Renal Hyperfiltration is a Determinant of Endothelial Function Responses to Cyclooxygenase 2 Inhibition in Type 1 Diabetes

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Submitted 22 December 2010 and accepted 10 March 2010.

Additional information for this article can be found in an online appendix at http://care.diabetesjournals.org

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Introduction: Our aim was to examine the effect of cyclooxygenase 2 (COX2) inhibition on endothelial function in subjects with type 1 DM analyzed on the basis of renal filtration status.

Methods: Flow mediated vasodilatation (FMD) was determined in type 1 DM subjects and hyperfiltration (glomerular filtration rate [GFR] ≥135 ml/min/1.73m², n=13) or normofiltration (GFR ≥135 ml/min/1.73m², n=11). Studies were performed before and after celecoxib (200 mg daily for 14 days) during euglycemia and hyperglycemia.

Results: Baseline parameters were similar in the two groups. Pre-treatment, FMD was augmented in normofiltering vs. hyperfiltering subjects during clamped euglycemia (10.2±5.3% vs. 5.9±2.3%, p=0.003). COX2 inhibition suppressed FMD in normofiltering (10.2±5.3% to 5.8±3.4%, p=0.006) vs. hyperfiltering subjects (ANOVA interaction, p=0.003).

Conclusions: Systemic hemodynamic function, including the response to COX2 inhibition, is related to filtration status in diabetic subjects, and may reflect general endothelial dysfunction.
Renal hyperfiltration is associated with an increased risk of progression to diabetic nephropathy in many, but not all, studies (1). Diabetic hyperfiltration may in part be due to cyclooxygenase 2 (COX2) upregulation (2; 3). We have previously identified a cohort of subjects with uncomplicated type 1 diabetes mellitus (DM) who exhibit hyperfiltration (glomerular filtration rate [GFR] ≥135 ml/min/1.73m$^2$) or normofiltration (GFR <135 ml/min/1.73m$^2$) during clamped euglycemia (4). In hyperfiltering subjects, COX2 inhibition reduces GFR, whereas in those with normofiltration, COX2 inhibition is associated with an opposite GFR rise and an exaggerated suppression of vasodilatory prostaglandins (4). Together with previous observations (4-6), these findings suggest that hyperfiltering and normofiltering individuals are physiologically distinct.

Previous studies have suggested that early type 1 DM is characterized by a state of generalized vasodilatation due to nitric oxide upregulation (7). The role of COX2 in the systemic vasculature in humans with early type 1 DM is, however, incompletely understood (8-11). Accordingly, our goal was to study the effect of COX2 inhibition on endothelial function in diabetic subjects with hyperfiltration or normofiltration. Our hypothesis was that renal hemodynamic differences would also be reflected in the systemic circulation.

RESEARCH DESIGN AND METHODS:
Recruitment, study protocols and renal hemodynamic data from a subset of this cohort have been previously described (Online Appendix Table A) (4). Experiments were carried out on 2 consecutive days before (clamped euglycemia [4-6 mmol/L] and hyperglycemia [9-11 mmol/L]) and repeated after 14 days of COX2 inhibition (celecoxib 200 mg daily) (4). Circulating insulin levels were measured on each study day (4). Endothelial function was determined by recording diameter changes in the brachial artery in response to increased blood flow generated during reactive hyperemia (flow-mediated dilatation [FMD]) and glyceryl trinitrate-induced dilatation (GTN).

Longitudinal, ECG-gated, end-diastolic images were acquired and the arterial diameter was determined using high resolution B-mode vascular ultrasound (Vivid 7, GE/Vingmed, Milwaukee, WI). After baseline images were recorded, the blood pressure cuff was inflated around the forearm distal to the elbow to >200 mmHg for 5 minutes. After deflation, the change in vessel diameter in response to reactive hyperemia (endothelium-dependent dilatation), was measured for a further 5 minutes. GTN (400 micrograms) was then administered sublingually, and the changes were measured over a further 5 minutes (endothelium-independent dilatation). FMD and GTN % changes were defined as the maximal percentage changes in vessel diameter after reactive hyperemia and administration of GTN, respectively, as we have previously described (12). The variability for repeated measurements of arterial diameters at flow mediated vasodilatation was 0.01±0.005 mm (absolute diameter) or 0.26±0.01% (% absolute value of brachial artery at FMD), which is similar to previous reports (13; 14).

