Remnant cholesterol predicts progression of diabetic nephropathy and retinopathy in type 1 diabetes

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Abstract. Jansson Sigfrids F, Dahlström EH, Forsblom C, Sandholm N, Harjutsalo V, Taskinen M-R, Groop P-H (Folkhälsan Institute of Genetics, Folkhälsan Research Center, Helsinki; University of Helsinki and Helsinki University Hospital, Helsinki; University of Helsinki, Helsinki; National Institute for Health and Welfare, Helsinki, Finland; Monash University, Melbourne, Australia). Remnant cholesterol predicts progression of diabetic nephropathy and retinopathy in type 1 diabetes. *J Intern Med* 2021; **290**: 632–645.

Background. We aimed to assess whether remnant cholesterol concentration and variability predict the progression of diabetic nephropathy (DN) and severe diabetic retinopathy (SDR) in type 1 diabetes.

Methods. This observational prospective study covered 5150 FinnDiane Study participants. Remnant cholesterol was calculated as total cholesterol – LDL cholesterol – HDL cholesterol and variability as the coefficient of variation. DN category was based on consensus albuminuria reference limits and the progression status was confirmed from medical files. SDR was defined as retinal laser treatment. For 1338 individuals, the severity of diabetic retinopathy (DR) was graded using the ETDRS classification protocol. Median (IQR) follow-up time was 8.0 (4.9–13.7) years for DN and 14.3 (10.4–16.3) for SDR.

Results. Remnant cholesterol (mmol L^{-1}) was higher with increasing baseline DN category ($P < 0.001$). A difference was also seen comparing non-progressors (0.41 [0.32–0.55]) with progressors (0.55 [0.40–0.85]), $P < 0.001$. In a Cox regression analysis, remnant cholesterol predicted DN progression, independently of diabetes duration, sex, HbA$_{1c}$, systolic blood pressure, smoking, BMI, estimated glucose disposal rate and estimated glomerular filtration rate (HR: 1.51 [1.27–1.79]). Remnant cholesterol was also higher in those who developed SDR (0.47 [0.36–0.66]) than those who did not (0.40 [0.32–0.53]), $P < 0.001$, and the concentration increased stepwise with increasing DR severity ($P < 0.001$). Regarding SDR, the HR for remnant cholesterol was 1.52 (1.26–1.83) with the most stringent adjustment. However, remnant cholesterol variability was not independently associated with the outcomes.

Conclusions. Remnant cholesterol concentration, but not variability, predicts DN progression and development of SDR. However, it remains to be elucidated whether the associations are causal or not.

Keywords: albuminuria, diabetic nephropathy, diabetic retinopathy, remnant cholesterol, type 1 diabetes.

Introduction

A growing body of evidence suggests a strong atherogenicity of the cholesterol in triglyceride-rich lipoproteins (TRLs), also termed remnant cholesterol. A causal relationship between remnant cholesterol and ischaemic heart disease has been suggested [1], and in addition, high remnant cholesterol concentrations have been linked to mortality

[2], ischaemic stroke [3] and chronic kidney disease (CKD) [4]. However, in the context of type 1 diabetes, the knowledge of remnant cholesterol is inadequate. Although we and others have previously shown that dyslipidaemia, hypertriglyceridaemia in particular, predicts diabetic nephropathy (DN) [5–7] and retinopathy (DR) [8, 9], the impact of remnant cholesterol on microvascular diabetic complications has been overlooked. Considering its strong

association with cardiovascular end-points and that diabetic micro- and macrovascular complications frequently coincide [10–12], the question has been raised whether remnant cholesterol also contributes to the development of renal and retinal lesions in individuals with diabetes.

Several assays for direct measurement of remnant cholesterol have been developed; however, few of them have been operational for clinical practice. [13] In contrast, remnant cholesterol calculation as total cholesterol – LDL cholesterol (LDL-C) – HDL cholesterol (HDL-C) serves as a clinically feasible tool. [14] Under non-fasting conditions, this surrogate marker represents the cholesterol in VLDL, IDL, chylomicrons and chylomicron remnant particles. Although the calculated remnant cholesterol tends to be higher than directly measured one, these two methods are correlated [2].

Moreover, an increasing interest in the association between vascular outcomes and the intraindividual variability of clinical parameters has emerged. With regard to diabetes, the variability of HbA_{1c} and its prognostic significance in predicting micro- and macrovascular complications has been devoted considerable research attention [15–17]. Recent evidence further suggests that high variability of triglycerides predicts incident microalbuminuria [18] and high variability of HDL-C the progression of DN [19] in individuals with type 2 diabetes. However, earlier studies on lipid variability have not included individuals with type 1 diabetes. Likewise, the variability of remnant cholesterol has previously not been assessed.

Based on these gaps in knowledge, we aimed to assess the relationship between remnant cholesterol concentration, kidney function, the progression of DN and DR in a multicentre Finnish cohort of individuals with type 1 diabetes. We further aimed to evaluate remnant cholesterol variability and to examine whether the variability adds to the DN and DR risk.

Materials and methods

The FinnDiane study

This study is part of the Finnish Diabetic Nephropathy (FinnDiane) Study, which was established to study micro- and macrovascular complications of type 1 diabetes [20]. It covers participants recruited from over 80 hospitals and primary healthcare centres throughout Finland.

FinnDiane is an observational prospective study. The first baseline visits were conducted in 1997, and since 2004, follow-up data have been obtained by re-examining the individuals and by reviewing their medical files. At the baseline visit, all participants underwent a thorough clinical examination and completed questionnaires regarding health and medical history with their attending physician. To assure correct diagnosis of type 1 diabetes, disease onset before the age of 40 and insulin treatment within 1 year of diagnosis were required.

Remnant cholesterol

Venous blood samples were drawn after a light breakfast and analysed for a serum lipid profile and other clinical markers. A recently introduced equation by *Sampson et al.* [21] was used to calculate LDL-C. The equation has appeared more precise than previous ones, particularly for individuals with hypertriglyceridaemia. Remnant cholesterol was calculated as total cholesterol – LDL-C – HDL-C. Of the 5264 study participants with manifest type 1 diabetes and at least one FinnDiane study visit by January 2021, baseline remnant cholesterol was available for a total of 5150 individuals (97.8%).

