

**The Effect of Direct Renin Inhibition on Renal Hemodynamic Function,
Arterial Stiffness and Endothelial Function in Humans with Uncomplicated
Type 1 Diabetes Mellitus: A Pilot Study**

David Z.I. Cherney MD, PhD¹, Vesta Lai RN¹, James W. Scholey MD¹, Judith A. Miller MD¹,
Bernard Zinman MD², Heather N. Reich MD, PhD¹

¹ Division of Nephrology, Toronto General Hospital, University of Toronto

² Samuel Lunenfeld Research Institute, University of Toronto

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Please address correspondence to:

David Z.I. Cherney, MD, PhD, FRCP(C)

Email: david.cherney@uhn.on.ca

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Introduction: Blockade of the renin angiotensin system (RAS) plays an important role in preventing end-organ injury associated with diabetes mellitus (DM). The recent development of direct renin inhibitors (DRI) provides a new approach to block the RAS, but the effect of DRIs on renal and systemic vascular function in uncomplicated type 1 DM have not been elucidated.

Methods: Renal hemodynamic function (inulin, paraaminohippurate clearance), augmentation index and pulse wave velocity, endothelial dependent vasodilatation (FMD) and endothelial independent vasodilatation (response to sublingual nitroglycerin) were evaluated before and after taking aliskiren (300 mg daily for 30 days) in 10 adult subjects with uncomplicated type 1 DM during clamped euglycemia (4-6 mmol/L) and hyperglycemia (9-11 mmol/L).

Results: In response to DRI, plasma renin activity decreased (0.40 to 0.13 ng/mL/h, $p < 0.05$) and plasma renin increased (5.2 to 75.0 ng/L, $p < 0.05$). Peripheral and central blood pressures decreased and effective renal plasma flow and glomerular filtration rate increased during clamped euglycemia and hyperglycemia ($p < 0.05$). Carotid augmentation index during clamped euglycemia decreased ($26 \pm 6\%$ to $20 \pm 5\%$, $p < 0.05$) as did pulse wave velocity during clamped hyperglycemia (7.8 ± 0.6 m/s to 6.8 ± 0.5 m/s, $p < 0.05$). In response to DRI, FMD increased during both clamped euglycemia ($1.92 \pm 1.13\%$ to $5.55 \pm 0.81\%$) and hyperglycemia ($1.86 \pm 0.98\%$ to 5.63 ± 0.62) as did the vasodilatory response to sublingual nitroglycerin.

Conclusions: DRI exerts a renal vasodilatory effect and improves parameters of systemic vascular function, suggesting that blockade of the RAS with this new class of agents has important functional effects in subjects with uncomplicated type 1 DM.

Diabetic renal complications are, in part, mediated by activation of the renin-angiotensin system (RAS), which leads to maladaptive renal and systemic hemodynamic responses including renal hyperfiltration, increased arterial stiffness, and endothelial dysfunction (1-3). More recently, a new class of RAS inhibitors has become available, called direct renin inhibitors (DRIs). DRIs block the generation of angiotensin I from angiotensinogen, thereby preventing the generation of angiotensin II (one of the main effectors of the RAS). DRIs also mitigate the cellular effects of prorenin and renin on the prorenin receptor thereby accounting for the prominent hemodynamic effects of DRIs in animals and humans (4). From a functional perspective, the addition of a DRI to an ARB or ACEi provides additional antihypertensive effects (4). In humans with type 2 DM and clinical evidence of diabetic nephropathy, DRIs such as aliskiren (Rasilez®, Novartis Pharmaceuticals Canada, Inc) exert anti-proteinuric effects that are additive to ARBs, suggesting that DRIs may specifically enhance blockade of the intrarenal RAS in humans (5). DRI is therefore an attractive strategy to block the RAS in conditions in which the RAS is activated, such as DM, and may provide incremental protective hemodynamic effects in humans when added to ACEi or ARB therapy (4).

However, the renal and peripheral vascular hemodynamic effects of DRI monotherapy have not been studied in human subjects with uncomplicated type 1 DM. Accordingly, the objective of this pilot study was to examine the renal hemodynamic and peripheral vascular effects of DRI using aliskiren in subjects with uncomplicated type 1 DM, when RAS activation may promote the cellular and hemodynamic changes that contribute to the development of diabetic nephropathy and vascular complications (6).