The data were analyzed based on renal filtration status. Between group baseline comparisons were made using parametric methods (unpaired t-test). Between group and within group differences in hemodynamic responses were determined by repeated measures ANOVA. All statistical analyses
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RESULTS: Baseline characteristics were similar in the two groups (Online Appendix Table B which is available at http://care.diabetesjournals.org). Renal hemodynamic function tests revealed expected differences in GFR. Within group blood pressure changes were not significant, nor were between group blood pressure differences (Table 1). Insulin concentrations were similar throughout the study. Before COX2 inhibition during clamped euglycemia, FMD was higher in normofiltering vs. hyperfiltering subjects (p=0.003, Table 1). In response to COX2 inhibition during clamped euglycemia, FMD declined in normofiltering subjects (p=0.006; ANOVA interaction term for between group effect, p=0.003). Differences were abolished by hyperglycemia. GTN responsiveness was similar in the two groups throughout the study.

DISCUSSION: While selective COX2 inhibition reduces hyperfiltration, the effect on FMD in humans with type 1 DM was until now unknown. Our first major finding was that during clamped euglycemia, hyperfiltering subjects exhibited evidence of impaired FMD compared with normofilterers. Our results suggest that in hyperfiltering type 1 DM subjects may be additionally at risk in that they demonstrated an impaired ability to induce arterial vasodilatation after an ischemic stimulus compared to those with normofiltration. Our second major observation was that in contrast with hyperfiltering subjects, COX2 inhibition suppressed FMD during clamped euglycemia in normofiltering subjects, without impairing GTN responsiveness. These findings in FMD and GTN responsiveness suggest that the observed differences may have been due to endothelial cell rather than vascular smooth muscle functional effects. While COX2 inhibition may reduce renal hyperfiltration in subjects with GFR≥135 ml/min/1.73m² (4), we have previously shown in normofiltering subjects an exaggerated suppression of vasodilatory prostaglandins and renal vasoconstriction (4), and in this study show impaired FMD.

Our study has limitations. We minimized the effect of the small sample size by using a homogeneous study cohort. Second, although our study offers physiologic insights into diabetic vascular dysfunction, the findings were significant during euglycemia and therefore have limited clinical applications in subjects who are frequently exposed to ambient hyperglycemia.

In conclusion, systemic hemodynamic function, including the response to COX2 inhibition, is related to filtration status in diabetic subjects, and may reflect general endothelial dysfunction.

ACKNOWLEDGEMENTS: The authors wish to thank the nurses in the Clinical Investigation Unit, Hospital for Sick Children, and in particular Ms. Maria Maione for her invaluable assistance with the protocol.

Sources of funding: This work was supported by an operating grant from the Juvenile Diabetes Research Foundation (to Drs. E.B. Sochett and J.A. Miller). Dr. D.Z.I Cherney was supported by a salary award from The Kidney Foundation of Canada and a KRESCENT-Ortho Biotech Fellowship, and operating funds from the Heart and Stroke Foundation of Canada, the Canadian Institutes of Health Research and the Canadian Diabetes Association. Dr. J.W. Scholey is the CIHR/AMGEN Canada Kidney Research Chair at the University Health Network, University of Toronto.

