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Markers of inflammation are cross-sectionally associated with microvascular complications and cardiovascular disease in type 1 diabetes—the EURODIAB Prospective Complications Study

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Abstract *Aims/hypothesis:* The pathogenesis of vascular complications in type 1 diabetes is poorly understood, but may involve chronic, low-grade inflammation. We investigated the association of markers of inflammation with vascular complications in type 1 diabetes. *Methods:* A cross-sectional nested case-control study of the follow-up data of the EURODIAB Prospective Complications Study. This study included 543 individuals (278 men) with type 1 diabetes diagnosed at <36 years of age. Cases (n=348) had complications of diabetes, controls (n=195) had no complications. *Results:* C-reactive protein, interleukin-6 and tumour necrosis factor-α levels, which were combined in an inflammatory marker Z-score, were associated with albuminuria, retinopathy and cardiovascular disease. Calculated means (95% confidence intervals) of the marker Z-score were −0.15 (−0.22 to −0.07), 0.10 (−0.05 to 0.25), and 0.28 (0.15 to 0.41), p for trend <0.0001, in individuals with normo-, micro- and macroalbuminuria; −0.23 (−0.33 to −0.13), 0.14 (0.02 to 0.25) and 0.20 (0.07 to 0.32), p for trend <0.0001, in individuals with no, non-proliferative and proliferative retinopathy; and −0.28 (−0.39 to −0.18) and 0.06 (−0.08 to 0.20), p<0.001, in individuals without and with cardiovascular disease. Per 1 SD increase of the inflammatory marker Z-score, GFR decreased by −4.6 (−6.6 to −2.6) ml per min per 1.73 m^2 (p<0.001). *Conclusions/interpretation:* We have shown that markers of inflammation are strongly and independently associated with microvascular complications and cardiovascular disease in type 1 diabetes. These data suggest that strategies to decrease inflammatory activity may help to prevent the development of vascular complications in type 1 diabetes.

Keywords Advanced glycation endproducts · Cardiovascular disease · C-reactive protein · Diabetes · Interleukin-6 · Microvascular complications · Tumour necrosis factor-α

Abbreviations CEL: N^{ε} -(carboxyethyl)lysine · CML: N^{ε} -(carboxymethyl)lysine · CRP: C-reactive protein

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The pathogenesis of vascular complications in type 1 diabetes is poorly understood. Glycaemic control and systolic blood pressure are important determinants in type 1 diabetes [1, 2], but cannot in themselves wholly account for disease occurrence.

Recent data on the pathogenesis of atherothrombosis and of (micro)albuminuria in non-diabetic and type 2 diabetic individuals have shown that inflammation plays an important role in their development [3]. As in type 2 diabetes, inflammatory activity is increased in type 1 diabetes [4]. We therefore hypothesised that inflammatory activity may be involved in the development of vascular complications in type 1 diabetes, both directly and by

mediating, in part, the effects of conventional risk factors such as blood pressure, HbA_{1c} and advanced glycation endproducts (AGEs) [5].

To test this hypothesis, we investigated the associations of markers of inflammation, i.e., C-reactive protein (CRP), interleukin-6 (IL-6), tumour necrosis factor-α (TNF-α), with the presence of microvascular complications and cardiovascular disease in type 1 diabetic patients of the EURODIAB Prospective Complications Study. In addition, we investigated whether the associations of HbA_{1c}, the AGEs pentosidine, N^{ε} -(carboxymethyl)lysine (CML) and N^{ε} -(carboxyethyl) lysine (CEL), systolic blood pressure and other vascular risk factors on the one hand with microvascular complications and cardiovascular disease on the other were mediated through inflammatory activity.

Methods

Subjects The EURODIAB Prospective Complications Study is a follow-up of the EURODIAB IDDM Complications study [6]. Baseline investigations (1988–1991) were performed on 3,250 men and women with type 1 diabetes drawn from 31 European centres. All subjects gave informed consent and the study was approved by local ethics committees. Sample selection was stratified by sex, age group and duration of diabetes, to ensure sufficient representation in all categories. Type 1 diabetes was clinically defined as a diagnosis made before the age of 36 years, with a continuous need for insulin therapy within 1 year of diagnosis. The follow-up was performed on average 7–9 years later. Of the 3,250 patients, 1,880 (57.8%) returned for examination [1, 2, 7]. At follow-up, a cross-sectional nested case-control study of markers and precipitators of inflammation and endothelial function and their associations with complications was performed (n=543). Markers of inflammation could not be determined at baseline because these samples have run out.

