

YKL-40, a Marker of Inflammation and Endothelial Dysfunction, Is Elevated in Patients With Type 1 Diabetes and Increases With Levels of Albuminuria

CAMILLA NOELLE RATHCKE, MD¹
 FREDERIK PERSSON, MD²
 LISE TARNOW, MD, DMSC²

PETER ROSSING, MD, DMSC²
 HENRIK VESTERGAARD, MD, DMSC^{1,3}

OBJECTIVE — The inflammation marker YKL-40 is elevated in patients with type 2 diabetes and is associated with atherosclerosis and increased cardiovascular mortality. In the present study, YKL-40 levels were examined in patients with type 1 diabetes with increasing levels of albuminuria, known to be associated with an increased risk of cardiovascular disease.

RESEARCH DESIGN AND METHODS — A total of 149 patients with type 1 diabetes attending Steno Diabetes Center were examined: 58 had normoalbuminuria (urinary albumin excretion rate <30 mg/24 h), 46 had persistent microalbuminuria (urinary albumin excretion rate 30–300 mg/24 h), and 45 had persistent macroalbuminuria/diabetic nephropathy (urinary albumin excretion rate >300 mg/24 h). The control group consisted of 55 healthy individuals. Groups were matched according to sex and duration of diabetes (>30 years).

RESULTS — Median levels [interquartile range] of serum YKL-40 were significantly higher in normoalbuminuria versus control (37 [29–52] vs. 53 [32–105] ng/ml, $P < 0.01$) and were increasing with increasing levels of albuminuria (microalbuminuria 74 [45–160] ng/ml and diabetic nephropathy 117 [68–215] ng/ml; $P < 0.001$ for all comparisons). YKL-40 levels correlated with the urinary albumin-to-creatinine ratio in the total group of participants ($r^2 = 0.25$, $P < 0.001$). Significant but weak intercorrelations of YKL-40 were found with age, diastolic blood pressure, A1C, and serum creatinine. After adjustment for significant covariates, albuminuria was significantly associated with YKL-40 levels ($P < 0.001$).

CONCLUSIONS — YKL-40 levels are elevated in patients with type 1 diabetes with an independent association between increasing YKL-40 levels and increasing levels of albuminuria. The present study is the first to suggest a role of YKL-40 in the gradually progressing vascular complications in patients with type 1 diabetes.

Diabetes Care 32:323–328, 2009

Persistent microalbuminuria is an established predictor of diabetic nephropathy leading to progressive renal insufficiency and end-stage renal disease and is associated with an increased risk of cardiovascular disease in patients with both type 1 and type 2 diabetes (1–3). Individuals with diabetes have, in general, a two- to fourfold in-

creased risk of subsequent cardiovascular disease (4). A large-scale study of patients with type 1 diabetes demonstrated up to a ninefold increased mortality risk from ischemic heart disease, which was excessively higher in patients <30 years of age (5). A substantial proportion of patients with type 1 diabetes will develop diabetic nephropathy years after onset of diabetes,

although studies from selected centers suggest a declining incidence (6,7).

Identification of predictors of cardiovascular disease and progression of nephropathy in patients with type 1 diabetes is important. The fact that the urinary albumin excretion rate is associated with an increased risk of cardiovascular morbidity and mortality (2), even in nondiabetic individuals and also at levels below the threshold of microalbuminuria (low-grade albuminuria) (8), suggests that an increasing urinary albumin excretion rate reflects vascular damage in the kidneys as part of systemic endothelial dysfunction (9). Endothelial dysfunction is the initial step in atherogenesis, which is largely responsible for the development of ischemic heart disease and thrombotic strokes (10).

YKL-40 is a marker of inflammation and endothelial dysfunction. It is a growth factor for several cell types and has an established role in extracellular matrix remodeling and angiogenesis (11). A substantial body of evidence indicates that YKL-40 participates in processes during the early stages of atherosclerosis, and it seems to be of pathogenic importance in the low-grade inflammation that precedes the development of cardiovascular disease (11–14). We have previously found significantly elevated levels of YKL-40 in patients with type 2 diabetes and an independent positive correlation with insulin resistance and parameters of the lipid profile (15,16).