Duality of interest: None.
REFERENCES:
1. Magee GM, Bilous RW, Cardwell CR, Hunter SJ, Kee F, Fogarty DG: Is hyperfiltration associated with the future risk of developing diabetic nephropathy? A meta-analysis. *Diabetologia* 52:691-697, 2009
2. Komers R, Lindsley JN, Oyama TT, Schutzer WE, Reed JF, Mader SL, Anderson S: Immunohistochemical and functional correlations of renal cyclooxygenase-2 in experimental diabetes. *J Clin Invest* 107:889-898, 2001
3. Komers R, Anderson S, Epstein M: Renal and cardiovascular effects of selective cyclooxygenase-2 inhibitors. *Am J Kidney Dis* 38:1145-1157, 2001
4. Cherney DZ, Miller JA, Scholey JW, Bradley TJ, Slorach C, Curtis JR, Dekker MG, Nasrallah R, Hebert RL, Sochett EB: The effect of cyclooxygenase-2 inhibition on renal hemodynamic function in humans with type 1 diabetes. *Diabetes* 57:688-695, 2008
5. Cherney DZ, Konvalinka A, Zinman B, Diamandis EP, Soosaipillai A, Reich H, Lorraine J, Lai V, Scholey JW, Miller JA: Effect of protein kinase Cbeta inhibition on renal hemodynamic function and urinary biomarkers in humans with type 1 diabetes: a pilot study. *Diabetes Care* 32:91-93, 2009
6. Sochett EB, Cherney DZ, Curtis JR, Dekker MG, Scholey JW, Miller JA: Impact of renin angiotensin system modulation on the hyperfiltration state in type 1 diabetes. *J Am Soc Nephrol* 17:1703-1709, 2006
7. Vervoort G, Wetzels JF, Lutterman JA, van Doorn LG, Berden JH, Smits P: Elevated skeletal muscle blood flow in noncomplicated type 1 diabetes mellitus: role of nitric oxide and sympathetic tone. *Hypertension* 34:1080-1085, 1999
8. Meeking DR, Browne DL, Allard S, Munday J, Chowienczyck PJ, Shaw KM, Cummings MH: Effects of cyclo-oxygenase inhibition on vasodilatory response to acetylcholine in patients with type 1 diabetes and nondiabetic subjects. *Diabetes Care* 23:1840-1843, 2000
9. Williams SB, Cusco JA, Roddy MA, Johnstone MT, Creager MA: Impaired nitric oxide-mediated vasodilation in patients with non-insulin-dependent diabetes mellitus. *J Am Coll Cardiol* 27:567-574, 1996
10. Verma S, Raj SR, Shewchuk L, Mather KJ, Anderson TJ: Cyclooxygenase-2 blockade does not impair endothelial vasodilator function in healthy volunteers: randomized evaluation of rofecoxib versus naproxen on endothelium-dependent vasodilatation. *Circulation* 104:2879-2882, 2001
11. Cuzzocrea S, Salvemini D: Molecular mechanisms involved in the reciprocal regulation of cyclooxygenase and nitric oxide synthase enzymes. *Kidney Int* 71:290-297, 2007
12. Cherney DZ, Lai V, Scholey JW, Miller JA, Zinman B, Reich HN: Effect of direct renin inhibition on renal hemodynamic function, arterial stiffness, and endothelial function in humans with uncomplicated type 1 diabetes: a pilot study. *Diabetes Care* 33:361-365
13. Kawano H, Motoyama T, Hirashima O, Hirai N, Miyao Y, Sakamoto T, Kugiyama K, Ogawa H, Yasue H: Hyperglycemia rapidly suppresses flow-mediated endothelium-dependent vasodilation of brachial artery. *J Am Coll Cardiol* 34:146-154, 1999
14. Hashimoto M, Akishita M, Eto M, Ishikawa M, Kozaki K, Toba K, Sagara Y, Taketani Y, Orimo H, Ouchi Y: Modulation of endothelium-dependent flow-mediated dilatation of the brachial artery by sex and menstrual cycle. *Circulation* 92:3431-3435, 1995
Table 1: Effects of COX2 inhibition and hyperglycemia in Type 1 DM

| Parameter                  | Before COX2 Inhibition |                     | After COX2 Inhibition |                     |
|----------------------------|------------------------|---------------------|-----------------------|---------------------|
|                            | Hyperfiltration Group  | Normofiltration Group | Hyperfiltration Group | Normofiltration Group |
|                            | Euglycemia              | Hyperglycemia        | Euglycemia             | Hyperglycemia        |
| **Biochemistry**           |                        |                     |                       |                     |
| Plasma insulin (pmol/L)    | 87±51                  | 101±52              | 98±99                 | 88±100              |
|                            | 103±69                 | 105±74              | 83±49                 | 77±73               |
| **Blood pressure**         |                        |                     |                       |                     |
| SBP                        | 114±10                 | 113±8               | 117±10                | 116±13              |
| DBP                        | 65±9                   | 62±5                | 60±5                  | 61±4                |
| MAP                        | 83±9                   | 79±6                | 79±7                  | 79±8                |
| HR                         | 70±11                  | 68±11               | 61±13                 | 61±10               |
|                            |                        |                     |                       |                     |
| **Endothelial Function**   |                        |                     |                       |                     |
| FMD (% change)             | 5.9±2.3                | 7.6±2.9             | 10.2±5.3*             | 8.3±4.23            |
| GTN (% change)             | 13.0±3.2               | 14.2±3.1            | 11.0±3.7              | 13.7±7.9            |
|                            | 8.3±3.9                | 8.2±2.54            | 5.8±3.4†‡             | 8.1±3.2             |
|                            | 12.1±4.2               | 14.7±4.9            | 11.1±4.7              | 13.9±7.0            |

SBP = systolic blood pressure in mmHg; DBP = diastolic blood pressure in mmHg; MAP = mean arterial pressure; HR = heart rate in beats per minute; clamped euglycemia = 4-6 mmol/L; clamped hyperglycemia = 9-11 mmol/L

* p=0.003 for FMD during clamped euglycemia in hyperfiltering group vs. normofiltering group
† p=0.006 for effect of COX2 inhibition on FMD in normofiltering group during clamped euglycemia
‡ p=0.003 for the change in FMD in hyperfilterers vs. normofilterers in response to COX2 inhibition during clamped euglycemia