b] OR (95% CI)
CC	126	382	1.00 referent	1.00 referent
CC/TT	75	222	1.07 (0.76, 1.50)	0.96 (0.67, 1.38)
CT	66	197	1.06 (0.74, 1.50)	0.93 (0.64, 1.35)
TT	9	25	1.17 (0.52, 2.63)	1.21 (0.52, 2.83)

[a]Adjusted for age (<55/55, 59/60, 64/65, ≥69/70 years)
[b]Adjusted for age, duration of diabetes, and the following lifestyle factors: alcohol consumption (non-drinker, <5.0, <10.0, >10.0 g/day), smoking (never, past, current), physical activity (quartile), and total energy (kcal/day)

cholesterol-lowering medications. The interaction was attenuated but remained statistically significant after adjustment for HDL-C concentration (p for interaction=0.01). Further adjustment for LDL-C, HbA_{1c}, family history of myocardial infarction, history of hypertension and hypercholesterolaemia, and use of aspirin did not appreciably alter the results (p for interaction=0.002).

Similarly, we observed a significant interaction between the *LIPC* −514 polymorphism and waist circumference (multivariate-adjusted p for interaction=0.023) in association with CHD risk. The *LIPC* −514 polymorphism was related to an elevation of CHD risk confined to diabetic men with greater waist circumference. Among 217 men whose waist circumference was equal to or greater than 105.2 cm (the highest tertile of the study participants), the CT or TT genotype was associated with approximately two-fold increased risk of CHD (multivariate-adjusted OR=1.99, 95% CI 0.98, 4.10). Corresponding ORs were 0.86 for men with waist circumference in the middle tertile and 0.57 for those in the lowest tertile.

Considering that altered LIPC activity has been related to lifestyle factors, including dietary fat intake, physical activity, and alcohol consumption, we further examined whether these factors modulated the association between the *LIPC* −514 polymorphism and CHD risk. No statistically significant interactions between these factors

Table 3 Association between *LIPC* genotypes and the risk of CHD according to BMI and waist circumference[a] among US diabetic men

LIPC genotype	Cases (201)	Controls (604)	Age-adjusted[c] OR (95% CI)	Multivariate-adjusted[d] OR (95% CI)
BMI (kg/m^2)				
<25.0				
CC	43	95	1.00 referent	1.00 referent
CT/TT	17	64	0.51 (0.26, 1.01)	0.36 (0.17, 0.79)
25.0–29.9				
CC	51	178	1.00 referent	1.00 referent
CT/TT	33	114	1.08 (0.65, 1.86)	0.99 (0.58, 1.69)
≥30				
CC	32	109	1.00 referent	1.00 referent
CT/TT	25	44	2.54 (1.27, 5.10)	2.52 (1.08, 5.90)
p for interaction			0.002	0.001
Waist circumference (cm)				
Low[b] (<96.0)				
CC	39	107	1.00 referent	1.00 referent
CT/TT	17	59	0.77 (0.39, 1.51)	0.57 (0.27, 1.22)
Medium[b] (96.0–105.2)				
CC	32	90	1.00 referent	1.00 referent
CT/TT	20	66	0.84 (0.43, 1.64)	0.86 (0.42, 1.75)
High[b] (≥105.2)				
CC	30	111	1.00 referent	1.00 referent
CT/TT	26	50	2.24 (1.15, 4.37)	1.99 (0.98, 4.10)
p for interaction			0.034	0.023

[a]Measurements of waist circumference were available for 164 cases and 483 controls
[b]Tertile
[c]Adjusted for age (<55/55, 59/60, 64/65, ≥69/70 years)
[d]Adjusted for age, duration of diabetes and the following lifestyle factors: alcohol consumption (non-drinker, <5.0, <10.0, >10.0 g/day), smoking (never/past/current), physical activity (quartile), and total energy (kcal/day)

and the *LIPC* −514 polymorphism in relation to CHD risk were observed.

Discussion

In this study of US diabetic men, the association between the *LIPC* C(−514)T polymorphism and CHD risk was modulated by both overall adiposity and central obesity. Among obese men, the CT or TT genotype was associated with significantly increased risk of CHD compared with those with the CC genotype. However, among lean men this polymorphism was associated with a reduction in CHD risk.

Previous data on the relationship between the *LIPC* C(−514)T polymorphism and CHD have been inconsistent. While several studies suggested a pro-atherogenic role of this polymorphism [24, 26–31], others reported no association or even an inverse association with CHD risk [20, 32–34]. For instance, in a Danish cohort, participants with the TT genotype were observed to have a 1.5-fold (95% CI 1.0, 2.2) increased risk of ischaemic heart disease compared with those with the CC genotype [29]. The extent of coronary atherosclerosis determined by angiography was reported to be higher in participants with the T allele [27]. The *LIPC* −514 T allele was also reported to be associated with a decrease in coronary flow reserve [28] and the presence of subclinical CHD [26]. In contrast, no association of the *LIPC* C(−514)T polymorphism with the risk for CHD or coronary artery disease was observed in a study of Finnish men [20]. In another cohort of Finnish men, men with the CC genotype had elevated risk of acute myocardial infarction compared with those having the CT or TT genotype (relative risk=1.5) [34]. Some of the inconsistencies may be due, at least in part, to different study population characteristics, such as underlying environmental and/or physiological characters, which can affect LIPC activity and HDL metabolism. In the present study we found that the association between the *LIPC* C(−514)T polymorphism and CHD risk was dependent on overall adiposity and central obesity status. Previous studies that did not account for these gene–environment interactions may have obscured the effect of this polymorphism on CHD risk.