Diabetic nephropathy

To assess the level of albumin excretion rate (AER), 24-h or overnight urine samples were collected. Additionally, all available pre-baseline timed and spot urine measurements were reviewed by Finn-Diane physicians, and the most advanced stage of albuminuria in two out of three consecutive measurements was recorded for each participant entering the study. Urine collections during menstruation, pregnancy, fever, infections or after heavy exercise were excluded when known. Normal AER was defined as an AER <20 µg min^{-1} or <30 mg/24 h or an albumin–creatinine ratio (ACR) <2.5 mg mmol^{-1} for men and <3.5 mg mmol^{-1} for women; microalbuminuria as an AER ≥20 and <200 µg min^{-1} or ≥30 and <300 mg/24 h or an ACR ≥2.5 and <25 mg mmol^{-1} for men or ≥3.5 and <35 mg mmol^{-1} for women; and macroalbuminuria as an AER ≥200 µg min^{-1} or ≥300 mg mmol^{-1} or an ACR ≥25 mg mmol^{-1} for men or ≥35 mg mmol^{-1} for women. End-stage renal disease (ESRD) was defined as ongoing dialysis/received kidney transplant. The Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula [22] was used to

estimate glomerular filtration rate (eGFR). CKD was defined as eGFR <60 mL min^{-1}/1.73 m^2.

Progression of DN was confirmed from medical files and defined as reclassification to a more advanced stage of AER or initiation of kidney replacement therapy. Of the 4500 individuals with normal AER, microalbuminuria, or macroalbuminuria at baseline, the progression status was available for 3879 (86.2%). Participants were followed until first progression or the most recent date of sustained DN category over a median (interquartile range; IQR) follow-up time of 8.0 (4.9–13.7) years.

Diabetic retinopathy

Severe diabetic retinopathy (SDR) was defined as retinal laser photocoagulation due to severe non-proliferative diabetic retinopathy (NPDR), proliferative diabetic retinopathy (PDR) or diabetic maculopathy. The history of SDR was assessed at the initial study visit by the attending physician or study nurse with the help of previous medical records. For the prospective phase of the study, data on laser treatment were retrieved from the National Care register for Health Care using the following Nordic Classification of Surgical Procedure codes: CKC10, CKC12, CKC15, CKC50, CKD40, CKD92 and CKD93. The study participants with a history of laser treatment preceding baseline were excluded from these analyses; hence, 3099 (60.2%) remained. These individuals were followed until the initiation of laser photocoagulation, 31 December 2015 or death, whichever occurred first. The median (IQR) follow-up time with respect to SDR was 14.3 (10.4–16.3) years.

In addition, in a subpopulation comprising 1338 participants, the severity of DR was graded at the initial study visit by an ophthalmologist, as reported previously [23]. The grading was based on fundus photographs and the 12-step ETDRS classification protocol, where level 10 represents no retinopathy, levels 20–53 NPDR of increasing severity and 61–85 PDR of increasing severity [24]. We further categorized NPDR into a mild (ETDRS levels 20 and 35), moderate (43 and 47) and severe (53) stage. Remnant cholesterol in relation to the early stages of DR was assessed within this subpopulation.

Remnant cholesterol variability

To assess intraindividual variability, all available lipids measured at local diabetes centres were studied together with lipids measured at the FinnDiane centre in Helsinki. Measurements from the first FinnDiane visit until the primary endpoint of interest or the most recent confirmed event-free date were included. Variability was assessed for individuals with 3 or more measurements – subsequently, 2768 individuals with a median of 8 (range 3–43) measurements per person were studied regarding DN over 9.1 (IQR 5.3–13.7) years. For SDR, 2079 individuals with no previous history of laser treatment were followed for 10.2 (IQR 5.8–12.4) years including a median of 8 measurements (range 3–59) each. To correct for the strong interference of the absolute mean on the standard deviation (SD), variability was defined as the coefficient of variation (CV), calculated as SD/intrapersonal mean. CVs are presented as percentages.

Ethical considerations

The research plan was approved by the Ethical Committee of the Helsinki and Uusimaa Hospital District, and the study was performed according to the Declaration of Helsinki. All participants provided their written informed consent.

Statistical analyses

Data were analysed using the R open-source software version 3.5.1 (http://www.r-project.org). A two-sided *P*-value <0.05 was considered statistically significant.

Variable distributions were assessed graphically. Continuous variables with symmetric distributions were analysed using Student's *t*-test (two groups) or ANOVA (several groups) and are presented as mean ± SD. When distributions were skewed, between-group differences were analysed using the Mann–Whitney *U* (two groups) or the Kruskal–Wallis test (several groups) and are presented as median (IQR). Categorical variables were analysed with Pearson's chi-squared test and are presented as *n* (%).

Associations between remnant cholesterol and progression of DN/SDR were analysed using Cox proportional hazards regression models. Four levels of adjustment were chosen: first, a univariate model including only logarithmic remnant cholesterol; second, adjusting for the non-modifiable covariates duration of diabetes and sex; third, further adjusting for well-established modifiable

risk factors for microvascular complications: HbA_{1c}, systolic blood pressure, smoking status, BMI and estimated glucose disposal rate (eGDR) as a proxy for insulin resistance (calculated using a formerly presented formula [20]); fourth, further adjusting for eGFR. Due to its previously shown nonlinear relationship with DN progression [25], BMI was included as restricted cubic splines with three knots. The number of knots was based on Akaike information criterion minimization to reduce overfitting.

To further evaluate the impact of remnant cholesterol on the development and progression of DN and SDR, the cohort was divided into quartiles of baseline remnant cholesterol concentration and cumulative incidences with 95% confidence intervals (CIs) for the groups were calculated at 5, 10 and 15 years of follow-up. The cumulative incidences were also illustrated using Kaplan–Meier curves and probabilities compared with the log-rank test.

Results

Clinical characteristics

Table 1 summarizes baseline-measured clinical characteristics of the studied individuals, stratified by quartiles of remnant cholesterol concentration. Individuals with remnant cholesterol in the higher quartiles were more often men, had higher blood pressure, as well as a poorer glycaemic control than those with lower remnant cholesterol. The presence of microalbuminuria or a more advanced DN category, as well as the presence of CKD (eGFR <60 mL min^{-1}/ 1.73 m^2), was also more frequent in the individuals with higher remnant cholesterol. Similarly, these study participants had more often undergone laser treatment due to severe stages of DR.

At baseline, 787 (15.5%) subjects were using a lipid-lowering agent, predominantly statins. Notably, this proportion increased with increasing quartile of remnant cholesterol, ranging from 7.6% in quartile 1 to 22.5% in quartile 4 (Table 1). The median (IQR) remnant cholesterol concentration was 0.52 (0.40–0.72) mmol L^{-1} in individuals with lipid-lowering medication and 0.42 (0.33–0.59) mmol L^{-1} in those without, $P < 0.001$.

Development and progression of diabetic nephropathy

In the cohort, remnant cholesterol concentrations calculated from the baseline-measured lipid values were higher with increasing DN category, as presented in Fig. 1 (P for trend <0.001). Notably, concentrations were lower amongst individuals with ESRD who had received a kidney transplant (0.57 [0.42–0.74]), than those who had not (0.63 [0.46–0.96]), $P = 0.008$. Likewise, remnant cholesterol concentrations were higher amongst the individuals with CKD at baseline (0.61 [0.45–0.90]) than those without (0.42 [0.33–0.57]), $P < 0.001$.