We assessed microvascular complications and cardiovascular disease; did a physical examination; measured height, weight, waist and hip circumference, and resting blood pressure; obtained information on smoking habits; and measured biochemical variables according to a standardised protocol [6]. Albumin excretion rates were measured centrally from 2×24-h urine collections as previously described [1]. Micro- and macroalbuminuria were defined as an albumin excretion rate of between 20 and 200, and above 200 μg/min, respectively. We estimated glomerular filtration rate (GFR) by the Cockroft–Gault formula. Retinopathy was assessed from retinal photographs according to the EURODIAB protocol [8]. Cardiovascular disease was defined as a positive medical history of a cardiovascular event, including myocardial infarction, angina, coronary artery bypass graft and/or stroke, and/or ischaemic changes on a centrally Minnesota coded ECG [9]. Hypertension was defined as systolic pressure ≥140 mmHg, diastolic pressure ≥90 mmHg and/or use of antihypertensive drugs.

Laboratory measurements Pentosidine levels were determined in unhydrolysed urine as previously described [10]. Urinary excretion of pentosidine was normalised for urine concentration by expressing it as nmol pentosidine/mmol urinary creatinine. CML and CEL were determined in plasma as previously described [11] with an inter-assay coefficient of variation of 6.0%. CML and CEL were normalised for lysine concentration by expressing it as μM/mM lysine.

Plasma levels of CRP (n=539), IL-6 (n=536) and TNF-α (n=528) were measured as previously described [12]. Intra- and inter-assay coefficients of variation as determined in our laboratory were 3.9 and 8.7%, 4.5 and 9.0%, 7.3 and 8.5%, respectively.

Selection of cases and controls Cases were selected to have the greatest complication burden as possible, to provide sufficient numbers for subgroup analyses. Controls were selected to be completely free of complications. Thus cases were all those with cardiovascular disease or proliferative retinopathy or macroalbuminuria at follow-up, and all those with microalbuminuria and some degree of retinopathy (n=348). Controls were all those who had no evidence of cardiovascular disease, retinopathy or neuropathy, and were normoalbuminuric at follow-up (n=195). This selection allowed us to compare people with and without complications. Cases and controls were unmatched, so that the impact of key variables, such as age, could still be assessed, and any adjustments were taken care of at the analysis stage.

Z-score Because we measured markers of inflammatory activity only once, the associations (if any) of inflammatory activity with outcomes will tend to be underestimated. To address this concern, we constructed an inflammatory marker *Z*-score that combined information on C-reactive protein, interleukin-6 and tumour necrosis factor-α. For each individual, the values of each inflammation marker were expressed as a *Z*-score, i.e. (value in the individual minus the mean value in the study population) divided by the standard deviation, a value that thus ranged from approximately −2.5 to +2.5. The inflammatory marker *Z*-score was then calculated as (*Z*-score of C-reactive protein +*Z*-score of interleukin-6+*Z*-score of tumour necrosis factor-α) / 3.

Statistical analysis For the subsequent analyses, we divided the patients into groups according to the level of albuminuria (normo-, micro- and macroalbuminuria), the presence and severity of retinopathy (no, non-proliferative and proliferative), and the presence or absence of cardiovascular disease. In the latter group, we excluded from the analyses those in whom cardiovascular disease was absent but who did have micro- or macroalbuminuria or retinopathy (227 of 348 individuals).

Primary analyses Variables with a skewed distribution were ln-transformed in all analyses. We used ANOVA to estimate mean values of CRP, IL-6, TNF-α and the inflammatory marker *Z*-score according to the presence or absence and severity of vascular complications. We then

adjusted these analyses for age, sex, HbA_{1c}, duration of diabetes and systolic blood pressure. We used linear regression analyses to investigate the association of inflammatory markers with GFR.

Secondary analyses We next investigated whether HbA_{1c}, pentosidine, CML, CEL, systolic blood pressure and other vascular risk factors were associated with albuminuria, GFR, retinopathy and cardiovascular disease independently of age, sex, HbA_{1c}, duration of diabetes and systolic blood pressure, and whether these associations were mediated by the level of inflammatory activity, by further adjusting these associations for the inflammatory marker *Z*-score. A change in the β of more than 33% was considered to be consistent with mediation by the inflammatory marker *Z*-score.