Studies of YKL-40 have never been performed in patients with type 1 diabetes. The objective of the present study was to evaluate serum YKL-40 levels in patients with type 1 diabetes and with increasing levels of albuminuria. On the basis of previous studies, we expected to find 1) higher serum YKL-40 levels in patients with type 1 diabetes and 2) increasing serum YKL-40 levels with increasing levels of albuminuria explained by a progressive systemic endothelial dysfunction.

From the ¹Department of Endocrinology, Herlev Hospital, University of Copenhagen, Copenhagen, Denmark; the ²Steno Diabetes Center, Gentofte, Denmark; and the ³Faculty of Health Sciences, University of Copenhagen, Copenhagen, Denmark.

Corresponding author: Camilla Noelle Rathcke, cnr@dadlnet.dk.

Received 25 June 2008 and accepted 22 October 2008.

Published ahead of print at <http://care.diabetesjournals.org> on 28 October 2008. DOI: 10.2337/dc08-1144.

The corresponding author had full access to all data and takes full responsibility for the integrity of the data and the accuracy of the analyses.

© 2009 by the American Diabetes Association. Readers may use this article as long as the work is properly cited, the use is educational and not for profit, and the work is not altered. See <http://creativecommons.org/licenses/by-nc-nd/3.0/> for details.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Table 1—Clinical data of the control group and the diabetic patients differentiated according to level of albuminuria

	Control group	Normoalbuminuria	Microalbuminuria	Macroalbuminuria	P
n, total	55	58	45	46	0.46
Male sex (%)	65.4	51.7	53.3	58.7	0.0001
Age (years)	50.5 ± 10.9	55.6 ± 10.8	54 ± 11.1	49 ± 9.6	0.005
Diabetes duration (years)		36.8 ± 10.5	35.5 ± 11.3	33.9 ± 10.5	0.39
BMI (kg/m ²)	25.8 ± 3.6	24.7 ± 2.9	25.1 ± 3.7	25.1 ± 4.3	0.46
Smoking	10 (18.2)	12 (20.7)	13 (28.9)	19 (41.3)	0.09
Use of antihypertensive medications	0	28 (48.3)	43 (95.6)	43 (93.5)	0.0001
Use of statins	2 (3.6)	16 (27.6)	18 (40)	23 (50)	0.0001
Use of aspirin	2 (3.6)	21 (36.2)	27 (60)	29 (63)	0.0001
YKL-40 (ng/ml)	37 (27–52)	53 (32–105)	74 (45–160)	117 (68–215)	0.0001
A1C (%)	5.5 ± 0.3	8.2 ± 1.1	8.8 ± 1.3	8.8 ± 1.1	0.0001
Creatinine (μmol/l)	95 (87–104)	92 (83–97)	89 (81–101)	125 (100–174)	0.0001
UACR (mg/g)	5 (3–8)	5 (4–8)	26 (11–63)	500 (208–1,155)	0.0001
eGFR (ml/min per 1.73 m ²)	70.5 ± 9.6	70.3 ± 10.3	70.7 ± 13.4	49.2 ± 19.2	0.0001
Cholesterol (mmol/l)	5.5 ± 0.9	4.8 ± 0.8	5.0 ± 1.0	4.9 ± 1.0	0.0001
Systolic blood pressure (mmHg)	132 ± 16	138 ± 21	141 ± 23	140 ± 24	0.94
Diastolic blood pressure (mmHg)	81 ± 10	74 ± 11	74 ± 12	76 ± 12	0.003
History of					
Myocardial infarction	0	3 (5)	5 (11.1)	3 (6.5)	0.11
Stroke	0	4 (6.9)	4 (8.9)	4 (8.7)	0.17
Intermittent claudication	0	4 (6.9)	8 (17.8)	8 (17.4)	0.005
Retinopathy					
None	—	6 (10.3)	1 (2.2)	2 (4.4)	0.0001
Simplex	—	19 (32.8)	11 (24.4)	10 (21.7)	0.0001
Proliferative	—	33 (56.9)	33 (73.3)	34 (73.9)	0.0001

Data are mean ± SD, median (interquartile range), or number (%) unless specified otherwise. *Some patients had UACR levels reduced by antihypertensive medication, which was not stopped when spot urine samples were collected for the study.

