

Low-density-lipoprotein cholesterol concentrations and risk of incident diabetes: epidemiological and genetic insights from the Framingham Heart Study

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Abstract

Aims/hypothesis Statins and niacin (nicotinic acid) reduce circulating LDL-cholesterol (LDL-C) levels by different mechanisms. Yet, both increase the risk of diabetes mellitus. Our objective was to relate blood LDL-C concentrations and a genetic risk score (GRS) for LDL-C to the risk of incident diabetes in individuals not treated with lipid-modifying therapy.

Methods We evaluated participants of the Framingham Heart Study who attended any of Offspring cohort examination cycles 3–8 and Third Generation cohort examination cycle 1 ($N=14,120$ person-observations, 6,011 unique individuals; mean age 50 ± 11 years, 56% women), who were not treated with lipid-modifying or antihypertensive medications and who were free from cardiovascular disease at baseline. Incident diabetes was assessed at the next examination.

Results The GRS was significantly associated with LDL-C concentrations (sex- and age-adjusted estimated influence 0.24, $p<0.0001$). On follow-up (mean 4.5 ± 1.5 years), 312 individuals (2.2%) developed new-onset diabetes. In multivariable models, a higher LDL-C concentration was associated with lower risk of diabetes (OR per SD increment 0.81, 95% CI 0.70, 0.93, $p=0.004$). The GRS was associated with incident diabetes in a similar direction and of comparable magnitude (OR per SD increment 0.85, 95% CI 0.76, 0.96, $p=0.009$).

Conclusions/interpretation Among individuals not treated with lipid-modifying therapy low LDL-C concentrations were associated with increased diabetes risk. These observations may contribute to our understanding of why lipid-lowering treatment may cause diabetes in some individuals. Additional studies are warranted to elucidate the molecular mechanisms underlying our observations.

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Keywords Diabetes risk · Low-density lipoprotein concentrations · Mendelian randomisation · Untreated individuals

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Abbreviations

GRS	Genetic risk score
IV	Instrument variable
HDL-C	HDL-cholesterol
LDL-C	LDL-cholesterol
SNP	Single-nucleotide polymorphism

Introduction

Statins and niacin (nicotinic acid) reduce circulating LDL-cholesterol (LDL-C) levels by different mechanisms. Yet, it is notable that both drugs have been associated with an

increased risk of diabetes mellitus in randomised controlled trials [1, 2]. These observations could suggest that low LDL-C concentrations per se (independent of off-target effects of lipid-lowering medications) may be associated with an increased diabetes risk. A Mendelian randomisation analysis (i.e. an analysis linking genetic variations in single-nucleotide polymorphisms [SNPs] to diabetes risk) recently suggested that the risk of diabetes observed with statin treatment could be attributable to ‘on-target’ effects of the drug, possibly supporting the idea that blood cholesterol concentrations may influence diabetes risk directly [3]. Consistent with this line of enquiry, another recent study reported that several lipid-related SNPs were oppositely associated with LDL-C concentrations and blood glucose levels [4] and a very recent cross-sectional study suggested that a genetic risk score (GRS) for LDL-C was associated with diabetes risk [5]. Additional supportive evidence linking low LDL-C concentrations to increased diabetes risk was suggested by a recent large cross-sectional study of individuals screened for familial hypercholesterolaemia [6]. The authors of that study reported a lower prevalence of diabetes in individuals with familial hypercholesterolaemia, compared with individuals without familial hypercholesterolaemia (with a graded association dependent on the mutation subtype) [6]. At least three smaller cross-sectional observational studies have similarly reported lower circulating LDL-C concentrations among people with insulin resistance and diabetes, compared with people without insulin resistance and diabetes [7–9]. Studies investigating the longitudinal relationship between blood LDL-C concentrations and incident diabetes are, however, lacking. Accordingly, we hypothesised that lower levels of LDL-C may be associated with greater risk of diabetes prospectively in individuals who are not on any lipid-lowering medications. We tested this hypothesis in the community-based Framingham Offspring and Third Generation cohorts. We also related a previously used GRS for LDL-C to the risk of developing diabetes [10] to assess whether individuals genetically predisposed to higher levels of LDL-C have a lower incidence of diabetes, thereby evaluating concordance between the two sets of epidemiological observations.