To compare the strength of the association of the inflammatory marker *Z*-score with vascular complications with that of established risk factors, we performed logistic regression analyses with the presence of vascular complications as dependent and the inflammatory marker *Z*-score and HbA_{1c} as independent variables.

A *p*-value of <0.05 was considered statistically significant.

Results

Table 1 shows the baseline characteristics of the study population.

Table 1 Characteristics of 543 type 1 diabetic individuals

Variable	Individuals with vascular complications (*n*=348)	Individuals without vascular complications (*n*=195)	*p* Value
Sex (men/women)	185/163	93/102	0.2
Duration of type 1 diabetes (years)	23.6 (19.0–30.3)	13.8 (10.8–18.5)	<0.001
Age (years)	41.8±10.6	36.1±8.1	<0.001
Body mass index (kg/m^2)			
Men/women	25.0±3.1/24.7±3.9	24.5±2.3/23.2±2.8	0.19/<0.001
Waist-to-hip ratio			
Men/women	0.93±0.10/0.84±0.13	0.91±0.13/0.85±0.19	0.2/0.9
Smoking status (%)			
Never/past/current	35.6/31.3/33.0	48.7/25.6/25.6	
Pack-years of smoking			
Past/current	9.0 (1.0–27.0)/ 15.0 (7.5–27.5)	3.3 (0.6–10.3)/ 9.6 (1.8–16.9)	
Retinopathy (%)			
No/non-proliferative/proliferative	12.4/42.8/44.8	–	0.04/<0.001
Normo-/micro-/macroalbuminuria (%)	38.9/24.2/36.9	–	
Cardiovascular disease (%)	34.8	–	
Systolic blood pressure (mmHg)	128±22	115±13	<0.001
Diastolic blood pressure (mmHg)	76±12	74±11	0.06
Hypertension (%)	57.5	13.4	<0.001
Serum creatinine (μmol/l)	75 (68–90)	72 (64–79)	<0.001
GFR (ml per min per 1.73 m^2)	97±27	113±18	<0.001
Total cholesterol (mmol/l)	5.49±1.20	4.97±1.09	<0.001
HDL cholesterol (mmol/l)	1.60±0.43	1.69±0.45	0.02
LDL cholesterol (mmol/l)	3.28±1.10	2.86±0.93	<0.001
Triglycerides (mmol/l)	1.14 (0.85–1.60)	0.84 (0.66–1.08)	<0.001
HbA_{1c} (%)	9.0±1.6	7.7±1.3	<0.001
Urinary pentosidine (nmol/mmol creatinine)	0.47 (0.34–0.68)	0.42 (0.32–0.55)	0.001
CML (μM/mM lysine)	0.058±0.019	0.055±0.015	0.14
CEL (μM/mM lysine)	0.030±0.011	0.031±0.011	0.38
CRP (mg/l)	1.32 (0.52–2.92)	0.69 (0.35–1.79)	<0.001
IL-6 (pg/ml)	2.14 (1.35–3.96)	1.55 (1.05–2.42)	<0.001
TNF-α (pg/ml)	3.17 (2.35–4.37)	2.23 (1.68–2.85)	<0.001
Inflammatory marker *Z*-score (SD)	0.21±0.72	−0.38±0.59	<0.001

Data are presented as number, percentage or mean±SD, except for duration, pack-years of smoking, serum creatinine, triglycerides, pentosidine, CRP, IL-6 and TNF-α, which are shown as median (interquartile range)

Table 2 CRP, IL-6, TNF-α and the inflammatory marker *Z*-score according to the level of albuminuria

	Normoalbuminuria (*n*=329)		Microalbuminuria (*n*=84)		Macroalbuminuria (*n*=128)		*p* (for trend)
	Mean	95% CI	Mean	95% CI	Mean	95% CI	
CRP (mg/l)							
Crude	0.91	0.79–1.04	1.18	0.91–1.55	1.37	1.11–1.71	0.004
Model 1	0.94	0.81–1.08	1.09	0.83–1.42	1.24	0.98–1.58	0.17
IL-6 (pg/ml)							
Crude	1.90	1.73–2.09	2.76	2.28–3.33	2.79	2.39–3.24	<0.0001
Model 1	1.96	1.77–2.17	2.62	2.15–3.19	2.62	2.21–3.12	0.009
TNF-α (pg/ml)							
Crude	2.42	2.31–2.53	3.11	2.84–3.41	3.93	3.65–4.23	<0.0001
Model 1	2.55	2.43–2.68	2.95	2.69–3.24	3.56	3.28–3.88	<0.0001
Inflammatory marker *Z*-score (SD)							
Crude	−0.20	−0.28 to −0.13	0.18	0.03–0.33	0.40	0.28–0.52	<0.0001
Model 1	−0.15	−0.22 to −0.07	0.10	−0.05 to 0.25	0.28	0.15–0.41	<0.0001