RESEARCH DESIGN AND METHODS

The present study was based on data used to identify biomarkers of diabetes and diabetic nephropathy by proteomic analyses. The participants were examined at the Steno Diabetes Center in 2004 and consisted of 55 Caucasian healthy individuals (control subjects) and three groups of Caucasian patients with type 1 diabetes attending the Steno Diabetes Center. On the basis of 24-h urine collections analyzed as part of the routine care of the patients before the present study, they were divided into 58 patients with normoalbuminuria (urinary albumin excretion rate <30 mg/24 h), 46 patients with persistent microalbuminuria (at least two of three consecutive urine samples with albumin excretion rate 30–300 mg/24 h), and 45 patients with persistent macroalbuminuria/diabetic nephropathy (albumin excretion rate >300 mg/24 h). Groups were matched at the group level by sex, age (±5 years), and duration of diabetes (±3 years) (>20 years, for normoalbuminuric patients). Control subjects were randomly selected from the general healthy population by

advertisement in the local news. They were enrolled in the study if they had plasma glucose levels <6 mmol/l after an overnight fast and had no prior history of cardiovascular disease including hypertension. Subjects referred to the hospital on the suspicion of diabetes or other endocrine diseases were not included even though a diagnosis was not confirmed. Matching of control subjects with individuals from all the diabetic groups at individual level was not possible.

Investigations were performed in the morning after an overnight fast. Arterial blood pressure was measured three times with an appropriate-sized cuff after at least 10 min supine rest. Urinary albumin concentration was measured by an enzyme immunoassay from early morning spot urine collections. Serum and urine creatinine concentration was assessed by a kinetic Jaffé method. Plasma samples were stored at –80°C until analysis. Glomerular filtration rate (GFR) was estimated using the four variable Modification of Diet in Renal Disease GFR formulas (age, sex, race, and serum creatinine) (<http://mdrd.com>). Diabetic reti-

nopathy was assessed in all patients by fundus photography after pupillary dilatation and graded as nil, simplex, or proliferative. Patients were interviewed using the World Health Organization cardiovascular questionnaire. Smokers were defined as individuals smoking ≥ cigarettes/cigars/pipes a day; all others were classified as nonsmokers.

The study was approved by the local ethics committee. All patients gave their informed consent.

Serum YKL-40 was analyzed with a commercial assay (ELISA; Quidel, San Diego, CA) on material frozen at the time of inclusion. The measuring range of the assay was 20–300 ng/ml, and the intra-assay and interassay coefficients of variation were 5.8 and 6.0%, respectively.

Statistical analysis

Comparisons between the groups were made with the Kruskal-Wallis test for ordinal data. Continuous data were compared with one-way ANOVA. If data had a non-Gaussian distribution as seen in a P plot, data were logarithmically transformed. Analyses of intercorrelations and

