The Effect of Direct Renin Inhibition Alone and in Combination With ACE Inhibition on Endothelial Function, Arterial Stiffness, and Renal Function in Type 1 Diabetes

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OBJECTIVE—Diabetes is associated with renin-angiotensin system (RAS) activation, leading to renal and systemic vascular dysfunction that contribute to end-organ injury and significant morbidity. RAS blockade with ACE inhibitors reduces, but does not abolish, RAS effects. Accordingly, our aim was to determine if direct renin inhibition alone, and in combination with an ACE inhibitor, corrects early hemodynamic abnormalities associated with type 1 diabetes.

RESEARCH DESIGN AND METHODS—Arterial stiffness (augmentation index), flow-mediated vasodilatation (FMD), and renal hemodynamic function (inulin and paraaminohippurate clearance) were measured at baseline under clamped euglycemic and hyperglycemic conditions (n = 21). Measures were repeated after 4 weeks of aliskiren therapy and again after aliskiren plus ramipril.

RESULTS—Blood pressure–lowering effects of aliskiren were similar during clamped euglycemia and hyperglycemia. Combination therapy augmented this effect under both glycemic conditions (P = 0.0005). Aliskiren reduced arterial stiffness under clamped euglycemic and hyperglycemic conditions, and the effects were augmented by dual RAS blockade (−3.4 ± 11.2 to −8.0 ± 11.5 to −14.3 ± 8.4%, respectively, during euglycemia, P = 0.0001). During clamped euglycemia, aliskiren increased FMD; dual therapy exaggerated this effect (5.1 ± 3.3 to 7.5 ± 3.0 to 10.8 ± 3.5%, repeated-measures ANOVA, P = 0.0001). Aliskiren monotherapy caused renal vasodilatation during clamped hyperglycemia only. In contrast, dual therapy augmented renal vasodilatory effects during clamped euglycemia and hyperglycemia.

CONCLUSIONS—In patients with uncomplicated type 1 diabetes, aliskiren-based dual RAS blockade is associated with greater arterial compliance, FMD, and renal vasodilatation.

Diabetes Care 35:2324–2330, 2012

Diabetic renal complications are partly mediated by renin-angiotensin system (RAS) activation, which leads to maladaptive renal and systemic hemodynamic responses (1). Experimental and clinical studies of diabetic nephropathy have demonstrated that blockade of the RAS with ACE inhibitors (ACEis) and angiotensin (Ang) II type 1 receptor blockers (ARBs) attenuates but does not prevent proteinuria or renal disease progression (2,3), which may be partly due to incomplete blockade of the RAS (4,5). For example, treatment with ACEis and ARBs leads to increases in circulating renin and prorenin levels, effects that may lead to increased generation of Ang I (6–8). Studies have also demonstrated that Ang II can be generated from Ang I, independently of ACE activity, and that long-term ACEi therapy subsequently leads to an increase in circulating levels of Ang II toward pretreatment values (9). The use of ACEi and ARB therapies is also ultimately limited by adverse side effects when these agents are combined to attempt to completely block RAS activity (10).

In addition to ACEi and ARB therapies, direct renin inhibitors (DRIs) also block the RAS and may have several advantages. DRIs block the generation of Ang I from angiotensinogen (11) and may also mitigate the direct cellular effects of prorenin and renin that are mediated by the prorenin receptor (12). These effects may account for the augmented hemodynamic effect of DRIs compared with ACEi or ARB monotherapy in animals and humans (11,13). In older patients with type 1 diabetes mellitus (DM) and in healthy subjects, DRIs induce renal vasodilatation (11,14). In humans with type 2 DM and proteinuria, DRIs such as aliskiren (Rasilez, Novartis Pharmaceuticals Canada, Inc.) exert blood pressure (BP)-independent antiproteinuric effects that are additive to ARBs, suggesting that DRI may enhance blockade of the intrarenal RAS in humans (15–17).