Data are expressed as estimated means with 95 % confidence intervals (95% CI). Model 1 was adjusted for age, sex, HbA_{1c}, duration of diabetes and systolic blood pressure

Primary analyses All inflammatory markers were significantly associated with albuminuria, GFR (inversely), retinopathy and cardiovascular disease in crude and adjusted analyses, except for CRP and albuminuria, CRP and GFR, and IL-6 and GFR in the adjusted analyses (Tables 2, 3, 4, 5 and Fig. 1).

Secondary analyses HbA_{1c}, systolic blood pressure, total cholesterol, triglycerides, LDL cholesterol, duration of diabetes and pack-years of smoking were independently associated with albuminuria and with retinopathy. Body mass index and HDL cholesterol were independently associated with retinopathy. Pentosidine was associated only with albuminuria; the association with retinopathy disappeared after adjustment for duration of diabetes. CML was independently associated only with albuminuria, while CEL was not associated with albuminuria, retinopathy or cardiovascular disease. Pentosidine, CML, CEL, body mass index, waist circumference, hypertension and duration of diabetes were independently associated with GFR (Table 5). None of the above associations changed markedly by adjustment for the inflammatory marker *Z*-score, except for the association of triglycerides with macroalbuminuria, and the associations of triglycerides, body mass index and HDL cholesterol with retinopathy (Table 6).

HbA_{1c}, HDL cholesterol, triglycerides, duration diabetes and pack-years of smoking were independently associated with cardiovascular disease, but these associations were not notably affected by adjustment for the inflammatory marker *Z*-score.

The association of the inflammatory marker *Z*-score with vascular complications was of a similar order of magnitude as that of HbA_{1c} (Fig. 2).

Additional adjustments The results were not changed materially by additional adjustments for body mass index, waist circumference, total cholesterol, triglycerides, HDL and LDL cholesterol, serum creatinine, pack-years of smoking, the presence of albuminuria, retinopathy or cardiovascular disease, the use of analgesics and anti-inflammatory drugs (*n*=59), oral contraceptives (*n*=110) or hormone replacement therapy (*n*=38), and the presence of peripheral artery disease as assessed by the ankle arm

Table 3 CRP, IL-6, TNF-α and the inflammatory marker *Z*-score according to the presence and severity of retinopathy

	No retinopathy (*n*=238)		Non-proliferative retinopathy (*n*=149)		Proliferative retinopathy (*n*=156)		*p* (for trend)
	Mean	95% CI	Mean	95% CI	Mean	95% CI	
CRP (mg/l)							
Crude	0.79	0.67–0.92	1.31	1.07–1.60	1.28	1.05–1.55	<0.0001
Model 1	0.86	0.72–1.02	1.24	1.02–1.51	1.12	0.90–1.39	0.033
Model 2	0.92	0.77–1.08	1.17	0.96–1.14	1.09	0.89–1.34	0.20
IL-6 (pg/ml)							
Crude	1.73	1.55–1.93	2.57	2.24–2.96	2.74	2.39–3.13	<0.0001
Model 1	1.85	1.63–2.10	2.51	2.18–2.91	2.50	2.14–2.93	0.01
TNF-α (pg/ml)							
Crude	2.27	2.15–2.40	3.06	2.86–3.29	3.60	3.37–3.85	<0.0001
Model 1	2.43	2.28–2.59	2.96	2.76–3.18	3.36	3.11–3.63	<0.0001
Inflammatory marker *Z*-score (SD)							
Crude	−0.32	−0.41 to −0.24	0.18	0.07–0.29	0.31	0.21–0.42	<0.0001
Model 1	−0.23	−0.33 to −0.13	0.14	0.02–0.25	0.20	0.07–0.32	<0.0001

Data are expressed as estimated means with 95 % confidence intervals (95% CI). Model 1 was defined as in the legend to Table 2; model 2 includes additional adjustment for body mass index