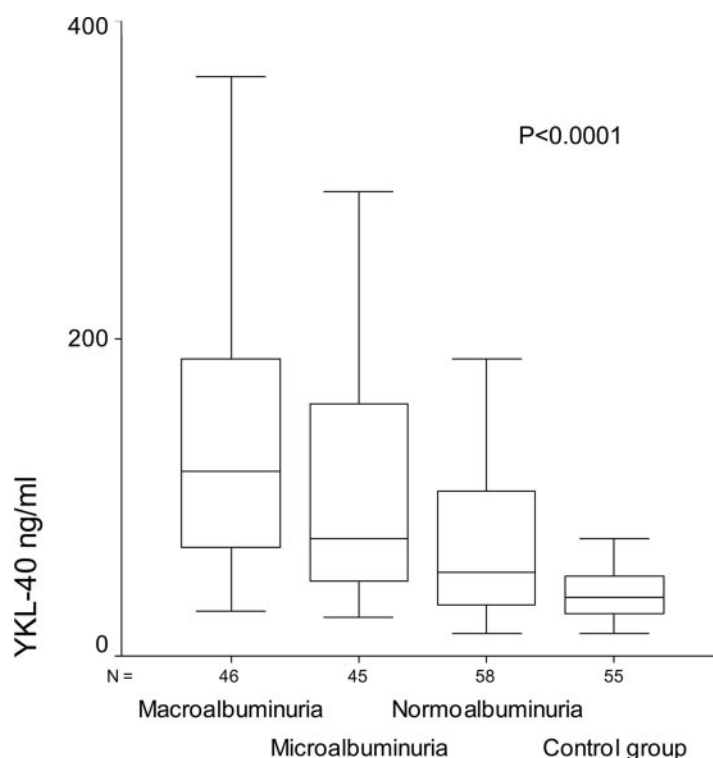


Figure 1—Median levels (interquartile range) of YKL-40 in the three diabetic groups compared with the control group: 37 (29–52) ng/ml in the control group, 53 (32–105) ng/ml in the patients with normoalbuminuria, 74 (45–160) ng/ml in patients with microalbuminuria, and 117 (68–215) ng/ml in patients with macroalbuminuria/diabetic nephropathy ($P < 0.0001$).

associations were performed using multivariate linear regression analysis.

P values were two-sided, and $P < 0.05$ was considered statistically significant. All analyses were made with the statistical software package SPSS (version 11.5; SPSS, Chicago, IL).

RESULTS— Clinical data for the control group and the diabetic patients differentiated according to level of albuminuria are shown in Table 1. YKL-40 levels according to level of albuminuria are illustrated in Fig. 1. The groups were well matched regarding sex and duration of diabetes, but the age-match was ruined because of a few patients with missing values and because of exclusion of a patient with nondiabetic nephropathy from the macroalbuminuria group.

Median YKL-40 levels were significantly different among all groups with increasing YKL-40 levels with increasing levels of albuminuria ($P < 0.001$). As expected, we found significantly lower estimated GFR (eGFR) in the macroalbuminuria group ($P < 0.0001$) but equivalent eGFR in the control, normoalbuminuria, and microalbuminuria groups. We found no significant difference in systolic blood pressure between

the groups for all comparisons or for comparisons of the diabetic groups, but there was a significant difference between the control subjects and the macroalbuminuria group ($P = 0.041$) and the control subjects and the microalbuminuria group ($P = 0.028$). The significant differences in diastolic blood pressure and serum cholesterol for all comparisons were due to differences between control subjects and the diabetic groups in total. No significant differences in diastolic blood pressure or serum cholesterol were found between the diabetic groups ($P = 0.68$ and $P = 0.39$, respectively). There was a significant difference in use of antihypertensive drugs between the groups ($P < 0.0001$). Almost half of the normoalbuminuric group (48.3%) and close to all subjects in the microalbuminuria (95.6%) and macroalbuminuria (93.5%) groups were using antihypertensive specimens.

Although we found a significant difference in A1C for all comparisons, no significant difference was found between the micro- and macroalbuminuria groups ($P = 0.94$).

Multiple regression analyses showed correlation of YKL-40 with the urinary albumin-to-creatinine ratio (UACR) in the total group of participants ($r = 0.50$, $P <$

Table 2—Intercorrelations of YKL-40

	Correlation coefficient	P
UACR	0.50	0.0001
A1C	0.36	0.001
Creatinine	0.33	0.001
Age	0.16	0.025
Diastolic blood pressure	0.14	0.049

Log-transformed data. Multiple regression analyses adjusted for sex, BMI, creatinine, cholesterol, and systolic blood pressure.

0.001) (Table 2). This correlation was not significant in any of the different subgroups. Significant intercorrelations of YKL-40 were also found with A1C, serum creatinine, age, and diastolic blood pressure, respectively, in the total group of participants (Table 2). No significant correlation was found between YKL-40 and systolic blood pressure, BMI, or total cholesterol, and YKL-40 levels were not predicted by GFR ($P = 0.73$).

In a multiple regression model adjusting for the significant covariates (UACR, A1C, serum creatinine, age, and diastolic blood pressure) and cholesterol, systolic blood pressure, and the presence of intermittent claudication and retinopathy, YKL-40 levels were significantly associated with the level of albuminuria ($P < 0.001$). Pairwise comparisons between the groups showed a significant association between YKL-40 levels and increasing levels of albuminuria to the level of microalbuminuria (Table 3). No significant difference in this association was found between the micro- and macroalbuminuria groups ($P = 0.08$).

