**Effect of Protein Kinase Cβ Inhibition on Renal Hemodynamic Function and Urinary Biomarkers in Humans With Type 1 Diabetes: A Pilot Study**

**OBJECTIVE** — The aim of this study was to examine the effect of protein kinase Cβ inhibition with ruboxistaurin on renal hemodynamic function and urinary biomarkers (monocyte chemoattractant protein-1 [MCP-1] and epidermal growth factor) in renin angiotensin system blockade-treated type 1 diabetic subjects.

**RESULTS** — Baseline clinical characteristics and eGFR were similar between groups. Hyperfiltration rate was higher in the ruboxistaurin group (96 ± 1 mmHg) than in the placebo group (91 ± 2 mmHg). Albuminuric subjects (n = 7) randomized to ruboxistaurin (32 mg daily for 8 weeks) or placebo (n = 4) for 8 weeks. Renal hemodynamic function was measured during clamped euglycemia or hyperglycemia before and after ruboxistaurin or placebo.

**CONCLUSIONS** — The effect of ruboxistaurin is modest and dependent, at least in part, on the level of ambient glycemia and baseline glomerular filtration rate.

**Research Design and Methods** — After giving informed consent, subjects (Table A1, with inclusion/exclusion criteria, is available in an online appendix at http://dx.doi.org/10.2337/dc08-1609) adhered to a diet that was Na replete and moderate in protein for 7 days before each experiment (6,7). Euglycemic (blood glucose 4–6 mmol/l) and hyperglycemic (blood glucose 9–11 mmol/l) conditions were maintained on two consecutive days using a modified glucose clamp technique, and renal hemodynamic function was measured using inulin and para-aminohippurate (6,7). Urinary biomarkers were measured by ELISA (Quantikine; R&D Systems, Minneapolis, MN) before and after treatment with ruboxistaurin or placebo, normalized for urinary creatinine. Subjects were then randomized (2:1) to ruboxistaurin (32 mg daily for 8 weeks) or a placebo in a double-blind fashion. All subjects were taking an ACE inhibitor, an angiotensin receptor blocker (ARB), or a combination throughout the study. The University Health Network Research Ethics Board approved the protocol.

The primary analysis examined hemodynamic responses during clamped euglycemia and hyperglycemia before and after treatment with ruboxistaurin or the placebo. In a post hoc analysis, we analyzed subjects on the basis of filtration status (hyperfiltration, glomerular filtration rate [GFR] ≥135 ml/min per 1.73 m²; normofiltration, <135 ml/min per 1.73 m²) (6,7). Between-group comparisons of all parameters at baseline were made using parametric methods (unpaired Student’s t test). Within-subject and between-group differences in the response to PKCβ inhibition were determined by repeated-measures ANOVA. All statistical analyses were performed using SPSS (version 14; SPSS, Chicago, IL).

**RESULTS** — Baseline clinical characteristics are shown in online appendix Table A2. At baseline, mean ± SEM arterial pressure was higher in the ruboxistaurin group (96 ± 1 mmHg) than in the pla-
The effect of ruboxistaurin (RBX) on GFR during euglycemia in hyperfiltration and normofiltration subjects (mean ± SEM). HF, hyperfiltration; NF, normofiltration. *P = 0.009 vs. baseline in hyperfiltration subjects. †P = 0.003 vs. response in normofiltration subjects.

CONCLUSIONS — The aim of this study was to determine the role of PKCB inhibition in humans with diabetes. Our major findings were that 1) during clamped hyperglycemia, ruboxistaurin lowered ERPF and renal blood flow, and 2) in a post hoc analysis based on filtration status, ruboxistaurin partially corrected hyperfiltration during clamped euglycemia, while MCP-1 decreased and the EGF–to–MCP-1 ratio increased in PKCB activity (1). Our first major finding was that during clamped euglycemia, ruboxistaurin did not significantly affect renal hemodynamic function. In contrast, during clamped hyperglycemia, ruboxistaurin lowered ERPF but there was no effect on GFR. Although from this experiment we could not determine why ruboxistaurin failed to lower GFR during hyperglycemia, the explanation may involve activation of redundant hemodynamic pathways, such as endothelin or cyclooxygenase-2, leading to the maintenance of GFR (10, 11).

We have previously reported that baseline GFR during clamped euglycemia is a determinant of renal hemodynamic responsiveness (6). Our second major finding was that ruboxistaurin was associated with reductions in GFR and filtration fraction without affecting ERPF in hyperfiltration subjects but was not associated with such reductions in normofiltration subjects. Furthermore, the change in the urinary biomarkers in hyperfiltration subjects was consistent with protection against renal injury (a decline in MCP-1 and a rise in the EGF–to–MCP-1 ratio (P = 0.041) in hyperfiltration versus normofiltration subjects (online appendix Figures A1–A3).

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

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