During follow-up, the stage of DN for a total of 642 individuals progressed: 266 from normal AER to microalbuminuria (9.9%), 110 from micro- to macroalbuminuria (20.2%) and 266 (41.0%) individuals with initial macroalbuminuria developed ESRD. Remnant cholesterol concentrations were not only higher amongst individuals with more advanced baseline stages of AER, but also amongst the individuals who progressed (0.55 [0.40–0.85] mmol L^{-1}) in comparison with those who did not (0.41 [0.32–0.55] mmol L^{-1}), $P < 0.001$.

Results from Cox regression analyses are shown in Table 2. With the most stringent level of adjustment, including both non-modifiable and modifiable DN risk factors as covariates, HR for all events of progression was 1.51 (1.27–1.79) for logarithmic remnant cholesterol ($P < 0.001$). Of note, independent associations were observed both for the development of microalbuminuria and for the progression from micro- to macroalbuminuria – HR: 1.61 (95% CI: 1.23–2.11), $P < 0.001$, and HR: 2.71 (95% CI: 1.83–4.01), $P < 0.001$, respectively. The HR for the initiation of kidney replacement therapy was 2.08 (95% CI: 1.63–2.65), $P < 0.001$, when adjusting for diabetes duration, sex, HbA_{1c}, systolic blood pressure, smoking status, BMI and eGDR, but was reduced to 1.21 (95% CI: 0.92–1.58), $P = 0.17$ when eGFR was added to the model.

Figure 2a illustrates the cumulative progression of DN stratified by quartiles of remnant cholesterol at baseline. As the incidence curves in Figure 2a and the cumulative incidences in Table 3 show, the quartiles started diverging with respect to progression at an early stage, with quartile 4 (Q4) expressing a 6.2-fold higher cumulative progression than quartile 1 (Q1) already after 5 years of follow-up. After 15 years, 41.2% (36.9–45.3%) of the individuals in quartile 4 had progressed, whereas the corresponding rates for Q1, Q2 and Q3 were 10.4% (7.8–13.0%), 16.0% (12.8–19.1%) and 24.1% (20.3–27.7%), respectively.

Table 1 *Clinical characteristics of the study population at baseline stratified by quartiles of remnant cholesterol concentration*

	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P
n	1288	1287	1288	1287	
Remnant cholesterol (mmol L^{-1})	0.14–0.33	0.34–0.43	0.44–0.60	0.61–5.27	
Sex (woman)	782 (60.7%)	676 (52.5%)	571 (44.3%)	467 (36.3%)	<0.001
Age (years)	38.1 ± 11.7	38.3 ± 12.5	39.2 ± 13.1	38.5 ± 12.0	0.09
Age at diabetes onset (years)	16.4 ± 9.4	16.3 ± 9.1	16.2 ± 9.6	15.5 ± 9.2	0.04
Diabetes duration (years)	21.7 ± 12.2	22.0 ± 12.7	23.1 ± 13.2	23.0 ± 11.8	0.005
DN category at baseline					<0.001
Normal AER	988 (79.2%)	901 (73.2%)	753 (61.5%)	531 (42.7%)	
Microalbuminuria	140 (11.2%)	149 (12.1%)	160 (13.1%)	178 (14.3%)	
Macroalbuminuria	77 (6.2%)	108 (8.8%)	190 (15.5%)	325 (26.1%)	
End-stage renal disease	42 (3.4%)	73 (5.9%)	121 (9.9%)	209 (16.8%)	
AER (mg/24 h)	8 (5–20)	10 (5–26)	11 (6–53)	24 (8–229)	<0.001
CKD (yes)	58 (4.5%)	107 (8.3%)	206 (16.0%)	379 (29.4%)	<0.001
eGFR (mL min^{-1}/1.73 m^2)	104 (88–115)	102 (87–116)	99 (75–115)	92 (49–114)	<0.001
SDR at baseline (yes)	311 (25.5%)	400 (32.5%)	493 (39.8%)	643 (51.1%)	<0.001
BMI (kg m^{-2})	24.2 ± 3.1	25.0 ± 3.5	25.4 ± 3.9	26.7 ± 4.3	<0.001
Systolic blood pressure (mmHg)	131 ± 17	133 ± 18	135 ± 19	140 ± 21	<0.001
Diastolic blood pressure (mmHg)	78 ± 9	78 ± 10	79 ± 10	82 ± 11	<0.001
RAAS inhibitor (yes)	290 (22.8%)	332 (26.1%)	441 (34.7%)	545 (42.9%)	<0.001
Lipid-lowering agent (yes)	96 (7.6%)	164 (12.9%)	242 (19.0%)	285 (22.5%)	<0.001
History of smoking					<0.001
Current	217 (17.8%)	253 (20.7%)	300 (24.5%)	353 (29.3%)	
Former	246 (20.2%)	279 (22.9%)	294 (24.0%)	335 (27.8%)	
Never	755 (62.0%)	688 (56.4%)	630 (51.5%)	518 (43.0%)	
HbA$_{1c}$ (mmol mol^{-1})	64.5 ± 14.0	66.4 ± 14.8	69.3 ± 16.1	73.8 ± 17.9	<0.001
eGDR (mg kg^{-1} min^{-1})	7.5 (5.4–9.1)	6.7 (4.8–8.8)	5.6 (4.0–8.1)	4.4 (3.0–6.4)	<0.001
Total cholesterol (mmol L^{-1})	4.66 ± 0.81	4.62 ± 0.83	4.83 ± 0.92	5.44 ± 1.16	<0.001
HDL cholesterol (mmol L^{-1})	1.55 ± 0.41	1.42 ± 0.38	1.34 ± 0.37	1.14 ± 0.36	<0.001
LDL cholesterol (mmol L^{-1})	2.82 ± 0.79	2.82 ± 0.81	2.98 ± 0.89	3.33 ± 1.02	<0.001
Triglycerides (mmol L^{-1})	0.64 (0.57–0.72)	0.90 (0.83–0.97)	1.22 (1.12–1.34)	1.99 (1.68–2.57)	<0.001

Data presented as n (%), mean ± SD or median (IQR).
DN, diabetic nephropathy; AER, albumin excretion rate; CKD, chronic kidney disease (eGFR <60 mL min^{-1}/1.73 m^2); eGFR, estimated glomerular filtration rate; SDR, severe diabetic retinopathy (defined as history of laser photocoagulation); BMI, body mass index; RAAS inhibitor, ACE inhibitor and/or angiotensin II receptor antagonist; eGDR, estimated glucose disposal rate.

Development and progression of diabetic retinopathy

Individuals with PDR at the initial study visit were characterized by higher remnant cholesterol concentrations than those with the earlier forms of DR, and the concentrations increased in a stepwise manner with increasing DR severity (P for trend <0.001). Figure 1 provides the median concentrations (IQR) of remnant cholesterol, stratified by stages of DR at baseline.

