Type 1 Diabetes Is Associated With Increased Cyclooxygenase- and Cytokine-Mediated Inflammation

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OBJECTIVE — The extent of involvement of cyclooxygenase (COX)-mediated inflammation in type 1 diabetes is unknown, and the association between the COX- and cytokine-mediated inflammatory responses in type 1 diabetes is not fully understood.

RESEARCH DESIGN AND METHODS — Plasma high-sensitivity C-reactive protein (CRP), 24-h urinary and plasma 15-keto-dihydro-prostaglandin $F_{2\alpha}$ (a metabolite of prostaglandin $F_{2\alpha}$ [$PGF_{2\alpha}$] and an indicator of COX-mediated inflammation), serum amyloid protein A (SAA), and interleukin (IL)-6 (indicators of inflammation) were measured in 38 subjects with type 1 diabetes and 41 healthy age- and sex-matched control subjects.

RESULTS — The inflammatory indicators (urinary 15-keto-dihydro-$PGF_{2\alpha}$, $P < 0.01$; IL-6, $P < 0.04$) were increased in men with diabetes. CRP and SAA did not show any significant difference between the diabetic and the control subjects. Urinary levels of 15-keto-dihydro-$PGF_{2\alpha}$ correlated with the degree of glycemic control, HbA_{1c} ($r = 0.42$, $P < 0.0005$). No correlation was found between the duration of diabetes and the inflammatory biomarkers or metabolic measurements.

CONCLUSIONS — These results suggest that an early low-grade inflammatory process reflected by elevated levels of $PGF_{2\alpha}$ and IL-6 is involved in type 1 diabetes. Thus, both COX- and cytokine-mediated inflammatory pathways are significantly related to type 1 diabetes.

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Compared with a control population, type 1 diabetes is associated with an increased risk of microvascular complications and premature atherosclerosis (1). Atherosclerosis is considered to be in part a consequence of a chronic low-grade inflammation (2,3), and it has been suggested that atherosclerosis and diabetes might share the mutual inflammatory phenomenon hypothesis (4,5). In recent years inflammation has been suggested to be involved in type 1 diabetes (6). Blood levels of interleukin (IL)-6 have been shown to be normal (7) or higher (8,9) in type 1 diabetic patients compared with levels in control subjects. Cytokine-mediated acute-phase proteins have also been studied (9,10), but their role in diabetes without atherosclerosis is inconclusive.

An ongoing inflammatory process can lead to gradual cell destruction followed by arachidonic acid release and its bioconversion by cyclooxygenase (COX) to various bioactive prostaglandins. Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) is a potent vasoconstrictive compound (11). Moreover, prostaglandins are important mediators of the inflammatory process (12,13), and 15-keto-dihydro-$PGF_{2\alpha}$, a major metabolite of $PGF_{2\alpha}$, has been shown to be a reliable indicator of in vivo COX-mediated inflammation (14,15). In a recent study, 15-keto-dihydro-$PGF_{2\alpha}$, high-sensitivity C-reactive protein (CRP), and serum amyloid protein A (SAA), which are all inflammatory indicators, were increased in men with type 2 diabetes in a population-based cross-sectional study (16). Cytokine-mediated CRP and SAA were more closely related to obesity and insulin than to the diagnosis of diabetes, suggesting that COX-mediated low-grade inflammatory activity is a central phenomenon in elderly type 2 diabetic patients. The association between type 1 diabetes and COX-mediated $PGF_{2\alpha}$ formation is unknown.

Thus, the goal of this study was to investigate the association between type 1 diabetes and COX-mediated $PGF_{2\alpha}$, inflammatory cytokine IL-6– and cytokine-mediated CRP, and SAA in type 1 diabetic subjects and compare it with age-matched healthy control subjects.

RESEARCH DESIGN AND METHODS

— Subjects with type 1 diabetes, all attending the clinic of diabetes and endocrinology at the Uppsala University Hospital, were asked to participate in the study. A total of 38 diabetic subjects accepted, of whom 36 were using meal-related rapid-acting insulin combined with bedtime NPH insulin, and two were treated with insulin pumps. Their mean duration of diabetes was 16.1 ± 10.3 years. A total of 41 apparently healthy nondiabetic control subjects were recruited from the staff of the Uppsala University Hospital and among medical students. The participants were between 20 and 45 years of age. The two groups

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Abbreviations: COX, cyclooxygenase; CRP, C-reactive protein; IL, interleukin; $PGF_{2\alpha}$, prostaglandin $F_{2\alpha}$; SAA, serum amyloid A.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Table 1—*Baseline clinical characteristics in type 1 diabetic and control subjects*