At baseline, only a limited number of patients had symptoms of intermittent claudication or previous episodes of myocardial infarction or stroke (Table 1). Patients with intermittent claudication and stroke had significantly higher YKL-40 levels than individuals without these macrovascular complications ($P = 0.021$ and $P = 0.05$, respectively), but in accordance with the multiple regression analyses adjusting for the presence of retinopathy and intermittent claudication, these associations became insignificant with adjustment for the significant covariates.

CONCLUSIONS— The micro- and macrovascular complications of diabetes remain a constant challenge to quality of life as well as to life expectancy. Intensive

Table 3—Associations between YKL-40 and levels of albuminuria

Pairwise comparisons of levels of albuminuria		Mean difference of YKL-40	P
Macroalbuminuria	Microalbuminuria	1.38 (0.96–1.98)	0.08
	Normoalbuminuria	2.02 (1.41–2.90)	0.0001
	Control subjects	4.31 (2.48–7.46)	0.0001
Microalbuminuria	Normoalbuminuria	1.46 (1.09–1.96)	0.012
	Control subjects	3.13 (1.85–5.28)	0.001
Normoalbuminuria	Control subjects	2.13 (1.35–3.38)	0.001

Data are ng/ml (95% CI). Multiple regression analysis after adjustment for UACR, age, A1C, creatinine, cholesterol, systolic and diastolic blood pressure, and the presence of retinopathy and intermittent claudication.

research has provided knowledge about the pathogenesis and about several potentially modifiable risk factors, including poor glycemic control, increased urinary albumin excretion, hypertension, and smoking. Despite improved and intensified treatment of diabetes and its vascular risk factors and complications, supplementary risk markers alone or in combination addressing other and earlier aspects of the pathogenesis are needed.

For the first time, YKL-40, a marker of inflammation and endothelial dysfunction, has been evaluated in patients with type 1 diabetes. We found elevated YKL-40 levels in patients with type 1 diabetes compared with control subjects and showed increasing YKL-40 levels with increasing levels of albuminuria. This finding is in accordance with previous studies showing that chronic low-grade inflammation is associated with the occurrence and progression of (micro)albuminuria (17) and that both micro- and macroalbuminuria are accompanied by increased levels of a variety of markers of endothelial dysfunction (18). Several studies have shown that biomarkers of endothelial dysfunction and inflammation are elevated in patients with type 1 diabetes without as well as with microvascular complications or diabetic nephropathy (18–20). Some studies also show an association with a decline in GFR (20), cardiovascular morbidity (19,20), and overall mortality (20). Chronic low-grade inflammation and endothelial dysfunction seem to be closely linked, and it seems that chronic low-grade inflammation can be both a cause and a consequence of endothelial dysfunction (18). It seems likely, therefore, that the high risk of cardiovascular disease in patients with type 1 diabetes could partly be described by increased inflammatory activity initiated by endothelial dysfunction and that an in-

flammatory state should be addressed as a risk factor in its own.

Dysfunction of the vascular endothelium is considered an important factor in the pathogenesis of diabetic micro- and macroangiopathy (18). Studies show that YKL-40 plays a role in endothelial dysfunction in relation to cell migration, reorganization, and tissue remodeling during atherogenesis (12–14). YKL-40 promotes chemotaxis, cell attachment, spreading, and migration of vascular endothelial cells, suggesting that YKL-40 promotes the process of atherosclerotic plaque formation, in which vascular smooth muscle cells (VSMCs) are induced to migrate through the intima in response to exogenous signals (13). YKL-40 also modulates vascular endothelial cell morphology by promoting the formation of branching tubules, indicating a role of YKL-40 in angiogenesis by stimulating the migration and reorganization of VSMCs (13). Furthermore, YKL-40 is produced and secreted by monocytes during differentiation to macrophages but is also secreted by activated macrophages (11), and YKL-40 protein expression is found in vivo in both macrophages and VSMCs in the atherosclerotic plaque (11). In accordance with this finding, normoalbuminuric type 1 diabetic patients have been found to have increased monocytic activity characterized by increased monocytic release of interleukin-6 and superoxide anion, which is accentuated in type 1 diabetic patients with microvascular complications (21). Therefore, substantial evidence indicates that YKL-40 participates in monocyte differentiation and macrophage activation as part of the endothelial dysfunction and the processes during early stages of atherosclerosis (11) and seems to be of pathogenic importance in the low-grade inflammation that precedes the development of cardiovascular