Of the 3099 individuals without SDR before entering the study, 561 (18.1%) underwent retinal laser treatment during the prospective phase of the study. These individuals had a higher baseline remnant cholesterol concentration (0.47 [0.36–
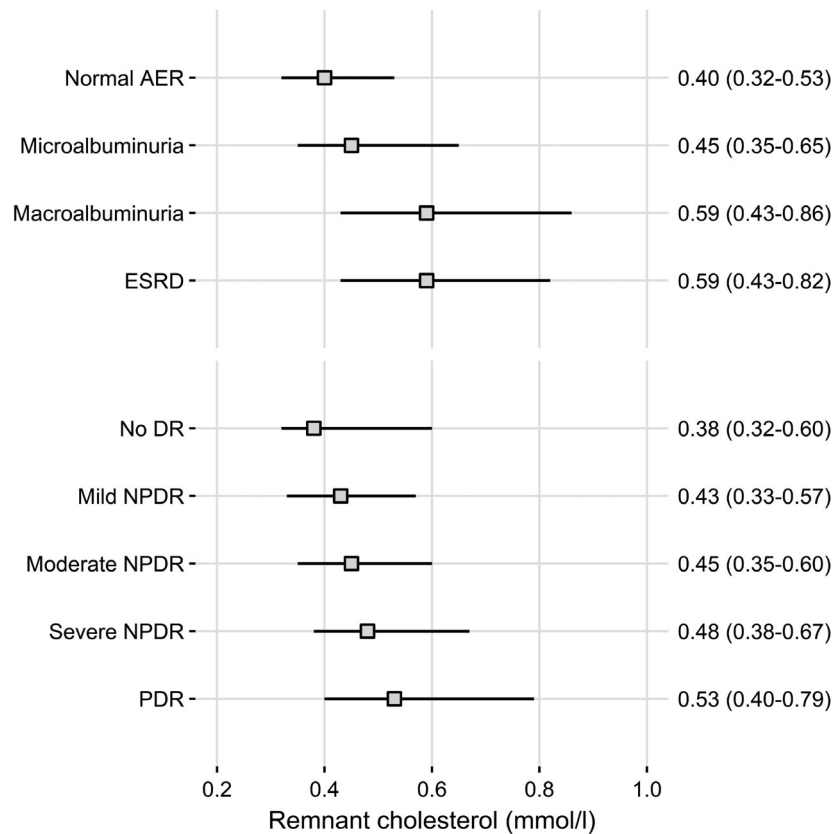
Fig. 1 *Median (IQR) of remnant cholesterol concentration stratified by diabetic nephropathy category and diabetic retinopathy stage at baseline. AER, albumin excretion rate; ESRD, end-stage renal disease; DR, diabetic retinopathy; NPDR, non-proliferative diabetic retinopathy; PDR, proliferative diabetic retinopathy.*

0.66]) than those who remained free of SDR (0.40 [0.32–0.53]), $P < 0.001$.

In the Cox regression analyses, the unadjusted HR (95% CI) for the development of SDR was 2.26 (1.94–2.64), $P < 0.001$, as presented in Table 2. Of note, the association remained independent at all levels of adjustment, even of the covariates in the fourth model including all non-modifiable and modifiable variables (HR: 1.52 [1.26–1.83], $P < 0.001$).

As observed for DN, the cumulative progression to SDR increased stepwise from the lowest to the highest quartile of baseline-measured remnant cholesterol. The calculated cumulative incidences at 5, 10 and 15 years of follow-up are presented in Table 3 and illustrated in Figure 2b. Already after a follow-up of 5 years, a 4.7-fold higher cumulative incidence of SDR was noted comparing Q4 with Q1. After 15 years, 29.8% (26.2–33.3%) of individuals

in Q4 had developed SDR, whereas for Q1, Q2 and Q3, the proportion was 11.9% (9.3–14.4%), 16.9% (13.9–19.7%) and 20.1% (16.9–23.1%), respectively.

Variability of remnant cholesterol

In line with the findings for remnant cholesterol concentration, also higher variability was noted at the more severe stages of DN: 23.1% (17.0–31.6%) in study subjects with normal AER, 24.2% (18.1–32.7%) in microalbuminuria and 25.6% (19.5–34.8%) in macroalbuminuria, $P < 0.001$. We further observed a difference in the variability between the DN progressors and the non-progressors when pooling the individuals (27.3% [19.4–37.4%] vs. 23.2% [17.3–31.5%], $P < 0.001$). This difference was mediated by the progression from normal AER to microalbuminuria, as the variability did not significantly differ at the other stages of

Table 2 *Cox regression analyses with different levels of adjustment for remnant cholesterol concentration with respect to progression of diabetic nephropathy (DN) and development of severe diabetic retinopathy (SDR)*

	Progression of DN	P	Normal AER to microalbuminuria	P	Micro- to macroalbuminuria	P	Macroalbuminuria to ESRD	P	Development of SDR	P
Number of individuals	3879		2686		544		649		3099	
Number of events (%)	642 (16.6%)		266 (9.9%)		110 (20.2%)		266 (41.0%)		561 (18.1%)	
Model 1 HR (95% CI)	3.15 (2.77–3.58)	<0.001	2.42 (1.93–3.04)	<0.001	3.17 (2.29–4.38)	<0.001	2.16 (1.75–2.66)	<0.001	2.26 (1.94–2.64)	<0.001
Model 2 HR (95% CI)	3.16 (2.77–3.61)	<0.001	2.40 (1.89–3.04)	<0.001	3.06 (2.19–4.28)	<0.001	2.19 (1.77–2.72)	<0.001	2.28 (1.95–2.67)	<0.001
Model 3 HR (95% CI)	2.23 (1.90–2.61)	<0.001	1.61 (1.23–2.11)	<0.001	2.73 (1.84–4.04)	<0.001	2.08 (1.63–2.65)	<0.001	1.51 (1.25–1.82)	<0.001
Model 4 HR (95% CI)	1.51 (1.27–1.79)	<0.001	1.61 (1.23–2.11)	<0.001	2.71 (1.83–4.01)	<0.001	1.21 (0.92–1.58)	0.17	1.52 (1.26–1.83)	<0.001