Variable	Type 1 diabetic subjects	Control subjects	P
n	38	41	—
Age (years)	32 ± 5	30 ± 6	NS
Sex (M/F)	14/24	22/19	NS
BMI (kg/m^2)	23.5 ± 2.7	23.1 ± 3.0	NS
Duration of diabetes (years)	16 ± 10	—	—
HbA_{1c} (%)	7.4 ± 1.7	4.0 ± 0.2	0.0001
Macrovascular complications	0/38	0/41	—
Microvascular complications	9/38	0/41	—
Hypertension	6/38	0/41	—
Albumin excretion rate (mg/h)	2.23 ± 5.83	0.32 ± 0.49	<0.05

Data are means ± SD.

were well matched for sex, age, and BMI. Clinical characteristics are shown in Table 1. None of the included subjects was pregnant or had a history of malignancy, liver disease, untreated hypothyroidism, alcohol abuse, intercurrent diseases, or use of lipid-lowering drugs. According to a self-administrated questionnaire, eight of the diabetic subjects and four of the control subjects reported daily smoking of cigarettes. Three diabetic and five control subjects reported that they took multivitamin supplements regularly.

Macrovascular disease was defined as clinical history of angina or myocardial infarction. Microalbuminuria was defined as a urinary albumin excretion rate of 30–300 mg/24 h and macroalbuminuria as >300 mg/24 h, respectively, in more than one urine collection. Proliferative retinopathy was considered present if observed by fundus photography, which all diabetic subjects had undergone, or if there was a history of laser treatment. Peripheral neuropathy was defined as the loss of ankle reflexes and/or the loss of lower-limb sensation to light touch (monofilament) or vibration on clinical testing. Four subjects had signs of multiple microangiopathic complications, here defined as a clinical history of retinopathy, neuropathy, and/or micro- or macroalbuminuria. One type 1 diabetic subject had a history of microalbuminuria and one patient had a history of macroalbuminuria. Three of the subjects had a history of proliferative retinopathy. Six diabetic subjects were using either ACE inhibitors or an angiotensin receptor antagonist. Informed consent was obtained from all participating subjects, and the study was approved by the ethical committee at the Medical Faculty of Uppsala University.

Sample collection and handling

Blood samples from all 79 participants were collected, after a 12-h overnight fast, into Vacutainer tubes containing Na-heparin, EDTA, or buffered sodium-citrate. HbA_{1c} was measured in fresh blood samples taken on the morning of the study.

The blood was stored on ice and thereafter centrifuged at 3,000 rpm for 10 min at 4 C°, and the plasma was separated. For determination of cholesterol and triglycerides, blood was collected into Vacutainer tubes without any addition and left to coagulate for 2 h. After low-speed centrifugation for 10 min, the serum was separated. One aliquot was stored in 4 C° before lipid analysis. A 24-h urine collection was delivered by 76 of the 79 participants at the time of the blood sampling. Urine samples were stored at −70°C until analysis for 15-keto-dihydro-$PGF_{2\alpha}$ and albumin were performed.

Biochemical assays (inflammatory biomarkers)

Radioimmunoassay of 15-keto-dihydro-$PGF_{2\alpha}$. The 24-h urinary and plasma samples were analyzed for 15-keto-dihydro-$PGF_{2\alpha}$ by a radioimmunoassay at our laboratory, as previously described by Basu (14). In brief, the cross-reactivity of the antibody with $PGF_{2\alpha}$; 15-keto-$PGF_{2\alpha}$; PGE_2; 15-keto-13,14-dihydro-PGE_2; 8-iso-15-keto-13,14-dihydro-$PGF_{2\alpha}$; 11β-$PGF_{2\alpha}$; 9β-$PGF_{2\alpha}$; thromboxane B_2; and 8-iso-$PGF_{3\alpha}$ was 0.02, 0.43, <0.001, 0.5, 1.7, <0.001, <0.001, <0.001, and 0.01%, respectively. The detection limit was 45 pmol/l. The levels of 15-keto-dihydro-$PGF_{2\alpha}$ in urine were adjusted for creatinine by division with the urinary creatinine concentration.

Assay of high-sensitivity CRP and SAA. High-sensitivity CRP and SAA measurements were performed by latex-enhanced reagent (Dade Behring, Deerfield, IL), using a BN ProSpec analyzer (Dade Behring). The intra-assay coefficient of variation (CV) of the CRP method was 1.4% at both 1.23 and 5.49 mg/l, and the intra-assay CV of the SAA method was 5.9% at 12.8 mg/l and 3.2% at 81.7 mg/l.