Methods

Participants We included participants who attended any of Framingham Offspring cohort examination cycles 3 through 8, or Framingham Third Generation cohort examination cycle 1 (combined $N=29,346$ person-observations) provided they were free from diabetes (n with diabetes = 1,644), not using lipid-modifying therapy (n using lipid therapy = 2,718) and attended the next examination cycle (where incident diabetes was determined, n attendees = 21,117). We chose a priori to exclude people with prevalent cardiovascular disease and

those using any antihypertensive medications (combined $n=3,788$ person-observations) because these conditions or the medications used to treat them have been associated with the risk of future diabetes in previous studies.[11–15] Additionally, we excluded people with missing data on key covariates (age, sex, blood pressure, antihypertensive medications, cholesterol treatment, lipid values and/or blood glucose concentrations; $n=3,209$).

At each examination cycle, blood was drawn from participants after an overnight fast (typically 10–12 h). Triacylglycerol, total cholesterol and HDL-cholesterol (HDL-C) concentrations were measured directly using standardised assays and LDL-C concentrations were calculated using the Friedewald formula [16]. We excluded people with blood triacylglycerol concentrations >10.34 mmol/l (>400 mg/dl) ($n=415$) because LDL-C concentrations cannot be calculated reliably above this value using the Friedewald formula. As a sensitivity analysis, we used directly measured total cholesterol concentrations to further ensure that any observed associations were not due to inaccurate calculations of LDL-C concentrations by the Friedewald formula. Diabetes was defined as a fasting plasma glucose concentration >3.23 mmol/l (>125 mg/dl) or the use of glucose-lowering medications. All participants provided written informed consent and the study protocol was approved by the institutional review board of the Boston University Medical Center.

Genotyping Participants from the Offspring and Third Generation cohorts had blood drawn for DNA analyses during their sixth and first examination cycles, respectively. DNA variants were genotyped on the Affymetrix 500K and MIPS 50K (Affymetrix, Santa Clara, CA, USA) platforms and imputations were based on MACH version 1.0 (<http://csg.sph.umich.edu/abecasis/MACH/>). The LDL-C GRS was calculated based on 37 SNPs (in accordance with previous work [10]) using a weighted summation of genotypes (coded additively for the risk allele; β estimates were derived from the genome-wide association study by Teslovich et al [17]). Since assembly of that risk score, more SNPs have been identified in larger meta-analyses but we used the ‘older’ 37 SNP score in order to maximise our sample size. Typically, the effect sizes of the newly discovered SNPs are small or have a low prevalence and, therefore, the contributions of the newly discovered SNPs to circulating LDL-C concentrations in community-based settings is modest. We used a corresponding weighted risk score of total serum cholesterol concentrations based on 52 SNPs from the study by Teslovich et al [17].

Statistical analyses Data from the different examination cycles were pooled to increase sample size (pooled repeated observations) [18]. Characteristics, including blood lipid concentrations, were updated at each examination cycle. All participants were followed until the next examination cycle for

incident diabetes. Because we had no diabetes events registered in between examination cycles (ascertainment was made at the examinations), we used multivariable logistic regression analyses with generalised estimating equations (accounting for repeated observations within participants) to calculate the ORs for development of diabetes in relation to blood LDL-C and the GRS. The interval between Framingham examinations was included as a covariate in the models (this method yields essentially similar results to time-dependent Cox models) [19]. We adjusted all statistical models for the following covariates (all covariates were included in the same model): examination cycle (as a categorical variable, which captured both calendar time period and the cohort status), age, sex, blood HDL-C and log(triacylglycerol) concentrations, BMI, systolic and diastolic blood pressure and fasting glucose level. Previous work based on the Framingham Offspring Study has demonstrated that these variables are important predictors of the risk of developing diabetes [20]. To facilitate comparison in OR estimates between LDL-C concentrations and GRS, we standardised these two variables prior to analyses (mean=0 and SD=1). We tested for effect modification by sex, age, BMI, fasting glucose concentrations, HDL-C and triacylglycerol concentrations (all but triacylglycerol concentrations were tested as continuous measures; the latter was tested as quintiles because of its wide distribution) by incorporating the interaction terms into multivariable models. Cubic regression splines were used to assess and graphically visualise the linearity/non-linearity of associations of LDL-C or GRS with incident diabetes. We performed an instrumental variable (IV) analysis (i.e. Mendelian randomisation analysis) for the binary outcome incident diabetes (Y) using the ratio method [21]. First, we regressed LDL-C (X, standardised) on