Model 1 = unadjusted.
Model 2 = Model 1 + non-modifiable variables (diabetes duration, sex).
Model 3 = Model 2 + modifiable variables (HbA_{1c}, systolic blood pressure, smoking status, body mass index, estimated glucose disposal rate).
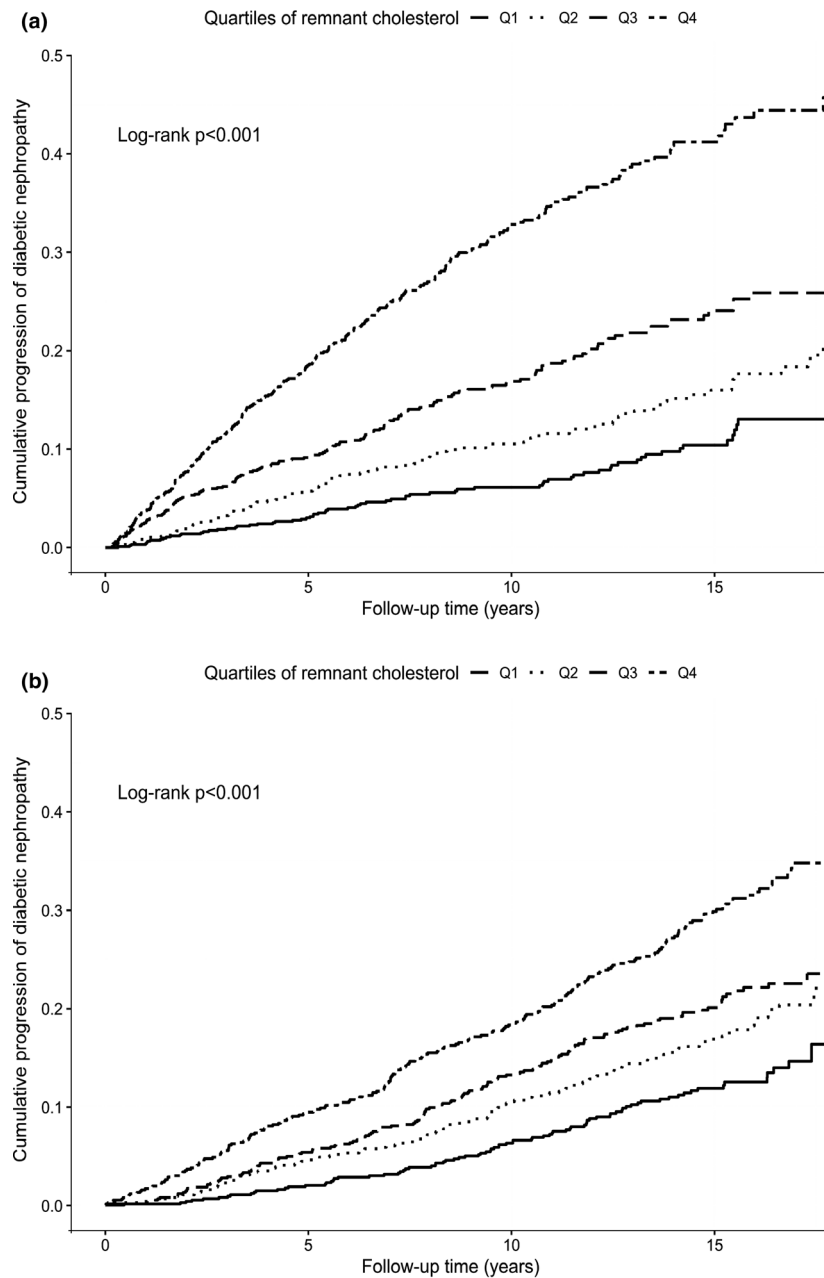Model 4 = Model 3 + estimated glomerular filtration rate.

(a)

(b)

Fig. 2 *Cumulative incidence curves for quartiles of remnant cholesterol concentration at baseline to illustrate (a) progression of diabetic nephropathy and (b) development of severe diabetic retinopathy. Log-rank p-values indicate the between-group differences in event probabilities. Quartile 1, solid line; quartile 2, dotted line; quartile 3, dashed line; quartile 4, dash-dotted line.*

progression (Table 4). In Cox regression analyses, the variability of remnant cholesterol predicted the development of microalbuminuria independent of sex and diabetes duration (model 2; HR 1.88 [95%

CI 1.31–3.00], $P < 0.001$), but the association was lost after further adjustment for modifiable covariates (Model 3; HR 1.48 [1.00–2.19], $P = 0.05$). The corresponding HRs for any progression (all events

Table 3 *Cumulative progression of diabetic nephropathy and development of severe diabetic retinopathy stratified by quartiles of remnant cholesterol at baseline*

	5 years of follow-up		10 years of follow-up		15 years of follow-up	
	Number of events	Cumulative incidence (95% CI)	Number of events	Cumulative incidence (95% CI)	Number of events	Cumulative incidence (95% CI)
Progression of diabetic nephropathy						
Quartile 1	27	3.0 (1.9–4.1)	48	6.1 (4.4–7.8)	65	10.4 (7.8–13.0)
Quartile 2	50	5.6 (4.1–7.1)	82	10.5 (8.3–12.7)	102	16.0 (12.8–19.1)
Quartile 3	84	9.3 (7.4–11.2)	131	16.9 (14.1–19.6)	157	24.1 (20.3–27.7)
Quartile 4	168	18.5 (15.9–21.0)	257	32.8 (29.3–36.2)	287	41.2 (36.9–45.3)
Development of severe diabetic retinopathy						
Quartile 1	15	2.0 (1.0–3.0)	45	6.3 (4.5–8.1)	77	11.9 (9.3–14.4)
Quartile 2	33	4.6 (3.1–6.1)	75	10.5 (8.2–12.7)	111	16.9 (13.9–19.7)
Quartile 3	40	5.4 (3.7–7.0)	95	13.2 (10.7–15.7)	136	20.1 (16.9–23.1)
Quartile 4	70	9.4 (7.3–11.5)	131	18.3 (15.4–21.1)	197	29.8 (26.2–33.3)

Cumulative incidences are presented as percentages.

pooled) were 1.51 (1.20–1.90), $P < 0.001$ (Model 2) and 1.15 (0.90–1.47), $P = 0.27$ (Model 3). Likewise, the remnant cholesterol variability was higher amongst those who developed SDR during the study than those who did not (24.8% [17.7–35.2%] vs. 22.6% [16.9–31.3%], $P = 0.03$). In Cox regression analyses, the HR for remnant cholesterol variability regarding SDR was 1.28 (1.03–1.60), $P = 0.03$, with adjustment for sex and diabetes duration, but was reduced to 1.00 (0.80–1.25), $P = 0.99$, when further covariates were added (Model 3).

Discussion

The triglycerides of TRLs are hydrolysed in the circulation, thus generating triglyceride-depleted remnant particles that are relatively enriched in cholesteryl esters and apolipoprotein(apo)E [13]. The major remnant cholesterol cargo is hepatic-derived (VLDL remnants) and less originate from intestinal, apoB-48-containing particles (chylomicron remnants) [14]. In recent years, remnant

cholesterol has emerged as a plausible culprit implicated in various vascular diseases, and the present study extends this view to cover microvascular complications in type 1 diabetes. We show that high calculated remnant cholesterol confers an increased risk of SDR and progression to more advanced stages of DN – independently of the initial category of kidney disease.