Measurement of IL-6. High-sensitivity IL-6 was analyzed by an enzyme-linked immunosorbent assay kit (IL-6 HS; R&D Systems, Minneapolis, MN). Samples and standards were pipetted in a microtiter plate coated with monoclonal antibody against IL-6. After incubation and washing, the enzyme substrate solution was pipetted and followed by the addition of anti–IL-6 antibody. The color reaction was proportional to the bound IL-6. The total CV of the method was 7%, and the interassay CV was 5%.

Other biochemical assays. Serum triglycerides and cholesterol were measured

Table 2—*Serum lipid concentrations in type 1 diabetic and control subjects*

Variable	Type 1 diabetic subjects	Control subjects	*P*
Cholesterol (mmol/l)	4.70 ± 0.94	4.79 ± 0.77	NS
Triglycerides (mmol/l)	0.82 ± 0.61	1.07 ± 0.54	$P < 0.05$
HDL cholesterol (mmol/l)	1.66 ± 0.47	1.58 ± 0.35	NS
LDL cholesterol (mmol/l)	2.67 ± 0.70	2.73 ± 0.71	NS
HDL triglycerides (mmol/l)	0.10 ± 0.10	0.10 ± 0.05	NS
LDL/HDL	1.71 ± 0.59	1.83 ± 0.70	NS

Data are means ± SD.

Table 3—*Inflammatory biomarkers in type 1 diabetes and control subjects*

Measurements	Type 1 diabetic subjects	Control subjects	*P*
n	38	39	
U-15-keto-dihydro-$PGF_{2\alpha}$ (pmol/mmol creatinine)	310 ± 100	260 ± 100	$P < 0.01$
P-15-keto-dihydro-$PGF_{2\alpha}$ (pg/ml)	20.1 ± 12.3	18.6 ± 9.9	NS
IL-6 (mg/l)	1.41 ± 0.64	1.19 ± 0.79	0.037
CRP (mg/l)	1.0 ± 1.16	1.15 ± 1.34	NS
SAA (mg/l)	3.02 ± 2.75	3.58 ± 2.71	NS

Data are means ± SD. P, plasma; U, urinary.

by enzymatic methods using IL Test Cholesterol Triander's Method 181618-80 and IL Test Enzymatic-Colorimetric Method 181709-00 for use in the Monarch apparatus (Instrumentation Laboratory, Lexington, MA). Apolipoprotein B–containing lipoproteins were precipitated with a sodium phosphotungstate, and magnesium chloride solution (17) LDLs were calculated according to Friedewald et al. (18). Albumin in urine was analyzed by rate nephelometry on an Array protein system (Beckman Instruments, Brea, CA). The assay was performed according to the recommendations by the manufacturer. HbA_{1c} level was measured by automated high-performance liquid chromatography (reference range 3.5–5.0%) (19).

Statistical analysis

Unpaired Student's *t* test was used to compare the two groups. Logarithmic transformations were applied on non–normally distributed parameters. The nonparametric Wilcoxon test was used on parameters, which were still not normally distributed after transformation. Linear relationships between variables were evaluated by Pearson's correlation test. Spearman correlation was used with parameters not normally distributed.

RESULTS

Basic metabolic variables

The urinary albumin excretion rate (means ± SD) was significantly higher among the diabetic subjects than the control subjects (2.23 ± 5.83 vs. 0.32 ± 0.49 mg/h, $P < 0.05$) (Table 1). In a subgroup analysis excluding patients with blood pressure–lowering drugs ($n = 6$), type 1 diabetic patients had significantly higher urinary albumin excretion rates than control subjects (10.8 ± 11.5 vs. 0.56 ± 1.2 mg/h, $P < 0.01$). No differences were noted in serum lipid and lipoprotein levels between diabetic and control subjects, except for serum triglycerides, which were higher in the control group than in the type 1 diabetic group (0.82 ± 0.61 vs. 1.07 ± 0.54 mmol/l, $P < 0.05$) (Table 2). Exclusion of subjects who were smokers and/or subjects who were taking antioxidant supplements from both groups did not change any of the relationships between diabetic and control subjects, except for serum triglycerides, which showed no significant difference after the exclusion (0.82 ± 0.68 vs. 0.99 ± 0.53 mmol/l, NS).