GRS (the IV variable, standardised) to estimate $\beta(\text{GX})$. Next, we ran logistic regression of diabetes incidence on GRS to estimate $\beta(\text{GY})$. We included age and sex in each model. We estimated $\beta(\text{IV})$ using the ratio $\beta(\text{IV})=\beta(\text{GY})/\beta(\text{GX})$ and we estimated variance, $\beta(\text{IV})$, as in Burgess [21]. As sensitivity analyses, all models were repeated using directly measured total cholesterol concentrations instead of estimated LDL-C. As another sensitivity analysis we also investigated the associations between GRS for HDL-C and for triacylglycerols and incident diabetes. A two-sided p value <0.05 was considered statistically significant for all analyses.

Results

A total of 14,120 person-observations were included (age [mean \pm SD] 50 ± 11 years, 56% women; 6,011 unique individuals) (Table 1). The mean concentration of LDL-C was 3.23 ± 0.89 mmol/l (limits 0.57–8.52 mmol/l; corresponding to 125 ± 34 mg/dl [limits 22–330 mg/dl]). The GRS varied from 47 to 110, with a mean of 78 ± 8 . The GRS was positively associated with LDL-C concentrations (age- and sex-adjusted estimated influence 0.24, $p<0.0001$). The GRS was also positively associated with circulating levels of triacylglycerols (age- and sex-adjusted estimated influence 0.08, $p<0.0001$) and negatively associated with circulating HDL-C concentrations (age- and sex-adjusted estimated influence -0.04 , $p<0.0001$).

On follow-up (mean 4.5 ± 1.5 years), 312 individuals (2.2%) developed diabetes. The number of diabetes events and total observational time by examination are presented in electronic supplementary material (ESM) Table 1. In

Table 1 Study sample characteristics

Characteristic	Offspring sample	Third Generation sample
<i>N</i>	11,364	2,756
Age, years	53 ± 10	39 ± 8
Female sex, <i>n</i> (%)	6,398 (56)	1,524 (55)
LDL-C concentration range, mmol/l	0.79–8.52	0.57–7.27
LDL-C concentration, mmol/l	3.32 ± 0.89	2.90 ± 0.81
HDL-C concentration, mmol/l	1.39 ± 0.41	1.44 ± 0.42
Triacylglycerol concentration, mmol/l	2.89 ± 1.60	2.61 ± 1.44
GRS 2010, sum mmol/l	2.02 ± 0.20	2.03 ± 0.20
GRS 2013, sum mmol/l	4.22 ± 0.27	4.24 ± 0.27
BMI, kg/m ²	26.4 ± 4.7	26.1 ± 5.1
Systolic blood pressure, mmHg	122 ± 17	115 ± 13
Diastolic blood pressure, mmHg	76 ± 10	74 ± 9
Fasting blood glucose concentration, mmol/l	2.41 ± 0.24	2.39 ± 0.21
Incident diabetes during follow-up, <i>n</i> (%)	257 (2.3)	55 (2.0)

Continuous variables are presented as mean \pm SD

Offspring sample comprises pooled observations from different examinations

multivariable-adjusted models, a higher LDL-C concentration was associated with a lower risk of diabetes (OR per SD increment 0.81, 95% CI 0.70, 0.93, $p=0.004$) (Fig. 1a). Models adjusted for fewer variables are available in ESM Table 2. The GRS was also associated inversely with incident diabetes (OR per SD increment of 0.85, 95% CI 0.76, 0.96, $p=0.009$). The relationship was, however, not linear for GRS (p for non-linearity=0.001) (Fig. 1b). Therefore, we performed additional analyses using tertiles of both GRS and LDL-C concentrations (Table 2). Compared with the lowest tertile, adjusted ORs were lower for the second and third tertiles without a clear dose–response relationship for either GRS or LDL-C. Formal Mendelian randomisation analysis supported a causal relationship between LDL-C concentrations and incident diabetes: the estimated OR of diabetes per 1 SD higher LDL-C concentration was 0.81 (95% CI 0.70, 0.93, $p=0.004$) for directly measured LDL-C levels and 0.49 (95% CI 0.29, 0.83, $p=0.008$) for LDL-C levels estimated from the GRS-based instrument variable.