Impaired postprandial metabolism of apoB-48 has been noted in type 1 diabetes [26, 27]. As each chylomicron carries a single molecule of apoB-48, it can be considered a specific marker of the number of chylomicron particles and their remnants [28]. However, comprehensive data on remnant cholesterol concentrations in type 1 diabetes are scarce, and to the best of our knowledge, no previous studies have evaluated the association between microvascular comorbidities and remnant cholesterol *per se* in this patient population. In the present study, we demonstrate that individuals with DN present with increased concentrations of remnant cholesterol, in comparison with their

Table 4 *Variability (as CV) of remnant cholesterol, stratified by the status of diabetic nephropathy (DN) and severe diabetic retinopathy (SDR) during follow-up*

	Number of individuals	Number of events	No progression	Progression	P
Development and progression of DN					
All events pooled	2768	355	23.2 (17.3–31.5)	27.3 (19.4–37.4)	<0.001
Normal AER to microalbuminuria	1951	150	22.7 (16.8–31.1)	27.8 (20.5–37.0)	<0.001
Micro– to macroalbuminuria	400	71	23.7 (18.1–31.8)	30.0 (18.1–40.3)	0.07
Macroalbuminuria to ESRD	417	134	25.6 (19.6–34.1)	25.6 (19.1 36.1)	0.71
	Number of individuals	Number of events	No SDR	SDR	P
Development of SDR					
All events pooled	2079	334	22.6 (16.9–31.1)	24.8 (17.7–35.2)	0.003

Data presented as median (IQR). CVs given as percentages.

counterparts with no signs of kidney disease. This applies not only to eGFR-based CKD but also to the earlier stages of kidney disease depicted by moderate-to-severe albuminuria. Analogous observations have been reported from the DCCT/EDIC intervention trial: a cross-sectional analysis of the cohort revealed that the study participants with type 1 diabetes and concurrent DN were characterized by high VLDL concentrations – particularly the smaller particle subclasses – as well as high IDL particle concentrations in men but not in women [29]. In type 2 diabetes, measured remnant cholesterol concentrations have been shown to correlate positively with urinary ACR and inversely with eGFR, which is also in line with our findings [30].

Consistent with several others [14], we calculated remnant cholesterol as total cholesterol – LDL-C – HDL-C, using lipid profiles measured after a light breakfast. As presented by *Jepsen et al.* [2], calculated remnant cholesterol tends to be higher than the directly measured one. However, a strong correlation ($R^2 = 0.74$) between these two methods exists and both metrics apply to the prediction of all-cause mortality risk [2]. The advantage of remnant cholesterol approximation is its wide availability and cost-effectiveness, as it is based on standard lipid measurements. Calculated remnant cholesterol serves as a suitable surrogate marker for measured remnant cholesterol and can, evidently, be used to assess the risk for various clinical outcomes [14]. Moreover, the recently introduced formula for LDL-C by *Sampson et al.*

[21] has appeared at least as accurate as other equations in normolipidaemic individuals and even more accurate when triglycerides are in the range of 4.0–9.0 mmol L^{-1}. The use of the updated LDL-C calculation, hence, enabled the inclusion of study participants who in the case of LDL-C estimation by the Friedewald formula would have been left out.

In the present study, remnant cholesterol predicted DN progression after adjustment for well-established risk factors such as diabetes duration, HbA$_{1c}$ and systolic blood pressure. The only exception was the progression from macroalbuminuria to ESRD, which was independent of all covariates apart from eGFR. Given the known link between triglycerides and DN in type 1 diabetes [5–7], the robust association between the cholesterol of TRLs and the progression of DN was presumable. However, whether it is the triglycerides or the remnant cholesterol that contribute to the DN progression, or whether both are just innocent bystanders acting as markers of a third pathway, remains an open question. This issue has been the focus of substantial research within the field of macrovascular disease. Large epidemiological analyses have linked elevated remnant cholesterol to heightened cardiovascular risk [14], and a Mendelian randomization study proposed that the association between calculated remnant cholesterol and ischaemic heart disease is causal [1]. *Lin et al.* recently reported that high remnant cholesterol concentration confers a significant atherosclerotic burden as assessed by computed tomography

coronary angiography, also in subjects with LDL-C within the normal range [31]. Even more importantly, triglyceride-rich nascent chylomicrons and VLDL particles are restricted by their large sizes (>70 nm) to penetrate the arterial endothelial barrier, whereas their remodelled triglyceride-depleted and cholesterol-enriched remnant particles can freely enter the intima [32, 33]. Hence, the contribution of triglycerides in atherosclerotic plaque formation is unlikely. Furthermore, it has been established that on their entrance to the intima, the remnant particles are trapped within the connective tissue matrix and – as opposed to LDL particles – can be taken up by macrophages without prior oxidative modification, turning these cells into foam cells [33–35]. Epidemiological, genetic and experimental evidence hence favours the atherogenicity of remnant cholesterol, leaving triglycerides as a surrogate marker of the TRL cholesterol content.

However, the potential link between remnant cholesterol and DN/DR is not as apparent. Despite the fact that diabetic micro- and macrovascular comorbidities tend to coincide [10–12] and individuals with diabetic microvascular disease carry an elevated risk of cardiovascular morbidity and mortality, atherosclerosis is not considered a key driver of diabetic microvascular complications. In experimental models, high VLDL enhances the expression of monocyte chemoattractant protein 1 in mesangial cells, facilitating the adhesion of monocytes to the mesangium [36]. Although glomerular accumulation of monocyte-derived cholesterol-loaded foam cells can be encountered in DN, the clinical relevance of these cells is unclear [37]. Another potential connection between remnant cholesterol and diabetic microvascular disease is inflammation. *Varbo et al.* have reported a close relationship between remnant cholesterol and low-grade systemic inflammation, depicted by elevated C-reactive protein (CRP) [38]. Hence, it is noteworthy that both albuminuria and declining eGFR are associated with CRP and inflammatory cytokines such as interleukin 6 and tumour necrosis factor-α in individuals with diabetes [39, 40]. Yet, whether glomerular foam cell accumulation, systemic inflammation or some other mediator between remnant cholesterol and diabetic microvascular complications exists remains to be further addressed.

An intriguing detail of the present study was that already after 5 years of follow-up, individuals in the highest quartile of remnant cholesterol presented with an almost fivefold cumulative incidence of SDR compared with those in the lowest quartile. The DN risk started diverging early as well. Considering the global burden of type 1 diabetes and its complications in particular, new strategies to narrow this gap are needed. A recent *post hoc* analysis of the TNT Trial [41] showed that atorvastatin therapy translates into a dose-dependent reduction of remnant cholesterol in individuals with clinically evident coronary heart disease. The analysis further revealed that the remnant cholesterol decline is followed by a decrease in the cardiovascular risk, independently of the change in LDL-C. These findings are promising, but also indicate that the detected associations between remnant cholesterol and the outcomes of our study are likely diluted by the effects of lipid-lowering therapy, which was more frequent in the top quartiles of baseline remnant cholesterol. Furthermore, recent findings suggest that the combination therapy of simvastatin and ezetimibe confers atheroprotective changes in remnant particles to a larger extent than statin therapy alone [42]. The combination therapy subsequently lowers the cardiovascular burden, also in individuals with reduced eGFR [43]. The numbers of individuals on ezetimibe therapy at the FinnDiane Study baseline were, unfortunately, too low to assess the potential benefits of the agent in comparison with statin monotherapy. Future clinical trials will, thus, have to determine whether lipid-lowering combination therapy modulates remnant cholesterol and the risk of microvascular complications in type 1 diabetes.