Unfortunately, the ALTITUDE (Aliskiren Trial in Type 2 Diabetes Using Cardio-Renal Endpoints) study, which examined the effect of DRI plus ARB therapy on cardiovascular and renal outcomes in older, high-risk type 2 DM patients with proteinuria, impaired renal function, or a history of cardiovascular disease, was discontinued because of a trend toward a greater risk of adverse events in the dual-therapy arm (unpublished). In light of data from ONTARGET (Ongoing Telmisartan Alone and in Combination with Ramipril Global Endpoint Trial) (10), which also included patients aged 55 years or older with established atherosclerotic disease or DM and end-organ

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Received 23 April 2012 and accepted 19 May 2012.
DOI: 10.2337/dc12-0773

damage, these results suggest that the risks of dual RAS blockade likely outweigh benefits in older patients with established renal or cardiovascular disease.

Despite what is known in high-risk patients, the physiologic effects of DRIs, alone and combined with ACEi, on renal and peripheral vascular hemodynamic have not been rigorously studied in young, low-risk patients with type 1 DM. This remains an important issue in the clinical management of young type 1 DM patients because a significant proportion of patients develop progressive renal disease, despite ACEi use (18–20). Accordingly, our objective was to examine the renal and peripheral vascular hemodynamic effects of aliskiren, alone and then combined with an ACEi, in subjects with uncomplicated type 1 DM. We hypothesized that the effects of DRI on arterial compliance, endothelial function, and renal function would be augmented by dual therapy and that dual RAS blockade would reverse the effect of clamped hyperglycemia on hemodynamic function.

RESEARCH DESIGN AND METHODS

The study recruited 24 men and women with uncomplicated type 1 DM (Table 1). Inclusion criteria were duration of type 1 DM >10 years, age >18 years, BP <140/90 mmHg, and 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). Women were studied during the late follicular phase of the menstrual cycle, determined by cycle day and measurement of 17-estradiol levels. None were using oral contraceptive medications. The local research ethics boards at the University Health Network and Hospital for Sick Children (Toronto, ON, Canada) approved the protocol, and all subjects gave informed consent. Of the 24 participants, 1 dropped out due to difficult intravenous access and 2 dropped out after the first set of experiments due to difficulty scheduling follow-up studies around work and schooling; therefore, 21 participants completed all three phases of the study.

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 2 consecutive days for 4 h preceding and during all investigations, a period of time demonstrated to be sufficient for detection of changes in vascular function (21).

In all phases of the experiment, blood glucose was maintained by a modified glucose clamp technique, as described previously (4). 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 min, 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 min of rest with the subject supine.

Renal and systemic vascular function studies were performed at baseline and then repeated in an identical fashion after 4 weeks of oral aliskiren therapy (150 mg/day for 2 weeks, followed by 300 mg/day for 2 weeks). Oral ramipril was added to aliskiren for a further 4 weeks (5 mg/day for 2 weeks, followed by 10 mg/day for 2 weeks), and the same studies were repeated.

Assessment of arterial stiffness and endothelial function

After the glucose clamp, peripheral BP was measured in the right brachial artery with an automated DINAMAP sphygmomanometer (Critikon, Tampa, FL). Right carotid artery waveforms were recorded with a high-fidelity micromanometer (SPC-301; Millar Instruments), and with use of the validated transfer function, corresponding central aortic pressure waveform and central BP data were generated (SphygmoCor, AtCor Medical Systems, Sydney, NSW, Australia). Mean arterial pressure and heart rate were determined using the integral software. The 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 bpm. The interoperator variability and reproducibility of the augmentation index have been validated to be 0.4 ± 6.4%. Our group has published and validated the use of the SphygmoCor device (14).

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]). In brief, the right brachial artery was scanned 2–5 cm above the antecubital fossa using high-resolution B-mode vascular ultrasound (Vividi, 7- to 15-MHz linear array transducer; GE/Vingmed, Waukesha, WI). Longitudinal, electrocardiogram-gated, end-diastolic images were acquired over six cardiac cycles, 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 (14).

After baseline images were recorded, the BP cuff was inflated around the forearm distal to the elbow to 200 mmHg for 5 min. The cuff was deflated, and 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 min. FMD was defined as the maximal percentage change in vessel diameter after reactive

Table 1—*Demographic characteristics at baseline, after aliskiren alone, and after combined therapy in type 1 DM patients and either normofiltration or hyperfiltration*

