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RESEARCH LETTER

Regulation of oxidative stress and inflammation by glycaemic control: evidence for reversible activation of the 5-lipoxygenase pathway in type 1 diabetes, but not in type 2 diabetes

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Abbreviations

CRP	C-reactive protein
CysLT	Cysteinyl leukotriene
ICAM-1	Intercellular adhesion molecule 1
PGF_2	Prostaglandin F_2
TXB_2	Thromboxane B_2

To the Editor: Additional studies are needed to clarify the impact of improved glycaemic control on cardiovascular diseases in both types of diabetes. Monnier et al. recently investigated the excretion rate of 8-iso-prostaglandin F_2 $(PGF_2)_\alpha$ in patients with type 2 diabetes. They concluded that this biomarker of oxidative stress is increased in patients with type 2 diabetes treated with oral hypoglycaemic agents vs patients with type 1 diabetes and that add-on insulin resulted in a decrease of 8-iso-$PGF_{2\alpha}$ excretion [1]. To address the impact of intensive therapy on the excretion of other arachidonic acid derivatives, we investigated the effect of a 3-month improvement of glycaemic control by intensive insulin treatment on the urinary excretion of leukotriene E_4 and 11-dehydro-thromboxane B_2 (11-dehydro-TXB_2) in consecutive patients with baseline HbA_{1c} >8.5%, 20 of whom had type 1 diabetes and 19 type 2 diabetes. Leukotrienes are arachidonic acid metabolites that are derived from the 5-lipoxygenase pathway and possess vasoactive, chemotactic and pro-inflammatory properties. Sufficient evidence suggests that 5-lipoxygenase pathway activation plays a role in the pathogenesis of cardiovascular diseases [2] and that leukotriene E_4 urinary excretion is a validated marker of in vivo cysteinyl leukotrienes (CysLTs) activation [3, 4]. Leukotriene E_4 urinary excretion is increased in poorly controlled patients with type 1 diabetes as compared with age-matched controls [4].

The study protocol was approved by the local Ethical Committee and all participants provided informed written consent. Patients with asthma or any pulmonary disease known to enhance leukotriene E_4 urinary excretion, heart failure, history of malignancy, recent infectious disease or plasma creatinine >180 μmol/l were excluded, as were patients treated with corticosteroids, pregnant women and smokers. Patient characteristics are detailed in Table 1; a more intensive treatment (participation in a structured education programme, insulin initiation or dosage revision) was justified in all patients. Interestingly, insulin therapy had been previously initiated in 47% of patients with type 2 diabetes, with duration of insulin treatment longer than 2 years in three of nine patients; it was applied to all

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Table 1 Baseline characteristics of the study population

Characteristic	Type 1 diabetes	Type 2 diabetes
n	20	19
Age (years)	37±12	58±12
Men (%)	45.0	68.4
Waist (cm)	82±11	109±14
Diabetes duration (years)	14.5 (5–22)	11 (10–15.5)
Retinopathy (%)	40	26.3
UAE >2.5 mg/mmol creatinine (%)	10	26.3
Hypertension (%)	20	73.7
Cardiovascular disease (%)	5	23.5

Unless otherwise indicated, data are presented as means ± SD or medians (interquartile ranges) according to the distribution of respective values, or percentages

UAE, urinary albumin excretion

patients entering this trial. Estimated glomerular filtration rate was >80 ml/min in all patients with type 1 (mean ± SD: 108±20) and >70 ml/min in all patients with type 2 diabetes (mean ± SD: 119±44). Patient follow-up included an early visit to check the efficacy and safety of the revised treatment and the end-of-study visit (25 to 35 and 80 to 100 days after inclusion, respectively). At baseline and end of study, each patient performed an overnight urine collection and blood was drawn after a 12 h overnight fast. Quantification of leukotriene E_4 and 11-dehydro-TXB_2 was performed by liquid chromatography tandem mass spectrometry in urine samples stored at −80°C until analysis using methods previously described [4]. The limits of detection were 10 and 100 pg/ml of urine for leukotriene E_4 and 11-dehydro-TXB_2 respectively; inter-and intra-assay variation were <6%.

Baseline urinary leukotriene E_4 and 11-dehydro-TXB_2 (pg/mg of creatinine), and high-sensitivity C-reactive protein (CRP) concentrations were not significantly different in type 1 and type 2 diabetes groups (Table 2). Baseline serum soluble intercellular adhesion molecule-1 (ICAM-1) was higher in patients with type 2 than in those with type 1 diabetes (p=0.017). Our data confirmed high leukotriene E_4 urinary excretion in patients with type 1 diabetes and showed similar values in poorly controlled patients with type 2 diabetes. Baseline urinary leukotriene E_4 and 11-dehydro-TXB_2 were similar in the type 2 diabetes subgroups receiving and not receiving insulin before the intensive treatment period (p=0.5). In a subset of 11 patients with type 1 diabetes, an additional sampling was possible 5 days after baseline in those in an ongoing education programme; leukotriene E_4 urinary excretion was not different at this stage vs baseline (data not shown).