During the past years, an interest in the association between intraindividual variability of clinical parameters and various outcomes has emerged. Within the field of diabetes, the impact of glycaemic instability on diabetic complications has received special focus. Both in the FinnDiane [15] and in the DCCT/EDIC cohorts [16], higher intraindividual HbA_{1c} variability has been observed amongst individuals experiencing progression of DN than their counterparts with sustained DN. However, lipid variability in type 1 diabetes has received less attention. We observed a median remnant cholesterol variability of 23–30%, depending on the baseline DN category and the DN progression/SDR status. Yet, despite some univariate differences, the associations between remnant cholesterol variability and the primary study outcomes lost significance in multivariable analyses.

Some limitations need to be considered when interpreting the results of this study. As previously stated, the accuracy and added knowledge would have been higher using measured, instead of calculated, remnant cholesterol. On the other hand, the study cohort is large and well-characterized, comprising participants with type 1 diabetes from multiple centres nationwide, and therefore provides a unique setting for biomarker studies such as this. There is, however, room for further research on this topic, and the association between microvascular diabetic complications and accurately assessed remnant cholesterol could be undertaken in the future. Another strength of this study is that the progression of DN was confirmed from medical files by the FinnDiane researchers and, hence, did not rely on self-reported questionnaires. Albeit the characterization of SDR was registry-derived, the severity of DR was comprehensively assessed in a subpopulation of the study participants and, therefore, we were able to study remnant cholesterol in relation to the earlier stages of DR as well.

Conclusion

In conclusion, this multicentre prospective study highlights the role of lipids in type 1 diabetes and adds to the previous knowledge by introducing remnant cholesterol to the context of microvascular diabetic complications. We show that by calculating remnant cholesterol from standard lipid profiles, the progression of DN and development of SDR can be predicted independently of several clinically meaningful risk factors. The increasing burden of diabetes and its complications is concerning and, therefore, an urge for more efficient treatment strategies exists. Whether lowering remnant cholesterol could translate into a reduction of microvascular complications of diabetes remains to be clarified, and for that purpose, it needs to be assessed whether the associations we present in this observational study are causal or not.

Conflict of interest

Per-Henrik Groop has received lecture fees from Astellas, Astra Zeneca, Boehringer-Ingelheim, Eli Lilly, Elo Water, Genzyme, Medscape, MSD, Mundipharma, Novartis, Novo Nordisk, Sanofi and Sciarc. He is an advisory board member for AbbVie, Astellas, Astra Zeneca, Bayer, Boehringer-Ingelheim, Eli Lilly, Janssen, Medscape, MSD, Mundipharma, Novartis, Novo Nordisk and Sanofi. None of these entities participated in the design or interpretation of the study. Other authors declare no conflicts of interest relevant to this article.

References

1 Varbo A, Benn M, Tybjærg-Hansen A, Jørgensen AB, Frikke-Schmidt R, Nordestgaard BG. Remnant cholesterol as a causal risk factor for ischemic heart disease. *J Am Coll Cardiol* 2013;**61:**427–36.

2 Jepsen A-MK, Langsted A, Varbo A, Bang LE, Kamstrup PR, Nordestgaard BG. Increased remnant cholesterol explains part of residual risk of all-cause mortality in 5414 patients with ischemic heart disease. *Clin Chem* 2016;**62:**593–604.

3 Varbo A, Nordestgaard BG, Tybjaerg-Hansen A, Schnohr P, Jensen GB, Benn M. Nonfasting triglycerides, cholesterol, and ischemic stroke in the general population. *Ann Neurol* 2011;**69:**628–34.

4 Nestel PJ, Fidge NH, Tan MH. Increased lipoprotein-remnant formation in chronic renal failure. *N Engl J Med* 1982;**307:**329–33.

5 Tolonen N, Forsblom C, Thorn L, *et al.* Lipid abnormalities predict progression of renal disease in patients with type 1 diabetes. *Diabetologia* 2009;**52:**2522–30.

6 de Boer IH, Rue TC, Cleary PA, *et al.* Long-term renal outcomes of patients with type 1 diabetes mellitus and microalbuminuria: an analysis of the diabetes control and complications trial/epidemiology of diabetes interventions and complications cohort. *Arch Intern Med* 2011;**171:**412–20.

7 Perkins BA, Bebu I, de Boer IH, *et al.* Risk factors for kidney disease in type 1 diabetes. *Diabetes Care* 2019;**42:**883–90.

8 Sjølie AK, Stephenson J, Aldington S, *et al.* Retinopathy and vision loss in insulin-dependent diabetes in Europe: The EURODIAB IDDM complications study. *Ophthalmology* 1997;**104:**252–60.

9 Tolonen N, Hietala K, Forsblom C, *et al.* Associations and interactions between lipid profiles, retinopathy and nephropathy in patients with type 1 diabetes: the FinnDiane Study. *J Intern Med* 2013;**274:**469–79.

10 Harjutsalo V, Thomas MC, Forsblom C, Groop P-H. Risk of coronary artery disease and stroke according to sex and presence of diabetic nephropathy in type 1 diabetes. *Diabetes Obes Metab* 2018;**20:**2759–67.

11 Tesfaye S, Chaturvedi N, Eaton SEM, *et al.* Vascular risk factors and diabetic neuropathy. *N Engl J Med* 2005;**352:**341–50.

12 Garofolo M, Gualdani E, Giannarelli R, *et al.* Microvascular complications burden (nephropathy, retinopathy and peripheral polyneuropathy) affects risk of major vascular events and all-cause mortality in type 1 diabetes: a 10-year follow-up study. *Cardiovasc Diabetol* 2019;**18:**159.

13 Chait A, Ginsberg HN, Vaisar T, Heinecke JW, Goldberg IJ, Bornfeldt KE. Remnants of the triglyceride-rich lipoproteins, diabetes, and cardiovascular disease. *Diabetes* 2020;**69:**508–16.

14 Varbo A, Nordestgaard BG. Remnant lipoproteins. *Curr Opin Lipidol* 2017;**28:**300–7.

15 Wadén J, Forsblom C, Thorn LM, Gordin D, Saraheimo M, Groop P-H. A1C variability predicts incident cardiovascular events, microalbuminuria, and overt diabetic nephropathy in patients with type 1 diabetes. *Diabetes* 2009;**58:**2649–55.