Baseline characteristics	Baseline	Aliskiren alone	Aliskiren plus ramipril
Sex			
Male	12	—	—
Female	9	—	—
Age (years)	22 ± 3	—	—
Diabetes duration (years)	17 ± 5	—	—
HbA_{1c} (%)	9.0 ± 1.2	9.0 ± 1.4	8.9 ± 1.2
BMI (kg/m^2)	24.5 ± 3.5	24.3 ± 3.4	24.7 ± 3.4
Protein excretion rate (μg/s)	0.063 ± 0.001	0.062 ± 0.001	0.062 ± 0.001
24-h Na^+ intake (mmol/day)	203 ± 57	171 ± 37	199 ± 43
Estimated protein intake (g/kg/day)	0.95 ± 0.29	0.93 ± 0.28	0.90 ± 0.26
Estrogen (pmol/L)	154 ± 76	174 ± 195	121 ± 51

Data are presented as *n* or mean ± SD.

hyperemia. FMD was also reported as "FMD/flow," defined as the maximal percentage change in vessel diameter divided by percentage change in flow (peak instantaneous flow − baseline instantaneous flow/baseline instantaneous flow × 100), where baseline instantaneous flow was averaged for 1 min before pressure cuff inflation, and peak instantaneous flow was calculated immediately after cuff deflation to create a stimulus-adjusted response measure. A single observer (D.Z.I.C.) obtained all measurements. The intraobserver variability for repeated measurements of arterial diameters at flow-mediated vasodilatation was 0.01 ± 0.005 mm (absolute diameter) or 0.26 ± 0.01% (percentage of the absolute value of the brachial artery at FMD), which is similar to that reported previously (14).

Assessment of renal hemodynamic function

After the assessment of arterial stiffness and endothelial function, a third intravenous catheter was inserted into the right arm and was connected to a syringe infusion pump for administration of inulin and paraaminohippurate (PAH). After collection of 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-min equilibration period, blood was collected for inulin, PAH, and hematocrit. Blood was further collected every 30 min for 60 min 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 (22).

Sample collection and analytical methods

Blood samples collected for inulin and PAH determinations were immediately centrifuged at 3,000 rpm for 10 min at 4°C. Plasma was separated, placed on ice, and 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 two baseline clearance periods represent GFR and ERPF, expressed per 1.73 m^2. Renal blood flow (RBF) was derived using ERPF/(1 − hematocrit), and renal vascular resistance was derived by dividing the mean arterial pressure by the RBF. All renal hemodynamic measurements were adjusted for body surface area.

Plasma aldosterone and Ang II were measured using previously described methods (23,24). To assess NO production, which can be influenced by RAS modulation (25), the plasma cyclic guanosine monophosphate (cGMP) level was measured at baseline, after aliskiren monotherapy, and after dual blockade under clamped euglycemic and hyperglycemic conditions. The assay for cGMP is based on the competition between cGMP in the standards or samples and a cGMP-acetylcholinesterase (AChE) conjugate (cGMP tracer) for a limited number of cGMP-specific rabbit antibody-binding sites. The rabbit antibody-cGMP complex (free or tracer) binds to the mouse monoclonal antibody IgG that is coated to the well. The plate is washed to remove the unbound reagent, and Ellman's Reagent (acetylthiocholine and 5,5′-dithiobis-2-nitrobenzoic acid, a substrate to AChE) is added to the well. The product of this enzymatic reaction, 5-thio-2-nitrobenzoic acid, has a distinct yellow color and absorbs strongly at 412 nm. The intensity of the color is proportional to the amount of cGMP tracer bound to the well, which is inversely proportional to the amount of free cGMP present in the standards or sample. Plasma samples are extracted with ethanol before analysis.

The urinary albumin excretion rate was determined using a 24-h urine collection by immunoturbidimetry. HbA_{1c} was measured by high-performance liquid chromatography, and plasma insulin levels were measured using standard techniques (26).

Statistical analysis

Changes in renal and systemic hemodynamic function in response to the DRI and DRI plus ACEi combination were assessed using a repeated-measures ANOVA. All statistical analyses were performed using SPSS 14.0 software (SPSS for graduate students). 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 demographic characteristics

At baseline, participants exhibited suboptimal HbA_{1c} values, and BMI was generally within the normal range (Table 1). Participants adhered to the prescribed sodium and protein dietary parameters. The HbA_{1c}, BMI, estimated dietary parameters, and estrogen levels in women were unchanged after the aliskiren monotherapy and dual-blockade phases of the study. As expected (27), the protein excretion rate was within normal limits and was unaffected by monotherapy or dual therapy. Plasma insulin levels were also stable under clamped euglycemic and hyperglycemic conditions at each of the three study periods (Table 2 and Table 3).