Levels of 15-keto-dihydro-$PGF_{2\alpha}$ (indicator of COX-mediated inflammation)

Type 1 diabetic patients had significantly higher levels ($P < 0.01$) of urinary 15-keto-dihydro-$PGF_{2\alpha}$ than control subjects (Table 3). In a subgroup analysis excluding patients with blood pressure–lowering drugs ($n = 6$), type 1 diabetic patients had significantly higher levels of urinary 15-keto-dihydro-$PGF_{2\alpha}$ than control subjects ($P = 0.02$). Type 1 diabetic patients with blood pressure–lowering drugs had no difference in urinary 15-keto-dihydro-$PGF_{2\alpha}$ compared with the normotensive diabetic patients (310 ± 90 vs. 310 ± 110 pmol/mmol creatinine). There was no difference in the levels of 15-keto-dihydro-$PGF_{2\alpha}$ between the subjects who have or do not have microvascular complications (microalbuminuria) (data not shown).

Levels of IL-6, CRP, and SAA (indicators of cytokine-mediated inflammation)

Type 1 diabetic patients had significantly ($P < 0.04$) higher plasma levels of IL-6 than control subjects (Table 3). Neither CRP nor SAA differed significantly between the diabetic patients and the control subjects (Table 3). In a subgroup analysis excluding patients with blood pressure–lowering drugs ($n = 6$), type 1 diabetic patients had significantly higher levels of IL-6 than control subjects ($P = 0.04$). Type 1 diabetic patients with blood pressure–lowering drugs had no difference in the levels of IL-6, CRP, and SAA compared with the normotensive diabetic patients, and there was no difference in the levels of IL-6, CRP, and SAA between the subjects who have or do not have microvascular complications (microalbuminuria) (data not shown).

Correlation between inflammatory indicators and metabolic measurements

Urinary levels of 15-keto-dihydro-$PGF_{2\alpha}$ correlated with the degree of glycemic control, as reflected by HbA_{1c} ($r = 0.42$, $P < 0.0005$) (Fig. 1). Plasma levels of 15-keto-dihydro-$PGF_{2\alpha}$ were positively correlated with IL-6 ($r = 0.24$, $P < 0.04$). No correlation was found between the duration of diabetes and any measured inflammatory or metabolic parameters.

CONCLUSIONS — In this case-control study, we compared type 1 diabetic patients with nondiabetic control subjects. The group was relatively heterogeneous with regard to duration of diabetes and diabetes-related complications, representing a random sample of an average Swedish diabetic population. With few exceptions type 1 diabetic subjects attend hospital clinics, whereby the sample can be considered to be population based in the hospital catchment area (population 220,000).

This study shows that COX-mediated urinary 15-keto-dihydro-$PGF_{2\alpha}$ and plasma IL-6 levels were significantly higher in patients with type 1 diabetes compared with healthy control subjects. Plasma levels of 15-keto-dihydro-$PGF_{2\alpha}$ and IL-6 were positively correlated ($r = 0.24$, <0.04). Although this correlation is not very strong, it is of potential biological importance because the pathways of formation of $PGF_{2\alpha}$ and IL-6 are novel and different. Furthermore, there was a fair degree of correlation ($r = 0.42$, $P < 0.0005$) between urinary levels of 15-keto-dihydro-$PGF_{2\alpha}$ and HbA_{1c}. Previously, we did not find any significant

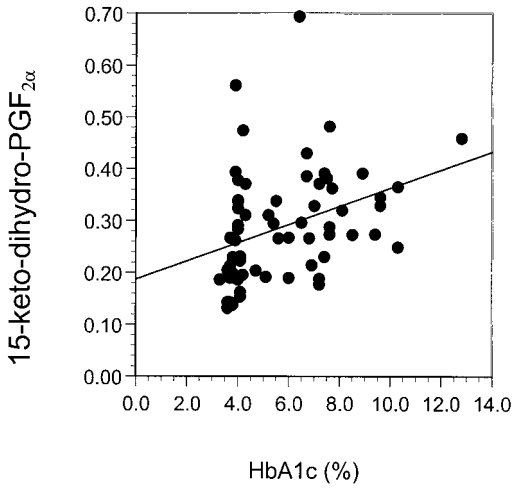

Figure 1—*Correlation between the urinary $PGF_{2\alpha}$ metabolite 15-keto-dihydro-$PGF_{2\alpha}$ (nmol/mmol creatinine) and HbA_{1c} (r = 0.42, P < 0.0005).*

association between the inflammatory biomarkers and HbA_{1c} levels in elderly men with type 2 diabetes (16). These subjects often suffer from long-lasting atherosclerotic macrovascular damage, in contrast to relatively young, well-controlled type 1 diabetic patients in this study without signs of any macrovascular complications (Table 1). Type 1 diabetic patients in this study do not show any signs of macrovascular disease, which is often related to inflammation, and it is reasonable to assume that it is easier to show a significant relationship between hyperglycemia and the levels of inflammatory biomarker $PGF_{2\alpha}$ metabolite in this group.