MATERIALS and METHODS

Subjects: Five men and five women with uncomplicated type 1 DM participated in this pilot study (Table 1). Inclusion criteria were: duration of type 1 DM ≥ 10 years, age ≥ 16 years, blood pressure $< 140/90$, no history of renal disease or macrovascular disease. No subjects had microalbuminuria (persistent urinary albumin to creatinine ratio > 2.1 mg/mmol in men or 2.8 mg/mmol in women). Female subjects were studied during the late-follicular phase of the menstrual cycle, determined by cycle day and measurement of 17β -estradiol levels. None were using oral contraceptives medications. The local Research Ethics Boards at the University Health Network and Hospital for Sick Children (Toronto, Canada) approved the protocol and all subjects gave informed consent.

Experimental Design: Subjects adhered to a high sodium (>150 mmol/day) and moderate protein (<1.5 g/kg/day) diet during the 7-day period before each experiment, as described previously (Table 1). Euglycemic (4-6 mmol/L) and hyperglycemic (9-11 mmol/L) conditions were maintained on two consecutive days for approximately 6 hours preceding and during all investigations, a period of time demonstrated to be sufficient for detection of changes in vascular function (7). In all phases of the experiment, blood glucose was maintained by a modified glucose clamp technique, as described previously (8). A 16-gauge peripheral venous cannula was inserted into the left antecubital vein for infusion of glucose and insulin and a second cannula was inserted for blood sampling more distally. Blood glucose was measured every 10-15 minutes and the insulin infusion was adjusted to maintain the desired glycemic level. All experiments were performed in the same warm (25°C), temperature-controlled room and in a dark,

quiet environment after 10 minutes of rest in the supine position. Renal and systemic vascular function studies were performed at baseline and then repeated in an identical fashion after 30 days of aliskiren therapy (300 mg orally per day).

Assessment of Arterial Stiffness and Endothelial Function: Following the glucose clamp, peripheral blood pressure was measured in the right brachial artery with an automated DINAMAP® sphygmomanometer (Critikon, Florida, USA). Right carotid artery waveforms were recorded with a high-fidelity micromanometer (SPC-301, Millar Instruments) and using the validated transfer function, corresponding central aortic pressure waveform and central blood pressure data were generated (SphygmoCor, AtCor Medical Systems Inc., Sydney, Australia). Mean arterial pressure and heart rate were determined using the integral software. Augmentation index, an estimate of systemic arterial stiffness was calculated as the difference between the second systolic peak and inflection point, expressed as a percentage of the central pulse pressure corrected to an average heart rate of 75 beats per minute. The aortic pulse wave velocity (PWV) was measured using the same device by sequentially recording ECG-gated right carotid and femoral artery waveforms. The inter-operator variability and reproducibility of the augmentation index have been validated to be $0.4 \pm 6.4\%$. Our group has published and validated the use of the SphygmoCor device (9).

Brachial artery 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). Briefly, the right brachial artery was scanned 2 to 5 cm above the antecubital fossa using high resolution B-mode vascular ultrasound (Vividi, 7-15 MHz

linear-array transducer, GE/Vingmed, WI, USA). Longitudinal, ECG-gated, end-diastolic images were acquired over 6 cardiac cycles, and the brachial arterial diameter was determined for each image using integrated software and the results were averaged. Diameter measurements were taken from the anterior to the posterior interface between the media and adventitia (7). 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 cuff deflation, the increase in blood flow was measured (reactive hyperemia) along with the change in vessel diameter (endothelium-dependent dilatation), which 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 was defined and reported as the maximal percentage changes in vessel diameter after reactive hyperemia. FMD and GTN were defined as the maximal percentage changes in vessel diameter after reactive hyperemia and administration of GTN, respectively. FMD was also reported as “FMD/flow”, defined as the maximal percentage change in vessel diameter divided by percentage change flow ($\text{peak instantaneous flow} - \text{baseline instantaneous flow} / \text{baseline instantaneous flow} \times 100$, where baseline instantaneous flow was averaged over 1 minute before pressure cuff inflation and peak instantaneous flow was calculated immediately after cuff deflation), to create a stimulus-adjusted response measure (10). A single observer (DZIC) obtained all measurements. The intra-observer variability for repeated measurements of arterial diameters at flow mediated vasodilatation was 0.01 ± 0.005 mm (absolute diameter) or $0.26 \pm 0.01\%$ (% absolute value of brachial artery at flow mediated vasodilatation), which is similar to that previously reported (7; 11).

Assessment of Renal Hemodynamic Function: Following the assessment of arterial stiffness and endothelial function, a third intravenous line was inserted into the right arm and was connected to a syringe infusion pump for administration of inulin and paraaminohippurate (PAH). After collecting blood for inulin and PAH blank, a priming infusion containing 25% inulin (60 mg/kg) and 20% PAH (8 mg/kg) was administered. Thereafter, inulin and PAH were infused continuously at a rate calculated to maintain their respective plasma concentrations constant at 20 and 1.5 mg/dl. After a 90 minute equilibration period, blood was collected for inulin, PAH and hematocrit (HCT). Blood was further collected every 30 minutes for 60 minutes for inulin and PAH, and glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) were estimated by steady state infusion of inulin and PAH, respectively (8).