Associations between LDL-C and diabetes risk were generally similar across age, for the two sexes, and for different BMIs (p for all interactions >0.2). Also, the association of LDL-C and incident diabetes was consistent across levels of HDL-C concentration (p for interaction=0.1) and fasting plasma glucose concentration (p for interaction >0.9) and did not differ significantly across quintiles of triacylglycerol concentration (p for interaction=0.4). There were also no effect modifications by age, sex, BMI, fasting glucose or HDL-C or triacylglycerol concentrations for the association between GRS and incident diabetes risk (p for all interactions >0.4).

Sensitivity analyses The association of a GRS underlying total cholesterol and circulating total cholesterol levels was of similar magnitude to that of LDL-C (sex- and age-adjusted estimated influence 0.24, $p<0.0001$). The GRS for total cholesterol was modestly associated with log-

triacylglycerol concentration (sex- and age-adjusted estimated influence 0.07, $p<0.0001$) and very modestly associated with HDL-C concentration (sex- and age-adjusted estimated influence -0.04 , $p<0.0001$). Analyses based on direct measurement of blood total cholesterol concentration yielded results consistent with those observed for LDL-C concentration, (Table 2 and ESM Fig. 1).

The correlation coefficients between the GRS for HDL-C and triacylglycerols with circulating concentrations of the corresponding lipid subtype were of similar magnitude to that of LDL-C (ESM Tables 3, 4). Neither the GRS for HDL-C nor the GRS for triacylglycerol was associated with incident diabetes (multivariable-adjusted OR for 1 SD increment in GRS 0.89 [95% CI 0.79, 1.02], $p=0.11$ and 0.88 [95% CI 0.77, 1.01], $p=0.064$ for HDL-C and triacylglycerol, respectively). When all three GRSs for LDL-C, HDL-C and triacylglycerol were included in the same model, the GRS for LDL-C remained borderline statistically significant (multivariable adjusted OR for 1 SD increment in GRS 0.89 [95% CI 0.79, 1.00], $p=0.060$), whereas neither the GRS for HDL-C nor the GRS for triacylglycerol was significantly associated with incident diabetes (OR 0.97 [95% CI 0.85, 1.11], $p=0.64$ and 0.94 [95% CI 0.81, 1.09], $p=0.38$, respectively).

When using a more extensive GRS based on the 2013 genome-wide association study (157 SNPs) [22] the associations with incident diabetes were similar (multivariable adjusted OR 0.86 [95% 0.75, 0.98], $p=0.020$ per 1 SD greater GRS).

Discussion

In the present investigation, we evaluated the association of blood LDL-C concentration with the risk of incident diabetes during a mean follow-up of 4.5 years in a large community-based sample of individuals who were not using

Fig. 1 Splines illustrating the OR of developing diabetes for LDL-C concentration (a) and the GRS underlying LDL-C concentration (b). Knots are placed at the 5th, 50th and 95th percentiles. Both models were adjusted for time between examinations, examination, HDL-C and triacylglycerol concentrations, sex, age, BMI, systolic and diastolic blood pressures and fasting blood glucose levels. p for overall association=0.0024 (a) and 0.0006 (b); p for non-linearity=0.22 (a) and 0.0013 (b). The y-axes are on a log scale. Grey lines represent 95% confidence intervals of the estimate