No participants experienced hyperkalemia or changes in serum creatinine during this series of experiments. One patient experienced transient orthostatic dizziness upon starting ramipril, which lasted less than 1 day and resolved spontaneously. All patients adhered to the study protocol medications for the prescribed duration.

Responses to aliskiren alone and combined with ramipril during clamped euglycemia

Hemodynamic response. Aliskiren monotherapy lowered BP significantly and the addition of ramipril further lowered systolic and diastolic BP (repeated-measures ANOVA, $P < 0.0001$; Table 2). Parallel effects were observed on arterial stiffness. Aliskiren lowered the carotid augmentation index and FMD, and these responses were exaggerated by the addition of ramipril (Table 2).

For renal hemodynamic function, aliskiren was associated with a rise in ERPF, although this change was not significant (repeated-measures ANOVA, P = NS). With the addition of ramipril, increases in ERPF and RBF were significant and accompanied by significant declines in RVR and filtration fraction (FF) (Table 2). The decline in RVR with dual blockade was significant compared with the response to aliskiren monotherapy (repeated-measures ANOVA, $P < 0.001$). Effects on GFR were not significant at any of the time points.

Neurohormonal response. In response to aliskiren monotherapy during clamped euglycemia, aldosterone levels tended to decrease, but this change did not reach statistical significance (Table 2). Circulating Ang II levels did decline after aliskiren monotherapy (repeated-measures ANOVA, $P = 0.006$) and remained suppressed at the end of the dual-blockade period. Effects on plasma cGMP were not significant.

Responses to aliskiren alone and combined with ramipril during clamped hyperglycemia

Hemodynamic response. Overall mean systolic and diastolic BP values were higher after aliskiren monotherapy

Table 2—*Hemodynamic responses to aliskiren alone and combined with ramipril during clamped euglycemia in type 1 DM patients*

	Baseline	Aliskiren	Aliskiren plus ramipril
BP			
Heart rate (bpm)	69 ± 11	67 ± 7	70 ± 10
Systolic BP (mmHg)	113 ± 8	107 ± 12*	102 ± 5*†
Diastolic BP (mmHg)	64 ± 7	59 ± 9*	52 ± 3*†
Vascular parameters			
Carotid augmentation index (%)	−3.4 ± 11.2	−8.0 ± 11.5*	−14.3 ± 8.4*†
Flow-mediated vasodilatation (%)	5.1 ± 3.3	7.5 ± 3.0*	10.8 ± 3.5*†
Flow-mediated vasodilatation/flow	0.080 ± 0.069	0.111 ± 0.068*	0.160 ± 0.092*†
Renal hemodynamic function			
ERPF (mL/min/1.73 m^2)	701 ± 132	796 ± 198	846 ± 171*
GFR (mL/min/1.73 m^2)	140 ± 32	147 ± 30	144 ± 29
FF	0.20 ± 0.01	0.20 ± 0.01	0.18 ± 0.01*
RBF (mL/min/1.73 m^2)	1,158 ± 217	1,259 ± 340	1,313 ± 284*
RVR (mmHg/L/min)	0.072 ± 0.014	0.068 ± 0.021	0.054 ± 0.009*†
Biochemistry			
Plasma levels			
Insulin (pmol/L)	101 ± 123	80 ± 96	124 ± 171
Aldosterone (pmol/L)	40.7 ± 6.5	33.5 ± 2.7	34.3 ± 3.4
Ang II (pmol/L)	2.39 ± 0.45	1.18 ± 0.07*	1.16 ± 0.21*
Plasma cGMP (pmol/mL)	5.15 ± 0.45	4.88 ± 0.46	4.47 ± 0.37

Data are mean ± SD. *$P \leq 0.025$ vs. baseline value. †$P \leq 0.025$ compared with value after aliskiren monotherapy.

during hyperglycemia conditions compared with mean values during clamped euglycemia (Table 2 and Table 3). Aliskiren monotherapy did not lower BP significantly during clamped hyperglycemia. However, similar to responses observed under euglycemic conditions, the addition of ramipril to aliskiren was associated with a reduction in BP during clamped hyperglycemia (Table 3). For arterial stiffness during clamped hyperglycemia, aliskiren monotherapy reduced the augmentation index (Table 3), and these effects were augmented by the addition of ramipril.