Sample Collection and Analytical Methods: Blood samples collected for inulin and PAH determinations were immediately centrifuged at 3000 rpm for 10 minutes at 4° C. Plasma was separated, placed on ice, and then stored at -70° C before the assay. Inulin and PAH were measured in serum by colorimetric assays using anthrone and N- (1-naphthyl) ethylenediamine respectively. The mean of 2 baseline clearance periods represent GFR and ERPF, expressed per 1.73 m². Renal blood flow (RBF) was derived using ERPF / [1-HCT] and renal vascular resistance (RVR) derived by dividing the MAP by the RBF. All renal hemodynamic measurements were adjusted for body surface area.

Active plasma renin was measured by 2 site immunoradiometric assay where 2 monoclonal antibodies to human active renin are used. One antibody was coupled to biotin while the second was radiolabeled for detection. The sample containing active renin was incubated simultaneously with both antibodies to form a complex. The

radioactivity of this complex was directly proportional to the amount of immunoreactive renin present in the sample. Plasma Renin Activity (PRA) was measured with a radioimmunoassay kit (GammaCoat[®] Plasma Renin Activity ¹²⁵I RIA Kit, CA-1533, Diasorin, Stillwater, Minnesota 55082-0285, USA). PRA determination involves an initial incubation of plasma to generate angiotensin I, followed by quantitation of angiotensin I by radioimmunoassay. In the GammaCoat[®] Plasma Renin Activity ¹²⁵I RIA Kit, the antibody is immobilized onto the lower inner wall of the GammaCoat tube. After incubation of standards, unknown samples and ¹²⁵I angiotensin I in the GammaCoat tube, the reaction mixture is removed by aspiration and the bound tracer counted in a gamma counter. A standard curve is constructed and the concentration of angiotensin I of the unknown sample obtained by interpolation.

Urinary albumin excretion rate was determined from three timed overnight urine collections. Urinary albumin concentration was determined by immunoturbidimetry. Hemoglobin A1C was measured by high-performance liquid chromatography.

Statistical Analysis: Changes in renal and systemic hemodynamic function in response to DRI were assessed using a repeated measures analysis of variance (ANOVA). All statistical analyses were performed using the statistical package SPSS (SPSS for graduate students, Version 14.0). The vascular data were obtained and analyzed by a single observer (D.Z.I.C.) who was blinded to the glycemic day and renal hemodynamic measurements.

RESULTS:

Baseline Clinical Characteristics and Hemodynamic Function: At baseline, subjects were normoalbuminuric and twenty-four urine collections demonstrated adherence to the prescribed sodium and protein dietary preparation. The mean serum estradiol level

was 138 ± 36.5 pmol/L, consistent with the late follicular phase of the menstrual cycle. Baseline peripheral and central blood pressure profiles, renal function, arterial stiffness and endothelial function are described in Table 2. Subjects were normotensive and had normal renal function. No subjects exhibited renal hyperfiltration (defined as $\text{GFR} \geq 135$ ml/min/m²) at baseline.

Renal and Systemic Hemodynamic Responses to DRI: As expected, circulating plasma renin activity decreased (0.40 to 0.13 ng/mL/h, $p=0.049$) and plasma renin increased (5.2 to 75.0 ng/L, $p=0.016$) after 30 days of DRI administration. Peripheral blood pressure decreased by 13/8 mmHg during clamped euglycemia and 12/4 mmHg during clamped hyperglycemia compared with baseline measurements ($p<0.05$), while heart rate did not change (Table 2). DRI administration also resulted in significant declines in central blood pressure during clamped euglycemia and clamped hyperglycemia ($p<0.05$). During clamped euglycemia and hyperglycemia, ERPF and GFR increased proportionately ($p<0.05$), so that net FF did not change

Administration of DRI resulted in both a significant reduction in carotid augmentation index during clamped euglycemia and a decline in pulse wave velocity during clamped hyperglycemia (all $p<0.05$). DRI also produced alterations in endothelial function. While the flow stimulus did not change significantly with DRI, FMD increased during both clamped euglycemia ($1.92 \pm 1.13\%$ to $5.55 \pm 0.81\%$) and hyperglycemia ($1.86 \pm 0.98\%$ to $5.63 \pm 0.62\%$, Table 2). Compared with baseline, FMD corrected for the flow stimulus (FMD/flow) and the response to GTN were also increased during clamped euglycemia and hyperglycemia (Table 2, Figure 1).