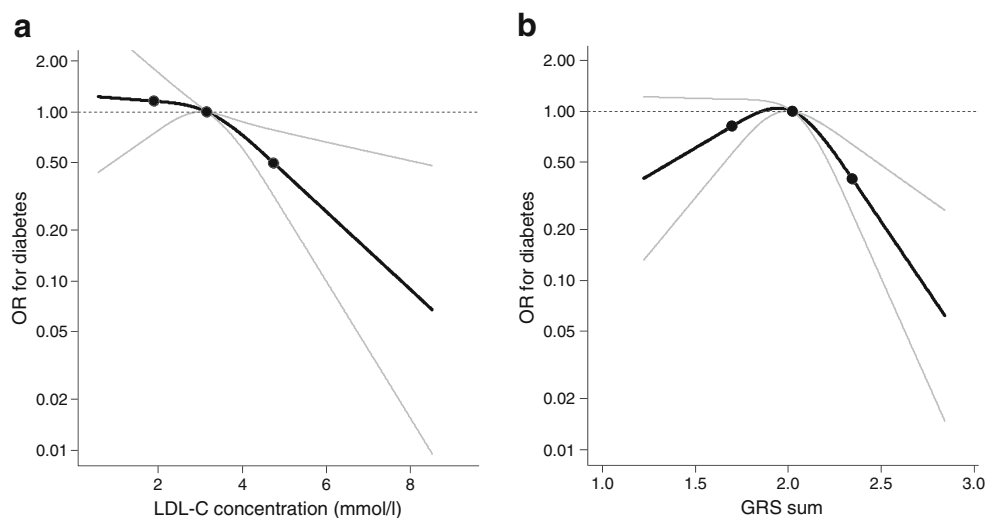


Table 2 Adjusted ORs of new-onset diabetes by tertiles of cholesterol concentration and GRS

Concentration/GRS	Adjusted OR ^a	<i>p</i> value
LDL-C concentration		
Tertile 1	1.42 (1.01, 2.00)	0.044
Tertile 2	1.48 (1.09, 2.00)	0.012
Tertile 3	1.00 (reference)	
GRS for LDL-C		
Tertile 1	1.56 (1.12, 2.24)	0.012
Tertile 2	1.77 (1.25, 2.49)	0.001
Tertile 3	1.00 (reference)	
Total cholesterol concentration		
Tertile 1	1.43 (0.98, 2.08)	0.064
Tertile 2	1.52 (1.12, 2.06)	0.007
Tertile 3	1.00 (reference)	
GRS for total cholesterol		
Tertile 1	1.52 (1.06, 2.18)	0.023
Tertile 2	1.76 (1.25, 2.46)	0.001
Tertile 3	1.00 (reference)	

Estimates were adjusted for time between examinations, examination, HDL-C and log(triacylglycerol) concentrations, sex, age, BMI, systolic and diastolic blood pressure and fasting blood glucose level

^a Compared with the third tertile

antihypertensive or lipid-lowering medications. Our study supports recent evidence that low LDL-C concentrations might be associated with increased diabetes risk both in the presence and absence of cholesterol-lowering drug treatment [6]. Although our observational study precludes any causal inference, the temporal relation of the association (blood LDL-C assessment pre-dated diabetes incidence), the dose-response observed across the range of values (splines, Fig. 1), the consistency of results in multiple analyses and the observation of a similar relationship between the GRS and incident diabetes all strengthen the likelihood that the observed association is causal [23]. To the best of our knowledge, previous studies that have investigated the association between blood LDL-C concentration and diabetes have been cross-sectional in design [3, 4, 6–9]. Therefore, we believe our prospective analyses are important and should encourage future studies to elucidate the biological underpinnings of the observed association, which may be important for future efforts to minimise the risk of diabetes with cholesterol-lowering treatment.

It is very likely that the observed inverse association of LDL-C with diabetes risk is more complex than our study was able to illuminate. For instance, diabetes risk may vary according to endogenous and exogenous cholesterol metabolic pathways, which may or may not have contributed to our observation of a non-linear relationship between GRS and diabetes risk (this possibly being due to the rather small sample size). Within this context, several cross-sectional studies