After a 3 month period of intensive glycaemic control (details on insulin therapy, see Table 2), HbA_{1c} and 11-dehydro-TXB_2 urinary excretion significantly decreased in

Table 2 Effect of intensive glycaemic control on BMI, and inflammatory and metabolic variables according to diabetes type

Variable	Type 1 diabetes		Type 2 diabetes	
	Baseline	After intensive treatment 1	Baseline	After intensive treatment 2
HbA_{1c} (%)	9.7±1.4	7.8±1.1[a]	10.1±1.3	7.3±1.4[a]
BMI (kg/m^2)	23.9±2.9	23.7±2.5	32.3±5.0	31.3±4.3
LTE_4 (pg/mg creatinine)[c]	72±48	49±31[a]	86±57	81±58
11-dehydro-TXB_2 (pg/mg creatinine)[c]	898±495	596±279[b]	1,037±820	564±531[a]
High sensitivity CRP (mg/l)	1.1 (0.7–2.0)	1.5 (0.5–2.2)	3.1 (2.5–4.8)	2.8 (1.2–5.4)
Fibrinogen (g/l)	2.9±0.4	3.1±0.6	3.5±0.9	3.6±0.8
Soluble ICAM-1 (μg/l)	220±38	214±79	298±131	295±121
Total cholesterol (mmol/l)	4.5±1.0	5.0±0.8	5.0±1.0	4.4±1.1
HDL-cholesterol (mmol/l)	1.8±0.4	2.0±0.4	1.2±0.3	1.2±0.4
Triacylglycerol (mmol/l)	0.9 (0.7–1.1)	0.7 (0.6–1.0)	2.2 (1.5–3.5)	1.7 (1.1–2.7)[a]

Unless otherwise indicated, data are presented as means ± SD or medians (interquartile ranges) according to the distribution of respective values

Intensive treatment 1: implementation of flexible insulin therapy: 0.67±0.29 IU kg^{-1} day^{-1}, 53±8% of the dose as bolus insulin (assumption: 210 g carbohydrate per day, normal pre-meal blood glucose and no extra bolus)

Intensive treatment 2: for patients not previously treated with insulin: 0.19±0.08 IU kg^{-1} day^{-1} as basal (n=10) and one multiple daily injection (MDI) regimen (0.78 IU kg^{-1} day^{-1}); for patients previously treated with insulin: 0.88±0.43 IU kg^{-1} day^{-1} (MDI, corrective pre-meal dose not included, n=9) vs 0.66±0.24 IU kg^{-1} day^{-1} at baseline

[a] p<0.001 vs baseline; [b] p<0.05 vs baseline

[c] To convert pg/mg creatinine to pg/mmol creatinine, multiply values by 113.12

LTE_4, leukotriene E_4

both groups, whereas body weight did not change. Leukotriene E_4 urinary excretion decreased by 32% in type 1 diabetes patients (p=0.008 vs baseline), but did not decrease in patients with type 2 diabetes (Table 2). In the type 1 diabetes group, baseline leukotriene E_4 and the change in HbA_{1c} were correlated to baseline HbA_{1c} (r=0.55, p=0.015 and r=0.77, p<0.001 respectively). The change in leukotriene E_4 urinary excretion was correlated to baseline leukotriene E_4 urinary excretion, baseline HbA_{1c} and to the change in HbA_{1c} (r=0.78, p<0.001, r=0.62, p=0.006 and r=0.46, p=0.050 respectively).

We had provided preliminary evidence of the link between hyperglycaemia and the CysLT pathway activation in an animal model of type 1 diabetes (insulin treatment in streptozotocin rats significantly reduced leukotriene E_4 urinary excretion) and also reported that leukotriene E_4 urinary excretion was significantly enhanced in patients with poorly controlled type 1 diabetes [3, 4]. The present investigation suggests that hyperglycaemia induces a reversible activation of the 5-lipoxygenase and thromboxane pathways in type 1 diabetes. Intensive diabetes management had no effect on other inflammatory biomarkers (soluble ICAM-1, high-sensitivity CRP) in this study population and did not reduce leukotriene E_4 urinary excretion in the type 2 diabetes group. Nevertheless, the decrease urinary excretion of 11-dehydro-TXB_2 in the type 2 diabetes group confirms the change previously reported after 4 weeks of intensive insulin therapy in patients with poorly controlled type 2 diabetes [5]. The reversible activation of the 5-lipoxygenase pathway related to chronic hyperglycaemia in type 1 is consistent with cardiovascular diseases appearing to be strongly glucose-mediated in this type of diabetes. Indeed, interventions targeting blood glucose could prevent cardiovascular diseases to a greater extent in type 1 than in type 2 diabetes; moreover, cardiovascular diseases might be more strongly glucose-mediated in type 1 diabetes [6, 7].

A similar 3 month improvement of hyperglycaemia did not change leukotriene E_4 urinary excretion in type 2 diabetes, which suggests that activation of the CysLT pathway was unchanged in this type of diabetes, a finding associated with multifactorial underlying inflammation (i.e. related to adipokines). Overall, the pathophysiology of oxidative stress and inflammation is complex and does not follow the same patterns according to the type of diabetes [8]. Multifactorial proinflammatory cell activation cannot be markedly modified by improved glycaemic control in type 2 diabetes. In contrast, in type 1 diabetes, hyperglycaemia-dependent reactive oxygen species (ROS) generation might be a crucial determinant of inflammation; moreover, flexible insulin therapy might reduce glucose variability.

We conclude that hyperglycaemia induced activation of the 5-lipoxygenase pathway in both types of diabetes, with a partial correction being achieved by intensive insulin therapy in patients with type 1, but not in those with type 2 diabetes.

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