DISCUSSION:

Blockade of the RAS is a critical approach to the management of patients with type 1 DM (12). The objective of this pilot study was therefore to examine the renal and peripheral vascular hemodynamic effects of DRI monotherapy using aliskiren in subjects with uncomplicated type 1 DM. Our major findings were that aliskiren: 1) decreases peripheral and central blood pressures, independent of glycemic status; 2) has a significant renal vasodilatory effect, regardless of ambient glycemia; 3) increases arterial compliance, and endothelial function as measured by FMD and GTN responsiveness.

DRI may block the RAS through a variety of mechanisms. First, in addition to conversion from angiotensin I by angiotensin converting enzyme, angiotensin II can be generated from angiotensin I via non-ACE, chymostatin-sensitive pathways, which are not affected by ACEi, but are blocked by DRI (13). Second, circulating levels of angiotensin peptides, including angiotensin II, increase after ARB treatment, leading to angiotensin type 2 receptor activation and possibly to vascular proliferative effects (14). This effect is not seen with DRI (14). Third, ACEi and ARB therapies increase circulating levels of renin and prorenin. These circulating factors bind to the (P)RR, increasing its catalytic activity and the generation of both angiotensin I and angiotensin II (12). In contrast, DRI may inhibit (P)RR activation (12). Fourth, direct binding of renin and/or prorenin to the (P)RR may stimulate signaling pathways associated with cell injury, ultimately leading to an increase in the expression of pro-fibrotic mediators by mesangial cells (12). This signaling cascade may be blocked using DRI (12). These mechanisms of action may be important when examining the additive effect of DRI to an ACEi or ARB, since DRI may mitigate compensatory pathways that limit the

long-term efficacy of ACEi or ARB-based monotherapies (4).

Our first major observation was the significant peripheral antihypertensive effect of DRI monotherapy in humans with type 1 DM during clamped euglycemia and hyperglycemia. ACEi and ARBs have well-described antihypertensive effects in healthy humans and in those with type 1 DM (15; 16). In human subjects with essential hypertension, DRI monotherapy is associated with a 12.7/9.7 mmHg reduction in blood pressure (17); the 13/8 mmHg reduction in systemic blood pressure that we observed in normotensive subjects with uncomplicated type 1 DM was similar to this previous report in non-diabetic subjects. Moreover, we observed a significant central blood pressure reduction after DRI therapy during clamped euglycemia and hyperglycemia. These changes in central blood pressures were similar in magnitude compared with previously reported effects of ACEi (18). Whether the effect of ACEi or ARB therapy combined with a DRI would have an additive antihypertensive effect on central blood pressure clearly requires future direct comparison studies. The antihypertensive effect that we observed during clamped hyperglycemia is of critical importance, since hyperglycemia can be a vasoconstrictive and hypertensive stimulus (19). Since patients with DM are frequently exposed to hyperglycemia, the ability of a DRI to control blood pressure during ambient hyperglycemic conditions is an important feature of this drug.

Our second major observation was that in subjects ingesting a carefully controlled high sodium diet, ERPF increased by 16% and 14% during clamped euglycemia and hyperglycemia, respectively. Previous studies examining the effect of ACEi and ARBs on renal hemodynamic function have demonstrated a wide spectrum of renal vasodilatory responses; the variability in the renal vascular response to these drugs has

been attributed to the influences of gender, renal filtration status, sodium intake, ambient glycemia and the presence or absence of DM (15; 16; 20). Fisher et al described the renal hemodynamic effects of DRI in healthy, salt-depleted individuals (20). In that study, administration of 300 mg of aliskiren was associated with a 29% increase in ERPF, which was greater than the effect observed in their historical control subjects who were treated with 25 mg of captopril. We have previously observed that dietary sodium restriction can paradoxically increase renal hemodynamic effects in uncomplicated type 1 DM, possibly through non-hemodynamic mechanisms (21). Whether the renal hemodynamic effect of DRI can be enhanced in DM subjects through the use of dietary sodium depletion is not known. Our results focus on DRI monotherapy, so future studies will be necessary to examine the additive effects of DRI to ACEi or ARB in this patient population.