disease. We have previously found elevated YKL-40 levels in patients with type 2 diabetes (15,16) in whom it correlated with insulin resistance and parameters of the lipid profile, but despite a known macrophage infiltration in adipose tissue, YKL-40 has never been found to be associated with BMI (15).

Because YKL-40 is excreted by the kidneys, we, not surprisingly, found a significant correlation between YKL-40 and UACR, but we did not find that increasing YKL-40 levels were predicted by a decline in eGFR. Despite the correlation between YKL-40 and UACR, we found a significant association between YKL-40 levels and level of albuminuria after adjustment for significant covariates, implicating increasing albuminuria with increasing YKL-40 levels. Patients with micro- and macroalbuminuria also had a higher prevalence of retinopathy and intermittent claudication, but adjustment for these complications did not attenuate the association between YKL-40 levels and level of albuminuria. The insignificant association when the macroalbuminuria level was reached is most likely explained by a systemically accentuated inflammatory state marginalizing the individual impact of YKL-40. This is in accordance with the finding of a larger proportion of individuals with proliferative retinopathy and intermittent claudication in the micro- and macroalbuminuria group and with the perception of YKL-40 as an early marker. We have previously shown an association between YKL-40 and an increased cardiovascular mortality rate in an elderly part of the general population without known diabetes and cardiovascular disease after adjustment for known cardiovascular risk factors and markers (16). In the same study, YKL-40 and UACR were independent markers of cardiovascular mortality with only weak intercorrelation and in accordance with the studies on low-grade albuminuria and risk of cardiovascular disease, YKL-40 and low-grade albuminuria synergistically predicted cardiovascular mortality. Other studies support this association between YKL-40 and cardiovascular morbidity and mortality, because YKL-40 levels are found to be associated with the presence and extent of coronary artery disease as assessed by coronary angiography (22), and, just recently, YKL-40 levels have been found to be elevated in patients with myocardial infarction (23).

Several studies have investigated the role of YKL-40 in relation to cancer, but

despite substantial evidence supporting a role of YKL-40 in cancer, newer results are conflicting (24,25). YKL-40 levels are particularly high in recurrent cancer states and highly differentiated cancers, which are characterized by high vascularization and a high turnover of extracellular matrix (24). One could hypothesize that YKL-40 may play a role in cancer because of its general role in extracellular tissue remodeling and its influence on proliferation and differentiation of vascular smooth muscle cells and vascular endothelial cells, but in vivo proof of this hypothesis is yet to be obtained. In our previous study, in which we found YKL-40 to be an independent predictor of overall and cardiovascular mortality, we did not find higher YKL-40 levels in individuals dying of cancer (16).

Limitations of the present study are the lack of investigation of other markers of endothelial dysfunction and inflammation, in particular, high-sensitivity C-reactive protein (hsCRP) because of a limited amount of biological material. However, previous studies either have not shown or have shown only a weak correlation between YKL-40 and hsCRP ($r = 0.17$, NS, and $r = 0.22$, $P < 0.0001$, respectively) (15,16), and hsCRP levels have not previously been found to influence the predictive value of YKL-40 in terms of overall or cardiovascular mortality (16). Therefore, we would not expect different outcomes if we included hsCRP in the analyses.

The perception of YKL-40 as an early marker (16) indicates that YKL-40 could possibly correlate with other early markers of endothelial activation and/or dysfunction. We found a trend toward higher YKL-40 levels in individuals with macrovascular complications, but because of a limited number of cases, the association was not statistically significant. The predictive value of YKL-40 with regard to albuminuria and the progression to nephropathy as well as the development of macrovascular complications and cardiovascular mortality is properly investigated in a prospective study, which is our next approach.