Table 4 CRP, IL-6, TNF-α and the inflammatory marker *Z*-score according to the presence of cardiovascular disease

	No cardiovascular disease (*n*=195)		Cardiovascular disease (*n*=121)		*p* (for trend)
	Mean	95% CI	Mean	95% CI	
CRP (mg/l)					
Crude	0.75	0.63–0.90	1.24	0.99–1.56	0.001
Model 1	0.83	0.68–1.00	1.07	0.83–1.38	0.16
IL-6 (pg/ml)					
Crude	1.70	1.51–1.91	2.60	2.24–3.02	<0.001
Model 1	1.83	1.61–2.08	2.31	1.95–2.74	0.05
TNF-α (pg/ml)					
Crude	2.18	2.05–2.31	3.22	3.00–3.46	<0.0001
Model 1	2.30	2.16–2.45	2.94	2.70–3.19	<0.0001
Inflammatory marker *Z*-score (SD)					
Crude	−0.37	−0.47 to −0.28	0.21	0.09–0.34	<0.0001
Model 1	−0.28	−0.39 to −0.18	0.06	−0.08 to 0.20	<0.001

Data are expressed as estimated means with 95% confidence intervals (95% CI). Model 1 was defined as in the legend to Table 2

index, or exclusion of individuals with CRP levels above 10 mg/l (*n*=16), except that the association between CRP and retinopathy decreased after adjustment for body mass index (Table 3, model 2).

Discussion

Our study shows that inflammatory activity is associated with vascular complications in Type 1 diabetic individuals. Due to the cross-sectional design, we cannot establish whether these associations are causal. However, our data are consistent with the hypothesis that inflammation is involved in the pathogenesis of both small and large vessel disease. Our results further suggest that inflammatory activity is involved in the pathway through which dyslipidaemia and obesity lead to microvascular damage.

Inflammation may contribute to increased urinary albumin excretion and decreased GFR by increasing glomerular permeability [13–15]. In support, anti-inflammatory therapy can prevent the development of albuminuria in experimental diabetic nephropathy [16]. In advanced nephropathy, which is often associated with a decline in GFR, renal tubular protein overload has been shown to elicit an inflammatory response that eventually results in renal interstitial remodelling and scarring [17]. In this phase, the kidney in diabetes may thus be both a target and a source of inflammation, a notion that is consistent with our observation of a stronger association of inflammation with macro- than with microalbuminuria.

Dyslipidaemia can damage glomerular podocytes, and mesangial and endothelial cells through exposure to oxidised LDL [18]. Activation of these cells results in an inflammatory response [19]. Our data suggest that the association of elevated triglyceride levels with albuminuria is, in part, mediated by inflammation.

Table 5 Associations of CRP, IL-6, TNF-α, the inflammatory marker *Z*-score and AGEs with GFR

	β	95% CI	Standardised *β*	*p* Value
CRP (mg/l)				
Crude	−0.2	−0.8 to 0.3	−1.0	0.41
Model 1	0.3	−0.2 to 0.8	1.3	0.25
Model 2	0.2	−0.4 to 0.7	0.5	0.59
IL-6 (pg/ml)				
Crude	−0.02	−0.09 to 0.05	−0.8	0.56
Model 1	−0.01	−0.07 to 0.06	−0.3	0.86
Model 2	0.01	−0.05 to 0.07	0.3	0.75
TNF-α (pg/ml)				
Crude	−7.3	−8.4 to −6.2	−12.5	<0.001
Model 1	−6.6	−7.7 to −5.6	−11.5	<0.001
Model 2	−6.0	−7.0 to −5.0	−10.7	<0.001
Inflammatory marker *Z*-score (SD)				
Crude	−10.7	−13.5 to −7.8	−7.9	<0.001
Model 1	−6.2	−9.0 to −3.5	−4.6	<0.001
Model 2	−7.1	−9.8 to −4.3	−5.1	<0.001
Urinary pentosidine (nmol/mmol creatinine)				
Crude	−10.8	−14.2 to −7.4	−6.6	<0.001
Model 1	−7.7	−10.7 to −4.7	−4.9	<0.001
Model 2	−5.9	−8.8 to −3.0	−3.6	<0.001
CML (0.01 mM/mM lysine)				
Crude	−4.1	−5.3 to −3.0	−7.4	<0.001
Model 1	−3.4	−4.4 to −2.4	−6.1	<0.001
Model 2	−2.5	−3.5 to −1.5	−9.4	<0.001
CEL (0.01 mM/mM lysine)				
Crude	−5.5	−7.4 to −3.6	−6.1	<0.001
Model 1	−4.1	−5.8 to −2.4	−6.4	<0.001
Model 2	−3.7	−5.3 to −2.1	−4.1	<0.001