We did not find any difference in the inflammatory biomarkers in patients with microvascular complications compared with those who do not have microvascular complications. Furthermore, we did not find any difference in the inflammatory biomarkers in type 1 diabetic patients who were treated with blood pressure–lowering drugs compared with the normotensive patients. It is known that angiotensin II may stimulate IL-6 and other inflammatory biomarkers (20,21). Thus, it is possible that administration of angiotensin II blockers, as given to six patients, would reduce the levels of inflammatory biomarkers. This might explain why there is no significant difference in inflammatory biomarkers among blood pressure–treated and nontreated patients.

This study is the first to report increased levels of COX-mediated $PGF_{2\alpha}$ as measured by urinary 15-keto-dihydro-$PGF_{2\alpha}$ in type 1 diabetic patients compared with control subjects. Prostaglandins are well-known mediators of inflammation, and 15-keto-dihydro-$PGF_{2\alpha}$, a major metabolite of bioactive $PGF_{2\alpha}$, is a potent indicator of in vivo inflammatory processes (14,15,22,23). Thus, the results from this study suggest ongoing COX-mediated low-grade inflammatory processes among patients with type 1 diabetes. $PGF_{2\alpha}$ is shown to be a potent vasoconstrictive compound (11), and this might have further contributed to the pathogenesis of diabetes complications via decreased blood flow (24) and endothelium dysfunction (25). Recently, it has been shown that 11-dehydro-thromboxane B_2, a metabolite of thromboxane A_2 and a COX-mediated product primarily formed in the thrombocytes, is associated with type 1 diabetes (26). This might reflect that $PGF_{2\alpha}$, being a major product of arachidonic acid through COX, could possibly induce platelet adhesion and aggregation, as has been demonstrated for an isomer of $PGF_{2\alpha}$, 8-iso-$PGF_{2\alpha}$ (27,28). Furthermore, we have also shown that a significant correlation exists between the glycemic status as reflected by HbA_{1c} and the urinary levels of 15-keto-dihydro-$PGF_{2\alpha}$.

This study also demonstrates enhanced levels of IL-6 in type 1 diabetic patients, which further indicates that type 1 diabetes is associated with an increased cytokine-mediated inflammatory response, which is corroborated by other studies (6,8). Furthermore, the levels of IL-6 correlated with COX-mediated $PGF_{2\alpha}$ metabolite. Cytokines have earlier been shown to stimulate prostaglandin formation in fibroblasts through induction of COXs (29). Both the cytokines and COXs are activated in the cells through the nuclear factor-κB pathway under inflammatory conditions elicited by various stress factors, such as reactive oxygen species and viral and bacterial infection (30). Together, these results indicate that the inflammatory mechanism might be responsible for enhanced atherogenesis and is possibly associated with common vascular complications among type 1 diabetic patients. Although these patients have an acceptable average glycemic status, it did not account for a lower inflammatory response, as reflected by increased levels of cytokines and cytokine- and COX-mediated products.

Acetylsalicylic acid is known to reduce cardiovascular risk through affecting inflammatory processes (2). Intake of low-dose aspirin or other anti-inflammatory drugs is considered to be effective as secondary prophylaxis of cardiovascular diseases and primary prophylaxsis of myocardial infarction (31). Aspirin inhibits COXs and thus the biosynthesis of parent prostaglandins and thromboxanes that are related to inflammation (12,32,33). Recently, it has also been shown that high doses of salicylates specifically inhibit the function of I-κ-kinase-β, a key protein involved in the regulation of inflammation that interferes with insulin signaling and contributes to both insulin resistance and diabetes (34). The current study shows that basal levels of $PGF_{2\alpha}$ and IL-6 are increased among type 1 diabetic patients, involving both COX and cytokine pathways. Thus, it is tempting to speculate that acetylsalicylic acid might have some effect in reducing the inflammatory condition in this patient group, but this has yet to be determined.

In conclusion, this study demonstrates an involvement of low-grade cytokine- and COX-mediated inflammation in type 1 diabetes. Furthermore, we found a significant correlation between the levels of $PGF_{2\alpha}$ metabolite and the degree of metabolic control, which suggests a potential link to fatty acid oxidation and insulin resistance. Thus, hyperglycemia is associated with increased production of these important inflammatory compounds. The association of these inflammatory biomarkers with type 1 diabetes indicates that an important association exists between type 1 diabetes and the future possibility of development of atherosclerosis.

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