In summary, YKL-40 levels are elevated in patients with type 1 diabetes and increase with levels of albuminuria. YKL-40 levels are independently associated with increasing levels of albuminuria to the level of microalbuminuria after adjustment for UACR, age and other significant covariates, and the

presence of retinopathy and intermittent claudication.

Taken together, these results suggest a role for YKL-40 in the gradually progressing vascular complications in patients with diabetes, with YKL-40 being a possible early marker of microvascular complications. Further studies to implicate other inflammation markers and cardiovascular follow-up are needed.

Acknowledgments—The study was supported by grants from the Research Foundation of Herlev Hospital, the Research Foundation of Copenhagen County, the Hede-Nielsen Foundation, the Aase and Ejnar Danielsens Foundation, the Foundation of Advancement of Medical Science, and the A.P. Moeller and wife Chastine McKinney Moeller Foundation.

No potential conflicts of interest relevant to this article were reported.

We thank Ulla Kjaerulf-Hansen, Tonni Love Hansen, and Debbie Nadelmann, Endocrine Research Laboratory 5404, Herlev Hospital, University of Copenhagen, Denmark, for skillful laboratory assistance with measurements of YKL-40 and Tina R. Juhl, Birgitte V. Hansen, Lotte Pietrzak, Ulla M. Smidt, and Berit R. Jensen for skillful laboratory assistance at Steno Diabetes Center.

Parts of this study were presented in abstract form at the 68th annual meeting of the American Diabetes Association, San Francisco, California, 6–10 June 2008, and at the 44th Annual Meeting of the European Association for the Study of Diabetes, Rome, Italy, 7–11 September 2008.

References

1. Borch-Johnsen K, Feldt-Rasmussen B, Strandgaard S, Schroll M, Jensen JS: Urinary albumin excretion: an independent predictor of ischemic heart disease. *Arterioscler Thromb Vasc Biol* 19:1992–1997, 1999
2. Gerstein HC, Mann JF, Yi Q, Zinman B, Dinneen SF, Hoogwerf B, Halle JP, Young J, Rashkow A, Joyce C, Nawaz S, Yusuf S: Albuminuria and risk of cardiovascular events, death, and heart failure in diabetic and nondiabetic individuals. *JAMA* 286:421–426, 2001
3. Rossing P, Hougaard P, Parving H-H: Progression of microalbuminuria in type 1 diabetes: ten-year prospective observational study. *Kidney Int* 68:1446–1450, 2005
4. Stamler J, Vaccaro O, Neaton JD, Wentworth D: Diabetes, other risk factors, and 12-yr cardiovascular mortality for men screened in the Multiple Risk Factor Intervention Trial. *Diabetes Care* 16:434–444, 1993
5. Laing SP, Swerdlow AJ, Slater SD, Burden