The FMD was similarly improved with aliskiren, and this effect was maintained after the addition of ramipril. The change in FMD in response to dual therapy (8.1 ± 3.2%) versus DRI monotherapy (8.3 ± 3.1%) was blunted during clamped hyperglycemia compared with the effect during clamped euglycemia (between-group effect, $P = 0.003$; Fig. 1). The change in FMD in response to dual therapy versus baseline responses (ΔFMD +3.73 ± 0.84%) was blunted during clamped hyperglycemia compared with the effect observed during clamped euglycemia (ΔFMD +5.75 ± 0.78%; between-group effect, $P = 0.025$; Fig. 1).

For renal hemodynamic function during clamped hyperglycemia, aliskiren monotherapy was associated with an increase in ERPF, whereas changes in RBF and RVR failed to reach significance. Changes in these renal hemodynamic parameters were all highly significant after the addition of ramipril, and the decline in RVR was exaggerated compared with the response to aliskiren monotherapy (repeated-measures ANOVA, $P < 0.001$; Table 3).

Neurohormonal responses. In response to aliskiren monotherapy during clamped hyperglycemia, circulating aldosterone declined (P = NS). The decline in aldosterone was statistically significant after dual RAS blockade (repeated-measures ANOVA, $P = 0.01$; Table 3). Declines in circulating Ang II observed after aliskiren monotherapy persisted after dual blockade. Effects on plasma cGMP were not significant.

CONCLUSIONS

—Blockade of the RAS is an essential component of the clinical management of patients with type 1 DM. Unfortunately, a significant proportion of type 1 DM patients treated with an ACEi still develop renal disease (18), potentially due to incomplete blockade of RAS effects using ACEi monotherapy. The aim of this study was to measure the effect of DRI, alone and combined with an ACEi, on renal and systemic vascular parameters, including arterial stiffness and FMD. Our major findings were that:

1. DRI increased arterial compliance and endothelial function during clamped euglycemia and hyperglycemia. Dual RAS blockade augmented these affects during clamped euglycemia. BP-lowering effects were similarly augmented by dual RAS blockade.
2. Effects of DRI monotherapy on renal hemodynamic function only reached significance during clamped hyperglycemia. Renal effects were enhanced by the addition of an ACEi under both glycemic conditions.
3. Under controlled conditions, dual RAS blockade with a DRI and ACEi was well tolerated by healthy individuals with type 1 DM.

Endothelial dysfunction and increased arterial stiffness are characteristic features of animals and humans with DM and contribute to the development of diabetic macrovascular disease, including coronary artery disease, cerebrovascular disease, and peripheral vascular disease (28–30). Peripheral vascular function abnormalities related to DM have been attributed to a lack of vasodilator activity (nitric oxide) as well as an increase in vasoconstrictive factors such as hyperglycemia-induced Ang II (28–30). Hyperglycemia further impairs endothelial function and arterial compliance and raises BP through activation of the RAS and protein kinase C-β (31,32).

Our observation that DRI increases arterial compliance and endothelial function during clamped euglycemia and hyperglycemia is consistent with previous studies using ACEi/ARB monotherapy. In older normotensive, normoalbuminuric patients with type 1 DM, we have previously demonstrated that aliskiren improves FMD and arterial compliance (14). In the present work, we were able to demonstrate similar findings in a young cohort of patients with type 1 DM. To further extend previous work and determine whether dual therapy may afford therapeutic advantages in this target population, we examined the effect of DRI-based dual therapy on endothelial function and arterial stiffness in type 1 DM. Previous dual RAS blockade studies have that ACEi/ARB combination therapy may exert additive effects on arterial compliance in hypertensive patients (33), suggesting that monotherapy with ACEi may not offer optimal potential hemodynamic

Table 3—*Hemodynamic responses to aliskiren alone and combined with ramipril during clamped hyperglycemia in type 1 DM patients*