Data are expressed as regression coefficient (*β*), 95% confidence intervals (95% CI) and standardised *β*. The standardised *β* was expressed by 1 SD of the independent variable, thus per 1 SD increase of CRP the GFR decreased by 1.0 ml per min per 1.73 m^2 (top right). Model 1 was defined as in the legend to Table 2; model 2 includes additional adjustment for body mass index, waist circumference, urinary albumin excretion, retinopathy and cardiovascular disease

AGEs are thought to contribute to the pathogenesis of diabetic complications in part through inflammatory mechanisms [20]. We measured pentosidine, a specific marker of glycation [21, 22], urinary levels of which have been shown to be closely related to serum concentrations [23], and CML and CEL, which are thought to be major AGE products but which can be formed independently of glycation [24]. However, the associations of pentosidine and CML with albuminuria appeared not to be mediated through inflammation. Other pathogenic pathways, such as those involving matrix function and oxidative stress, may thus be involved [24]. The inverse associations of pentosidine, CML and CEL with GFR suggest that these AGEs are important in the pathogenesis of the decline in GFR in diabetic nephropathy, and evidence exists that inhibitors of AGE formation have renoprotective effects [25]. Alternatively, we cannot exclude the possibility that levels of AGEs were increased due to the decrease in GFR.

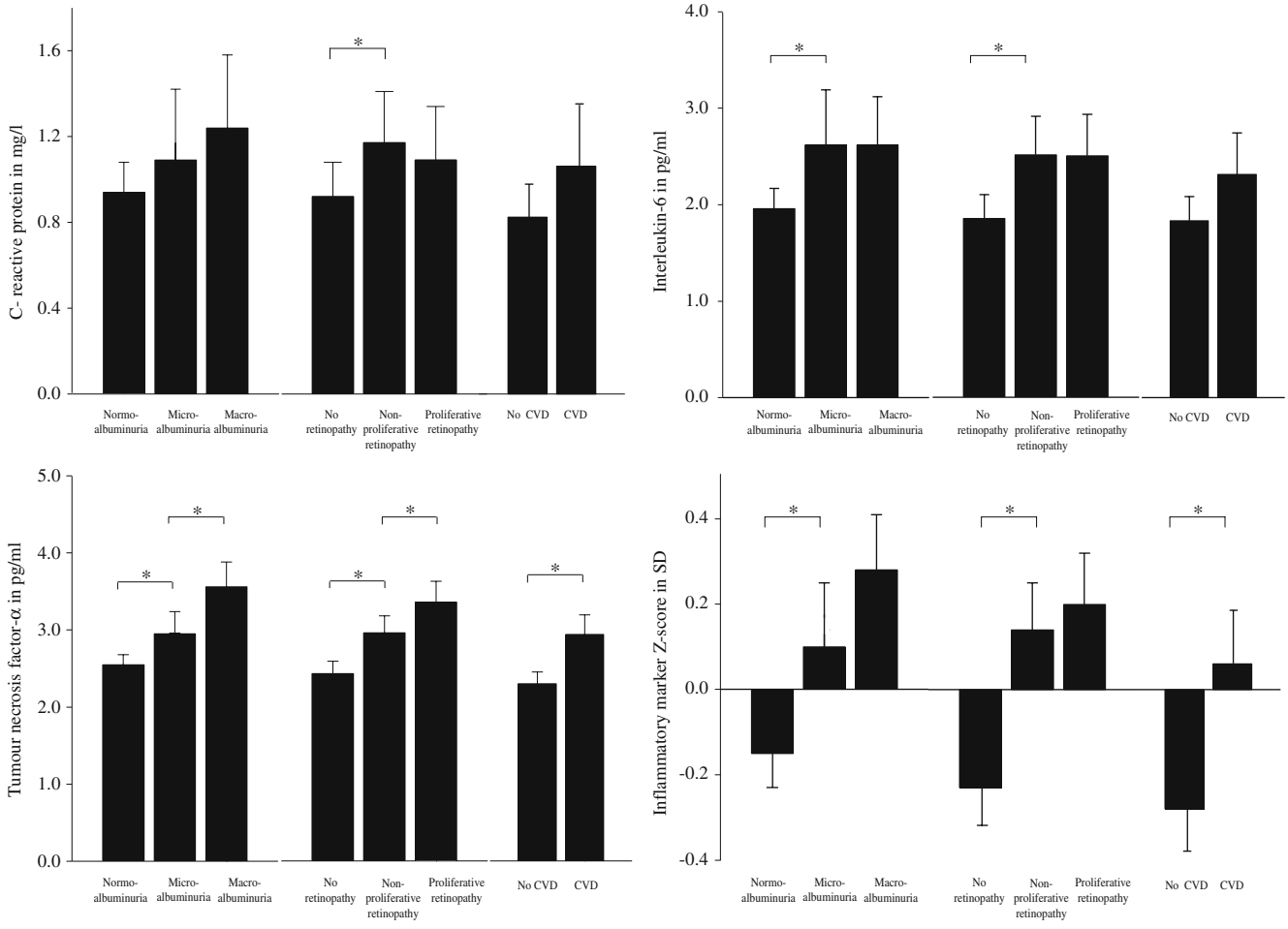

Fig. 1 Levels of CRP, IL-6, TNF-α and the inflammatory marker *Z*-score according to the severity of albuminuria, the presence and severity of retinopathy, and the presence of cardiovascular disease (CVD), *$p<0.05$. The *bars* represent the 95% confidence intervals