16 Kilpatrick ES, Rigby AS, Atkin SL. A1C variability and the risk of microvascular complications in type 1 diabetes: data from the diabetes control and complications trial. *Diabetes Care* 2008;**31:**2198–202.

17 Hietala K, Wadén J, Forsblom C, *et al.* HbA1c variability is associated with an increased risk of retinopathy requiring laser treatment in type 1 diabetes. *Diabetologia* 2013;**56:**737–45.

18 Bardini G, Innocenti M, Rotella CM, Giannini S, Mannucci E. Variability of triglyceride levels and incidence of microalbuminuria in type 2 diabetes. *J Clin Lipidol* 2016;**10:**109–15.

19 Chang Y-H, Chang D-M, Lin K-C, Hsieh C-H, Lee Y-J. High-density lipoprotein cholesterol and the risk of nephropathy in type 2 diabetic patients. *Nutr Metab Cardiovasc Dis* 2013;**23:**751–7.

20 Thorn LM, Forsblom C, Fagerudd J, *et al.* Metabolic Syndrome in Type 1 Diabetes: Association with diabetic nephropathy and glycemic control (the FinnDiane study). *Diabetes Care* 2005;**28:**2019–24.

21 Sampson M, Ling C, Sun Q, *et al.* A new equation for calculation of low-density lipoprotein cholesterol in patients with normolipidemia and/or hypertriglyceridemia. *JAMA Cardiol* 2020;**5:**540–8.

22 Levey AS, Stevens LA, Schmid CH, *et al.* A new equation to estimate glomerular filtration rate. *Ann Intern Med* 2009;**150:**604.

23 Hietala K, Harjutsalo V, Forsblom C, Summanen P, Groop P-H, Group OB of the FS. Age at onset and the risk of proliferative retinopathy in type 1 diabetes. *Diabetes Care* 2010;**33:**1315–9.

24 Davis MD, Fisher MR, Gangnon RE, *et al.* Risk factors for high-risk proliferative diabetic retinopathy and severe visual loss: Early Treatment Diabetic Retinopathy Study Report #18. *Invest Ophthalmol Vis Sci* 1998;**39:**233–52.

25 Todd JN, Dahlström EH, Salem RM, *et al.* Genetic evidence for a causal role of obesity in diabetic kidney disease. *Diabetes* 2015;**64:**4238–46.

26 Su JW, Lambert JE, Clandinin MT, Proctor SD. Impaired postprandial metabolism of apolipoprotein B48–containing remnant particles in normolipidemic subjects with brittle type 1 diabetes. *Diabetes Care* 2009;**32:**e21.

27 Lassenius MI, Mäkinen V-P, Fogarty CL, *et al.* Patients with type 1 diabetes show signs of vascular dysfunction in response to multiple high-fat meals. *Nutr Metab* 2014;**11:**28.

28 Sniderman AD, Thanassoulis G, Glavinovic T, *et al.* Apolipoprotein B particles and cardiovascular disease: a narrative review. *JAMA Cardiol* 2019;**4:**1287–95.

29 Jenkins AJ, Lyons TJ, Zheng D, *et al.* Lipoproteins in the DCCT/EDIC cohort: associations with diabetic nephropathy. *Kidney Int* 2003;**64:**817–28.

30 Sonoda M, Shoji T, Kimoto E, *et al.* Kidney function, cholesterol absorption and remnant lipoprotein accumulation in patients with diabetes mellitus. *J Atheroscler Thromb* 2014;**21:**346–54.

31 Lin A, Nerlekar N, Rajagopalan A, *et al.* Remnant cholesterol and coronary atherosclerotic plaque burden assessed by computed tomography coronary angiography. *Atherosclerosis* 2019;**284:**24–30.

32 Nordestgaard BG, Stender S, Kjeldsen K. Reduced atherogenesis in cholesterol-fed diabetic rabbits. Giant lipoproteins do not enter the arterial wall. *Arterioscler Off J Am Heart Assoc Inc* 1988; **8:** 421–8.

33 Borén J, Chapman MJ, Krauss RM, *et al.* Low-density lipoproteins cause atherosclerotic cardiovascular disease: pathophysiological, genetic, and therapeutic insights: a consensus statement from the European Atherosclerosis Society Consensus Panel. *Eur Heart J* 2020;**41:**2313–30.

34 Goldstein JL, Ho YK, Brown MS, Innerarity TL, Mahley RW. Cholesteryl ester accumulation in macrophages resulting from receptor-mediated uptake and degradation of hypercholesterolemic canine beta-very low density lipoproteins. *J Biol Chem* 1980;**255:**1839–48.

35 Nakajima K, Nakano T, Tanaka A. The oxidative modification hypothesis of atherosclerosis: The comparison of atherogenic effects on oxidized LDL and remnant lipoproteins in plasma. *Clin Chim Acta* 2006;**367:**36–47.

36 Lynn EG, Siow YL, O K. Very low-density lipoprotein stimulates the expression of monocyte chemoattractant protein-1 in mesangial cells. *Kidney Int* 2000;**57:**1472–83.

37 Eom M, Hudkins KL, Alpers CE. Foam cells and the pathogenesis of kidney disease. *Curr Opin Nephrol Hypertens* 2015;**24:**245–51.

38 Varbo A, Benn M, Tybjærg-Hansen A, Nordestgaard BG. Elevated remnant cholesterol causes both low-grade inflammation and ischemic heart disease, whereas elevated low-density lipoprotein cholesterol causes ischemic heart disease without inflammation. *Circulation* 2013;**128:**1298–309.

39 Navarro-González JF, Mora-Fernández C, de Fuentes MM, García-Pérez J. Inflammatory molecules and pathways in the pathogenesis of diabetic nephropathy. *Nat Rev Nephrol* 2011;**7:**327–40.

40 Saraheimo M, Teppo A-M, Forsblom C, Fagerudd J, Groop P-H. Diabetic nephropathy is associated with low-grade

inflammation in Type 1 diabetic patients. *Diabetologia* 2003;**46:**1402–7.

41 Vallejo-Vaz AJ, Fayyad R, Boekholdt SM, *et al.* Triglyceride-rich lipoprotein cholesterol and risk of cardiovascular events among patients receiving statin therapy in the TNT trial. *Circulation* 2018;**138:**770–81.

42 Ahmed O, Littmann K, Gustafsson U, *et al.* Ezetimibe in combination with simvastatin reduces remnant cholesterol without affecting biliary lipid concentrations in gallstone patients. *J Am Heart Assoc* 2018;**7:**e009876.

43 Baigent C, Landray MJ, Reith C, *et al.* The effects of lowering LDL cholesterol with simvastatin plus ezetimibe in patients with chronic kidney disease (Study of Heart and Renal Protection): a randomised placebo-controlled trial. *Lancet* 2011;**377:**2181–92.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Physicians and nurses at health care centers participating in the collection of FinnDiane patients.