	Baseline	Aliskiren	Aliskiren plus ramipril
BP			
Heart rate (bpm)	66 ± 8	71 ± 9	70 ± 121
Systolic BP (mmHg)	115 ± 10	112 ± 12‡	103 ± 2*†
Diastolic BP (mmHg)	64 ± 9	62 ± 9‡	56 ± 1*†§
Vascular parameters			
Carotid augmentation index (%)	0.3 ± 11.8	−7.1 ± 12.4*	−14.1 ± 11.7*†
Flow-mediated vasodilatation (%)	4.6 ± 3.0	8.1 ± 3.2*	8.3 ± 3.1*
Flow-mediated vasodilatation/flow	0.071 ± 0.052	0.113 ± 0.054*	0.147 ± 0.089*
Renal hemodynamic function			
ERPF (mL/min/1.73 m^2)	747 ± 150	842 ± 215*	945 ± 423*
GFR (mL/min/1.73 m^2)	142 ± 26	155 ± 42	149 ± 34
FF	0.20 ± 0.01	0.19 ± 0.01	0.18 ± 0.01
RBF (mL/min/1.73 m^2)	1,193 ± 250	1,319 ± 356	1,481 ± 167*
RVR (mmHg/L/min)	0.070 ± 0.013	0.061 ± 0.012	0.054 ± 0.015*†
Biochemistry			
Plasma levels			
Insulin (pmol/L)	112 ± 140	99 ± 124	117 ± 132
Aldosterone (pmol/L)	39.8 ± 4.5	32.0 ± 3.0	27.9 ± 1.3*
Ang II (pmol/L)	2.48 ± 0.32	1.33 ± 0.17*	1.16 ± 0.16*
cGMP (pmol/mL)	5.09 ± 0.43	5.13 ± 0.53	5.04 ± 0.44

Data are mean ± SD. *$P \leq 0.025$ vs. baseline value. †$P \leq 0.025$ vs. value after aliskiren monotherapy. ‡$P \leq 0.025$ vs. BP after aliskiren monotherapy during clamped euglycemia. §$P < 0.01$ vs. diastolic BP after dual blockade during clamped euglycemia.

benefits of RAS blockade. To our knowledge, this is the first study to examine the effect of dual RAS blockade with a DRI on these vascular parameters in young patients with uncomplicated type 1 DM.

In our cohort, dual RAS blockade augmented effects on FMD and arterial stiffness during clamped euglycemia. During clamped hyperglycemia, the decline in the carotid augmentation index was further enhanced using dual RAS blockade. The effect of aliskiren on FMD during clamped hyperglycemia was also significant, but this effect was not enhanced by the addition of ramipril. Contrary to our hypothesis, the further rise in FMD after the addition of ramipril during euglycemia (ΔFMD +3.23 ± 0.31%) was greater than the response during hyperglycemia (ΔFMD +0.21 ± 0.32%), suggesting that hyperglycemia still blunted FMD responses. This observation suggests the presence of persistent activation of hyperglycemia-induced factors despite maximum RAS blockade, leading to macrovascular dysfunction such as prooxidant and inflammatory pathways (3).

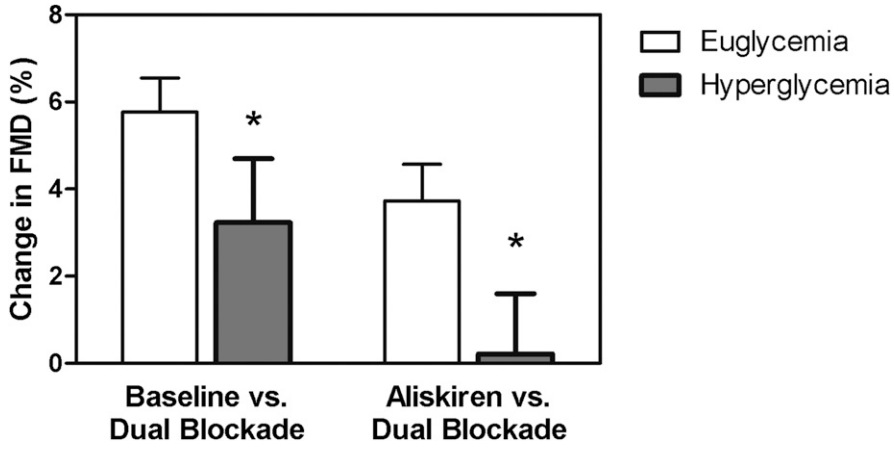

Figure 1—*FMD responses to aliskiren and dual therapy during clamped euglycemia and clamped hyperglycemia (mean ± SD).* *P *< 0.025 for response during clamped hyperglycemia vs. clamped euglycemia.*