Inflammatory markers were associated with both early and advanced retinopathy, which suggests that inflammation plays a role in both early and late stages of diabetic retinopathy. The interpretation that the retina is a source of inflammation is unlikely, as the retina is unable to increase protein synthesis to the extent that plasma protein levels increase [26]. Our results are in accordance with previous studies that suggest a role for inflammation and endothe-

Table 6 Associations of selected risk factors and vascular complications: effect of adjustment for inflammatory activity

	Normo- vs. microalbuminuria			Normo- vs. macroalbuminuria			No vs. non-proliferative retinopathy			No vs. proliferative retinopathy		
	β	*p* Value	% Change	*β*	*p* Value	% Change	*β*	*p* Value	% Change	*β*	*p* Value	% Change
Triglycerides (mmol/l)												
Model 1	0.04	0.876		0.57	0.051		1.31	<0.001		1.02	0.003	
Model 2	−0.10	0.742	–	0.29	0.332	−49	0.97	0.003	–	0.67	0.066	−35
Body mass index (kg/m^2)												
Model 1							0.10	0.016		0.10	0.058	
Model 2						–	0.07	0.115	–	0.06	0.268	−33
HDL cholesterol (mmol/l)												
Model 1							−0.78	0.008		−0.87	0.024	
Model 2						–	−0.40	0.200	−48	−0.39	0.331	−55

Data are expressed as *β* with *p* values as resulted from logistic regression analyses. Model 1 was adjusted for age, sex, HbA1c, duration of diabetes and systolic blood pressure, unless this was the variable under consideration. Model 2 was identical to model 1 plus adjustment for the inflammatory marker *Z*-score. Triglycerides and pentosidine were ln-transformed in all analyses, *β* are given for the ln-transformed variable

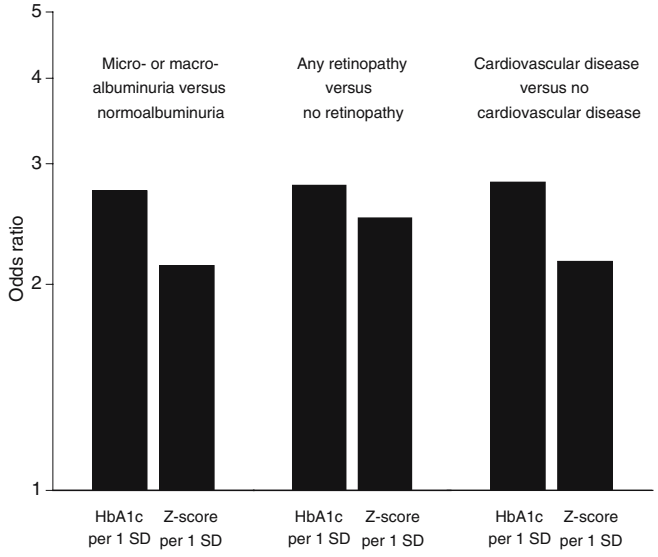

Fig. 2 The odds ratios of the associations of HbA_{1c} and the inflammatory marker *Z*-score with albuminuria, retinopathy and cardiovascular disease. The association of the inflammatory marker *Z*-score with the presence of vascular complications was somewhat smaller than, but of a similar magnitude as, the association of HbA1c with the presence of vascular complications. Odds ratios were calculated per standard deviation (SD)