Our third major observation was the significant effect of DRI on arterial stiffness and endothelial function. In the present set of experiments using a typical therapeutic dose of DRI, we observed reductions in carotid augmentation index (23.5%) and pulse wave velocity (12.8%) that were similar to the effects of ACEi that have been reported by others (22). In addition to effects on arterial stiffness, we observed significant positive effects on endothelial function. The effect of ACEi therapy on endothelial function is variable; in obese, non-DM humans and in some studies involving subjects with type 1 DM, ACEi has no effect on endothelial function (23; 24). In hypertensive individuals, ACEi improves flow-mediated dilatation by 30-40% (24). In the present study, we observed a 65% increase in endothelial function whether or not FMD was corrected for the flow stimulus. DRI also improved GTN responsiveness, independent of glycemic status, suggesting that this class

of agents increases nitric oxide bioavailability, similar to the effect of ACEi. As reviewed elsewhere, the physiologic impact related to the compensatory rise in plasma renin after DRI is not known (4). While the small sample size precludes any definitive conclusions, this pilot study suggests that the positive blood pressure and renal hemodynamic effects of DRI therapy extend to arterial stiffness and endothelial function in subjects with uncomplicated type 1 DM.

This pilot study has important limitations. First, the sample size was small, and may have limited our ability to detect some differences in renal hemodynamic parameters (such as renal vascular resistance) and arterial stiffness (such as carotid augmentation index during clamped hyperglycemia). We attempted to minimize the effect of the small sample size by utilizing homogeneous study groups and by careful pre-study dietary preparation. We also decreased variability by using a study design that allowed each subject to act as his or her own control.

FIGURE LEGEND:

Figure 1: Effect of DRI on Endothelial Function in type 1 DM (mean±SEM)

*p=0.04 vs. baseline during clamped euglycemia

†p=0.0001 vs. baseline during clamped hyperglycemia

In conclusion, DRI has significant beneficial hemodynamic effects in the renal and systemic circulations, leading to reductions in peripheral and central blood pressures, renal vasodilatation, and augmented arterial compliance and endothelial function in subjects with uncomplicated type 1 DM. The magnitude of these effects is similar to that seen in previous studies using ACEi and ARBs. This work highlights the need for future physiologic and clinical studies in humans that examine the effect of dual RAS blockade using DRIs and ACEi or ARB therapy.

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Table 1: Baseline Clinical Characteristics (mean \pm SEM)

Parameter	n=10
Males/Females	5/5
Age (years)	43.1 \pm 3.9
Hemoglobin A _{1C} (%)	7.93 \pm 0.40
Diabetes duration (years)	23.2 \pm 2.2
Body mass index (kg/m ²)	26.01
Albumin excretion rate (μ g/min)	0.233 \pm 0.053
24 hour Na ⁺ intake (mmol/day)	199 \pm 25
Estimated protein intake (gm/kg/day)	1.03 \pm 0.15
Weight (kg)	79 \pm 5

n = no. of subjects

Table 2: Blood Pressure, Arterial Stiffness and Endothelial Function Responses to DRI in Type 1 DM (mean ± SEM)

Parameter	Before DRI		After DRI	
	Euglycemia	Hyperglycemia	Euglycemia	Hyperglycemia
HR (bpm)	63±2	61±3	64±3	62±2
Blood pressure				
Peripheral SBP (mmHg)	120±4	123±4	107±3*	111±4†
Peripheral DBP (mmHg)	70±2	72±2	62±3*	68±3†
Renal hemodynamic function				
Effective renal plasma flow	558±27	606±29	665±24*	704±35†
Glomerular filtration rate	105±3	117±5	114±3*	124±2†
Filtration fraction	0.19±0.01	0.20±0.001	0.18±0.01	0.18±0.01
Renal blood flow	914±58	984±65	1049±59*	1102±76†
Renal vascular resistance	0.082±0.013	0.086±0.010	0.080±0.013	0.078±0.007
Applanation Tonometry				
Central SBP (mmHg)	104±3	109±4	97±3*	103±3†
Augmentation index (%)	21.7±16.6	22.0±5.0	16.6±4.8*	18.7±4.6
Aortic PWV (m/s)	7.4±0.4	7.8±0.6	6.7±0.4	6.8±0.5†
Endothelial Function				
Flow (% change)	74.58±5.63	81.01±7.32	75.54±9.69	69.59±8.19
FMD (% change)	1.92±1.13	1.86±0.98	5.55±0.81*	5.63±0.62†
FMD / flow	0.030±0.017	0.036±0.015	0.084±0.015*	0.094±0.016†
GTN (% change)	9.52±2.01	11.22±1.36	14.22±1.49*	15.85±1.46†

SBP = systolic blood pressure; DBP = diastolic blood pressure; PWV = pulse wave velocity; FMD = flow-mediated dilatation; Flow = % change in instantaneous flow; GTN = nitroglycerin

*p<0.05 vs. pre-DRI parameter during clamped euglycemia

†p<0.05 vs. pre-DRI parameter during clamped hyperglycemia