Interestingly, the additive beneficial effects on FMD and arterial stiffness after dual blockade in our study were accompanied by greater declines in systemic BP in response to dual therapy. In previous studies, dual RAS blockade using ACEi, ARB, and DRI therapies exerted variable effects on systemic BP, with some studies demonstrating synergistic effects (15,33–38). The variable effects of dual RAS blockade on BP observed in other studies may have resulted from inconsistent dietary sodium intake, variable ambient glycemic conditions, concomitant medications, or patient demographic characteristics at the time of the BP measurements. Our results suggest that the addition of an ACEi to a DRI augments the BP effect of DRI monotherapy through effects on macrovascular function including FMD and arterial stiffness.

The effects of dual RAS blockade with DRI and ACEi on renal hemodynamic function are not well described in healthy young individuals with type 1 DM. In the renal microcirculation of healthy, salt-depleted humans, the intravenous infusion of the DRI enalkiren is associated with significant renal vasodilatation (39). Treatment of healthy volunteers with aliskiren is also associated with a significant rise in ERPF, with no change in GFR, resulting in a decline in FF (11). In an older cohort of patients with uncomplicated type 1 DM, we previously demonstrated that aliskiren induces renal vasodilatation with a rise in ERPF of approximately 100 mL/min/1.73 m^2, which is greater than the effect observed in healthy non-DM study participants (11,14). To extend this work, we examined the effect of aliskiren, alone and combined with an ACEi on renal hemodynamic function in a younger cohort. As suggested by previous work from our laboratory and from others, under circumstances in which the intrarenal RAS is activated, such as in DM, blockade of additional steps in the RAS activation cascade should enhance efferent arteriolar vasodilatation (4).

Our second major observation was that aliskiren monotherapy induced a renal vasodilatory response that was similar to values seen in previous work (14). This effect was augmented with the addition of ramipril. Synergistic effects on renal hemodynamic parameters have been noted after dual RAS blockade using ARB/ACEi combinations (40). Our renal hemodynamic

results are similar to these previous findings, because effects on RVR were exaggerated by the addition of ramipril. Previous work has suggested that augmented renal hemodynamic effects of dual RAS blockade may be mediated by suppression of aldosterone escape, leading to greater vasodilatation (40). Our results are in agreement with this previous work, because declines in aldosterone were significant after the dual-blockade phase of the study.

This study has important limitations. First, the sample size was small and may have limited our ability to detect differences in renal hemodynamic parameters such as RVR. We attempted to minimize the effect of the small sample size by using homogeneous study groups and by careful prestudy dietary preparation. We also decreased variability by using a study design that allowed each subject to act as his or her own control. In addition, although we were unable to account for the effect of prior long-term RAS blockade on vascular parameters, that was not the goal of this set of experiments. Rather, our aim was to elucidate the effect of the DRI-based dual RAS blockade during controlled experimental conditions.

In conclusion, dual RAS blockade using DRI-based therapy exerts significant effects on arterial compliance and endothelial function compared with DRI monotherapy in subjects with uncomplicated type 1 DM. Although safe in this young patient cohort, the effects of DRI on renal vasodilatation and systemic vascular function are likely similar to effects observed with combination ACEi/ARB therapy.

Acknowledgments—This work was supported by operating grants from the Heart and Stroke Foundation of Canada to D.Z.I.C. and H.N.R. D.Z.I.C. was also supported by a Kidney Foundation of Canada Scholarship and a Canadian Diabetes Association-Kidney Research Scientist Core Education and National Training Program (KRESCENT) Joint New Investigator Award and receives operating support from the Canadian Institutes of Health Research (CIHR). H.N.R. was a recipient of a KRESCENT Program New Investigator Award. J.W.S. is the CIHR/AMGEN Canada Kidney Research Chair at the University Health Network, University of Toronto. No other potential conflicts of interest relevant to this article were reported.

D.Z.I.C., S.J., R.H., and V.L. researched data and wrote the manuscript. J.W.S., E.B.S. and H.N.R. contributed to study design and discussion and reviewed and edited the manuscript. D.Z.I.C. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

The authors thank Dr. Paul Yip and Jenny Cheung-Hum, University Health Network, for their invaluable assistance with biochemical assays included in this work and are grateful to the study participants whose time and effort is critical to the success of their research program.

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