lial dysfunction in the pathophysiology of diabetic retinopathy [27, 28]. Inflammatory mediators may increase retinal vascular permeability [29], possibly by upregulation of adhesion molecules on endothelial cells, and may cause changes in endothelial cell contact with pericytes, resulting in pericyte loss and the development of microaneurysms [30]. In the pathogenesis of proliferative retinopathy, retinal ischaemia and subsequent neovascularisation play a pivotal role. Increased inflammatory activity has been shown to stimulate leucocytes to adhere to retinal endothelial cells [30], which causes leucostasis and temporary ischaemia upstream of the adhering leucocytes. Reperfusion may damage the endothelium due to the generation of oxidative stress [31], setting the stage for a vicious cycle of endothelial dysfunction, ischaemia and reperfusion. Alternatively, inflammation may reduce endothelial production of nitric oxide [14]. Together with an increased release of vasoconstrictors, this may lead to vasospasm and subsequent retinal ischaemia [32]. In addition, treatment with anti-inflammatory agents may decrease the elevated retinal TNF-α levels in diabetes [33].

The association of body mass index with retinopathy appeared, in part, mediated by inflammation. Fat cells are a potential source of inflammatory mediators [34]. Importantly, our data suggest that even at low body mass indexes, as in our population, body fat and its distribution play a role in the development of microvascular complications.

Low HDL cholesterol levels are associated with retinopathy [2]. Our data suggest that this association is, in part, mediated by inflammation. Dyslipidaemia may lead to a systemic increase of the inflammatory state by glomerulosclerotic and atherosclerotic processes as described above [19]. However, we clearly cannot exclude the reverse interpretation that increased inflammatory activity *causes* changes in lipid profile [35], as prospective studies are needed to do this.

We observed an association between inflammation and the presence of cardiovascular disease in type 1 diabetic individuals. Inflammatory markers may reflect low-grade vessel wall inflammation, which plays a pivotal role in the pathogenesis of cardiovascular disease [19]. The reverse interpretation, that cardiovascular disease leads to an increased inflammatory activity is unlikely, since there is much evidence that inflammation is involved in the pathophysiology of cardiovascular disease [19]. Alternatively, body fat [34] or, in the presence of advanced nephropathy, the kidney may be sources of inflammatory mediators in diabetes. In any case, low-grade inflammation increases the expression of vascular adhesion molecules on endothelial cells, enhances the invasion of monocytes into the vascular wall, and eventually leads to the formation of atherosclerosis and cardiovascular disease [19].

Due to the cross-sectional design of this study, we cannot establish the time course of the associations between inflammation and vascular complications. However, longitudinal studies in non-diabetic and type 2 diabetic individuals have demonstrated that the increase in inflammatory activity is progressive and precedes the occurrence of vascular complications [36]. At this time, no longitudinal data of this type exist in type 1 diabetes. Therefore this cross-sectional study may serve as a starting point to explore these associations in type 1 diabetes. The probability of confounding in the associations we explored has been minimised by adjustments for many possible confounders. However, interference by factors we did not measure, such as other AGEs, cannot be ruled out. In addition, concentrations of inflammatory markers were only measured once, which might have diluted the associations we found, which thus may to some extent have been underestimated. The validity of our study design is supported by the finding that conventional risk factors were, as expected [37, 38], associated with microvascular complications and cardiovascular disease.

In conclusion, inflammation is associated with microvascular complications and cardiovascular disease in type 1 diabetes. These associations were of a similar order of magnitude as the association HbA_{1c} with vascular complications (Fig. 2). In addition, the associations of obesity and dyslipidaemia with vascular complications appear dependent on inflammatory activity. This suggests that inflammation itself is involved in the pathophysiological pathway leading to vascular complications and that it is involved in the pathways through which obesity and dyslipidaemia lead to vascular complications. Therefore, strategies to decrease inflammatory activity may help to prevent the development of vascular complications in type 1 diabetes.

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