The precise mechanism by which obesity modifies the association between the *LIPC* C(−514)T polymorphism and CHD risk has yet to be elucidated. The *LIPC* −514 polymorphism is a key determinant of LIPC activity. The T allele has been shown to decrease transcriptional activity of the *LIPC* promoter in vitro [21, 22] and to account for up to 38% of the variation in LIPC activity [23]. LIPC activity is also affected by body adiposity; both BMI [10] and intra-abdominal fat [45] were strongly and positively associated with LIPC activity. We speculated that the observed interaction between the *LIPC* polymorphism and adiposity in association with CHD risk may act through their influence on LIPC activity and be related to the dual roles of LIPC activity in lipoprotein metabolism. LIPC activity has both pro-atherogenic and anti-atherogenetic properties [4]. LIPC catalyses the conversion of large HDL2 particles to small dense HDL by modulating the triglyceride and phospholipids content of these particles. It can also act on large LDL and IDL particles to form small dense LDL particles [3–5]. On the other hand, as a ligand, LIPC can be potentially anti-atherogenic through stimulating reverse cholesterol transport and clearing IDL [4]. The *LIPC* C(−514)T polymorphism associated with lower hepatic lipase activity may, therefore, have both anti-atherogenic and pro-atherogenetic effects. Which effect prevails may be dependent on other factors. It is possible that, in the presence of obesity-related metabolic changes among diabetic patients the pro-atherogenic property related to the *LIPC* polymorphism outweighs the anti-atherogenic property, which may consequently lead to an elevated risk of CHD. Conversely, among lean diabetic men, the anti-atherogenic effect may prevail. The relationship among the *LIPC* −514 polymorphism, adiposity and CHD could be complicated by pathophysiological and metabolic changes associated with diabetes. We are unaware of experimental data on the relationship among diabetic patients.

In agreement with the findings from the present study, an interaction of similar direction between the *LIPC* polymorphism and adiposity in association with HDL-C concentrations was observed in some studies [35, 46]. In a study of French-Canadian men [46], only lean carriers of the T allele had higher plasma HDL-C levels compared with lean CC homozygotes. The beneficial effect of the T allele was abolished in the presence of visceral obesity. Similarly, among 780 US diabetic men who were free of cardiovascular diseases at the time of drawing blood (the source cohort of the controls in the present study), we observed that overall adiposity significantly modified the association between the *LIPC* polymorphism and plasma HDL-C concentrations [35]. Only among lean men (BMI <25 kg/m^2) was the T allele associated with significantly elevated plasma HDL-C levels; there was no association in overweight or obese men. In the present study, the interaction between adiposity and the *LIPC* polymorphism was attenuated but remained significant after controlling for plasma HDL concentrations, which indicated that the observed interaction was due in part, but not entirely, to a differential effect of the *LIPC* −514 polymorphism on HDL concentrations by obesity status. Experimental studies with measurements of the *LIPC* polymorphism, LIPC activity and lipoprotein profiles are warranted to explore the physiological and functional significance of this polymorphism for atherosclerosis and CHD risk in the context of obesity.

One limitation of our study is that the HPFS does not represent a random sample of US diabetic men. The variant allele frequency (0.20) in this study, however, is in agreement with that reported in previous studies among people of European extraction [24, 47]. Secondly, we cannot exclude the possibility that the observed association with CHD is due to linkage disequilibrium with other, undiscovered variants in this gene or to its interaction with other genes important in determining plasma HDL-C levels or CHD risk; for example, the apolipoprotein AI/CIII/AIV,

cholesteryl ester transfer protein and lipoprotein lipase loci. Thirdly, we cannot rule out the possibility that the observed significant interaction may be due to residual confounding by unmeasured factors. We controlled for major measured risk factors for CHD and the findings did not change appreciably. Lastly, we cannot completely exclude the possibility that our results may be due to chance. It should be noted, however, that we conducted the present study based on our a priori finding of significant interactions between the *LIPC* −514 polymorphism and adiposity in determining plasma HDL concentrations [35]. Nonetheless, it will be important to confirm these findings with additional investigations among large samples of diabetic patients.

In conclusion, we observed heterogeneity of the association of the *LIPC* C(−514)T polymorphism with CHD risk according to obesity status among diabetic men. The increase in CHD risk associated with the polymorphism was observed only for obese men. These data support a role for gene–environment interaction in susceptibility to CHD among diabetic patients and, if confirmed, may provide an additional impetus for reducing obesity to prevent CHD.

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