have noted a direct association between insulin resistance and high endogenous cholesterol synthesis coupled with low intestinal cholesterol absorption [7–9]. Yet, statin treatment inhibits endogenous cholesterol synthesis but increases the risk of future diabetes [3]. Hepatic endogenous cholesterol synthesis is linked to glucose metabolism via common metabolic intermediates including acetyl coenzyme A. It could be hypothesised that one way to adapt to subtle impairments in glucose metabolism is by means of increasing the production of endogenous cholesterol and triacylglycerol. A genetically reduced capacity to produce endogenous cholesterol (marked by lower LDL-C levels) would, in such case, perhaps be associated with increased diabetes risk in predisposed individuals, as suggested by previous case reports describing clustering of diabetes in individuals with familial hypobetalipoproteinaemia [23]. Further, in a recent study examining SNP variants relating to different lipid-associated genes, most SNPs showed opposite directionality of relations between LDL-C levels and fasting glucose/HbA_{1c}/HOMA-IR [4]. Also, interestingly, because the rate of intestinal cholesterol absorption is reciprocally related to the rate of endogenous cholesterol synthesis, genetic variation related to higher intestinal cholesterol absorption pathways may perhaps lead to lower endogenous cholesterol production and a greater risk of diabetes. Consistent with this premise, an SNP variant within the Niemann–Pick C1-like 1 gene (*NPC1L1*) was shown to be associated with LDL-C concentration and insulin resistance in similar directions (i.e. low LDL-C and low HOMA-IR) [4]. This gene codes for a transmembrane protein located in the apical part of the intestinal enterocytes and canicular membrane of hepatocytes [24]. The net effect of blocking this channel is lower intestinal cholesterol uptake and perhaps to some extent higher endogenous cholesterol production (the channel is the target of the lipid-lowering drug ezetimibe) [24].

Another proposed mechanism is that pancreatic cholesterol uptake may be of importance for beta cell function and insulin secretion. Higher intracellular cholesterol concentrations have been suggested to be detrimental for pancreatic beta cell function [6, 25]. Such a hypothesis is consistent with the study linking familial hypercholesterolaemia (with defective LDL-receptors) to a lower prevalence of diabetes. Clearly, more studies are needed to delineate the relative importance of different cholesterol metabolism pathways and determine their impact on the risk of diabetes.

Limitations Our study sample was modest in size and thereby precluded exploratory pathway-based genetic analyses or analyses aiming to address the relationship between individual SNPs in lipid genes and incident diabetes. We linked an aggregated risk score including SNPs that have previously been associated with LDL-C and total cholesterol concentrations to

the risk of diabetes, but acknowledge that this may have been a simplification of the true relationship between lipids and diabetes risk [17]. Because the LDL-C GRS was also weakly associated with concentrations of triacylglycerols (positively) and HDL-C cholesterol (negatively) it cannot be excluded that our observations might have been biased towards the null (because high triacylglycerol and low HDL-C concentrations are markers of diabetes risk). However, consistent with prior analyses, we did not observe any evidence of causal influence of HDL-C or triacylglycerols on diabetes risk (i.e. the GRSs for these two lipid measures were not associated with diabetes) [5]. Further, the magnitude of the association between GRS and LDL-C concentrations was rather weak (age- and sex-adjusted estimated influence was 0.24). The GRS (which was based on rather fewer selected SNPs), therefore, left a proportion of the circulating LDL-C concentration unexplained. Finally, our sample mainly comprised white people of European ancestry and the generalisability to other races is unknown.

Clinical implications and future directions As noted above, a recent community-based study showed that people with familial hypercholesterolaemia had a substantially lower prevalence of diabetes than people without familial hypercholesterolaemia [6]. This finding is interesting and is in agreement with our observations where especially individuals with LDL-C concentrations or GRS above the median values had a lower risk of diabetes (estimated from the splines). Further, based on our observations it may be hypothesised that people with relatively low baseline cholesterol levels have the greatest risk of developing diabetes with statin treatment; however, this premise warrants further studies. Despite a plausible association of low LDL-C concentrations with diabetes risk, it is important to emphasise that the net effect of lowering cholesterol is cardioprotective among high-risk individuals. Thus, cholesterol-lowering treatment should not be withheld in individuals with prevalent, or at high risk of, cardiovascular disease. More research is warranted to confirm our observations and to elucidate the molecular mechanisms underlying the observed associations. Such studies may also help to guide future efforts to lower cholesterol levels without increasing diabetes risk.

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