- AC, Morris A, Waugh NR, Gatling W, Bingley PJ, Patterson CC: Mortality from heart disease in a cohort of 23,000 patients with insulin-treated diabetes. *Diabetologia* 46:760–765, 2003
6. Hovind P, Tarnow L, Rossing K, Rossing P, Eising S, Larsen N, Binder C, Parving H-H: Decreasing incidence of severe diabetic microangiopathy in type 1 diabetes. *Diabetes Care* 26:1258–1264, 2003
7. Bojestig M, Arnqvist HJ, Hermansson G, Karlberg BE, Ludvigsson J: Declining incidence of nephropathy in insulin-dependent diabetes mellitus. *N Engl J Med* 330:15–18, 1994
8. Laing SP, Swerdlow AJ, Slater SD, Botha JL, Burden AC, Waugh NR, Smith AW, Hill RD, Bingley PJ, Patterson CC, Qiao Z, Keen H: The British Diabetic Association Cohort Study, II: cause-specific mortality in patients with insulin-treated diabetes mellitus. *Diabet Med* 16:466–471, 1999
9. Deckert T, Feldt-Rasmussen B, Borch-Johnsen K, Jensen T, Kofoed-Enevoldsen A: Albuminuria reflects widespread vascular damage: the Steno hypothesis. *Diabetologia* 32:219–226, 1989
10. Libby P, Ridker PM, Maseri A: Inflammation and atherosclerosis. *Circulation* 105:1135–1143, 2002
11. Rathcke CN, Vestergaard H: YKL-40, a new inflammatory marker with relation to insulin resistance and with a role in endothelial dysfunction and atherosclerosis. *Inflamm Res* 55:221–227, 2006
12. Millis AJ, Hoyle M, Kent L: In vitro expression of a 38,000 dalton heparin-binding glycoprotein by morphologically differentiated smooth muscle cells. *J Cell Physiol* 127:366–372, 1986
13. Malinda KM, Ponce L, Kleinman HK, Shackelton LM, Millis AJ: Gp38k, a protein synthesized by vascular smooth muscle cells, stimulates directional migration of human umbilical vein endothelial cells. *Exp Cell Res* 250:168–173, 1999
14. Shackelton LM, Mann DM, Millis AJ: Identification of a 38-kDa heparin-binding glycoprotein (gp38k) in differentiating vascular smooth muscle cells as a member of a group of proteins associated with tissue remodeling. *J Biol Chem* 270:13076–13083, 1995
15. Rathcke CN, Johansen JS, Vestergaard H: YKL-40, a biomarker of inflammation, is elevated in patients with type 2 diabetes and is related to insulin resistance. *Inflamm Res* 55:53–59, 2006
16. Rathcke CN, Raymond I, Kistorp C, Faber J, Vestergaard H: The inflammation marker YKL-40 is elevated in patients with type 2 diabetes and is associated with an increased mortality rate in normal individuals after adjustment for risk factors of cardiovascular events (Abstract). *Diabetologia* 50:s103, 2007

17. Jager A, van HV, Kostense PJ, Emeis JJ, Nijpels G, Dekker JM, Heine RJ, Bouter LM, Stehouwer CD: C-reactive protein and soluble vascular cell adhesion molecule-1 are associated with elevated urinary albumin excretion but do not explain its link with cardiovascular risk. *Arterioscler Thromb Vasc Biol* 22:593–598, 2002
18. Schalkwijk CG, Stehouwer CD: Vascular complications in diabetes mellitus: the role of endothelial dysfunction. *Clin Sci (Lond)* 109:143–159, 2005
19. Schram MT, Chaturvedi N, Schalkwijk CG, Fuller JH, Stehouwer CD: Markers of inflammation are cross-sectionally associated with microvascular complications and cardiovascular disease in type 1 diabetes: the EURODIAB Prospective Complications Study. *Diabetologia* 48:370–378, 2005
20. Astrup AS, Tarnow L, Pietraszek L, Schalkwijk CG, Stehouwer CD, Parving H-H, Rossing P: Markers of endothelial dysfunction and inflammation in type 1 diabetic patients with or without diabetic nephropathy followed for 10 years: association with mortality and decline of glomerular filtration rate. *Diabetes Care* 31:1170–1176, 2008
21. Devaraj S, Cheung AT, Jialal I, Griffen SC, Nguyen D, Glaser N, Aoki T: Evidence of increased inflammation and microcirculatory abnormalities in patients with type 1 diabetes and their role in microvascular complications. *Diabetes* 56:2790–2796, 2007
22. Kucur M, Isman FK, Karadag B, Vural VA, Tavsanoğlu S: Serum YKL-40 levels in patients with coronary artery disease. *Coron Artery Dis* 18:391–396, 2007
23. Nojgaard C, Host NB, Christensen IJ, Poulsen SH, Egstrup K, Price PA, Johansen JS: Serum levels of YKL-40 increases in patients with acute myocardial infarction. *Coron Artery Dis* 19:257–263, 2008
24. Johansen JS: Studies on serum YKL-40 as a biomarker in diseases with inflammation, tissue remodelling, fibrosis and cancer. *Dan Med Bull* 53:172–209, 2006
25. Roslind A, Knoop AS, Jensen MB, Johansen JS, Nielsen DL, Price PA, Balslev E: YKL-40 protein expression is not a prognostic marker in patients with primary breast cancer. *Breast Cancer Res Treat* 112:275–285, 2008