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Direct renin inhibition improves parasympathetic function in diabetes

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Abstract

Aim—The renin-angiotensin-aldosterone system (RAAS) and autonomic nervous system regulate the cardiovascular system. Blockade of the RAAS may slow progression of end-organ damage. Direct renin inhibition offers a means for blocking the RAAS. The objective of this study was to examine the effect of direct renin inhibition on cardiovascular autonomic function.

Methods—In this double-blind, placebo-controlled trial, 60 individuals with diabetes were randomly assigned to 300 mg of aliskiren or placebo once daily for 6 weeks. The primary end point was a change in tests of cardiovascular autonomic function. Autonomic function was assessed by power spectral analysis and RR-variation during deep breathing (i.e., mean circular resultant (MCR), expiration/inspiration (E/I) ratio). The MCR and E/I ratio assess parasympathetic function. Secondary measures included change in biochemical parameters (e.g., plasma renin activity, leptin, interleukin-6). Change in cardiovascular autonomic function and blood analytes were analyzed by a mixed effects model for repeated measures.

Results—Baseline characteristics were similar between treatment groups. In response to aliskiren compared with placebo, blood pressure was reduced as well as plasma renin activity (from 2.4 ± 3.8 (mean $\pm$ SD) to 0.5 ± 0.4 μ g/l/h, $p < 0.001$). There was a significant interaction (aliskiren $\times$ visit) for MCR ($p = 0.003$) and E/I ratio ($p = 0.003$) indicating improvement in MCR and E/I ratio for those on aliskiren. MCR means, baseline vs. follow-up, were 41.8 ± 19.7 vs. 50.8 ± 26.1 (aliskiren) and 38.2 ± 23.6 vs. 37.5 ± 24.1 (placebo).

Conclusions—Parasympathetic function (i.e., MCR and E/I ratio) was enhanced by down-regulation of the RAAS.

Keywords

Cardiovascular autonomic function; renin-angiotensin-aldosterone system; renin inhibition; parasympathetic function

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Introduction

Cardiovascular autonomic neuropathy (CAN), impairment of autonomic control of the cardiovascular system, is prevalent in approximately 20% of individuals with diabetes but prevalence rates up to 65% have been reported with increased age and duration of diabetes [1]. The earliest indicator of cardiovascular autonomic dysfunction is a reduction in heart rate variability (HRV) [2]. Reduced HRV may reflect enhanced sympathetic and/or reduced parasympathetic modulation.

The autonomic nervous system (ANS) and the renin-angiotensin-aldosterone system (RAAS) play an integral role in the regulation of the cardiovascular system. Activity of the RAAS is controlled by renin [3], which is secreted by the kidney. Renin cleaves angiotensinogen to form angiotensin I with angiotensin-converting enzyme (ACE) converting angiotensin I to angiotensin II. Established physiological and pathophysiological effects of angiotensin II include vasoconstriction, increased blood pressure (BP), promotion of sympathetic outflow and stimulation of aldosterone synthesis.

The RAAS is activated, or dysregulated, by hyperglycemia in individuals with diabetes [4]. Increased activity of the RAAS both systemically and locally plays a role in diabetic complications (e.g., nephropathy, cardiovascular disease) with modulation of the RAAS potentially slowing the progression [5]. Experimental evidence has shown clear interactions between the RAAS and the sympathetic nervous system (SNS) [6, 7]. It is well known that the SNS can stimulate renin release [3] and stimulation of renin release increases the formation of angiotensin II [6]. Thus the SNS is a key factor in the concentration of circulating angiotensin II. Angiotensin II in turn exerts several actions on the SNS [6]. For example, angiotensin II acts at presynaptic sympathetic nerve endings facilitating sympathetic influences on the systemic circulation [8]. Conversely, angiotensin II, as well as BP and salt intake, regulates renin release by negative feedback-loops [3]. Thus, it appears that the RAAS and SNS do not operate independently but there is crosstalk between the two systems as they interact with each other in regulatory functions [8]. To date most of the evidence of an association of the RAAS and ANS has been with the SNS but interactions of the RAAS and parasympathetic nervous system (PNS) are also possible. A previous study in patients with heart failure suggested an interaction between the RAAS and PNS as they demonstrated a negative association between baroreflex sensitivity and plasma renin activity (PRA) [9].

Given that there is an interaction between the RAAS and ANS and increased activity of the RAAS may contribute to ANS dysfunction, interventions to ameliorate autonomic dysfunction using ACE-inhibitors and angiotensin receptor blockers (ARBs) have been utilized. Studies, however, have found conflicting results. Whereas 3 to 12 months use of quinapril showed some improvement in autonomic function [10-12], no improvement was shown after 12 months of treatment with trandolapril [13]. Conflicting results using ARBs have also been reported [12, 14]. One explanation for inconsistent results could be due to incomplete blockade of the RAAS. Aliskiren, a direct renin inhibitor, is the first in this class of antihypertensives. Aliskiren interferes with the enzymatic activity of renin by blockage of the catalytic site of the molecule [15]. This upstream blockade [15] produces down-regulation of the RAAS. In the present study, we hypothesized that blocking the RAAS at the first point of the pathway may offer an advantage over other RAAS inhibitors by inhibiting angiotensin II induced facilitation of sympathetic neurotransmission with subsequent improvement in parasympathetic function. Thus, we investigated the effect of direct renin inhibition on cardiovascular autonomic function in individuals with diabetes. The pathogenesis of diabetic neuropathy is complex. Vascular dysfunction driven by metabolic changes [16] along with other biochemical perturbations as a result of

hyperglycemia (e.g., oxidative and nitrosative stress [17, 18], activation of the polyol pathway [19], advanced glycation end products, elevated inflammatory markers [20]) may contribute to the dysfunction of nerves. Therefore, we also explored the effect of direct renin inhibition on biochemical perturbations that may be associated with autonomic dysfunction.

Materials and Methods

Study design and subjects

This study was a randomized, double-blind, placebo-controlled trial using aliskiren or placebo ([Clinicaltrials.gov: NCT00935064](https://clinicaltrials.gov/ct2/show/study/NCT00935064)). Aliskiren 300 mg or placebo were administered once daily for 6 weeks. This study had approval of the Institutional Review Board of Christiana Care Corporation and each participant gave written informed consent before participating in the study.

Participants were eligible if they were ≥ 18 years old with type 1 or type 2 diabetes mellitus. Other inclusion criteria included: (a) untreated patients with prehypertension or stage 1 hypertension as defined by the Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC 7) [21]; and (b) patients who were currently being treated with any antihypertensive medication other than aliskiren and whose systolic BP (SBP) was 115-159 mmHg and diastolic BP (DBP) was 60-99 mmHg at screening. Exclusion criteria included: (a) use of the maximum dose of an ACE-inhibitor or an ARB; (b) history of a myocardial infarction, percutaneous coronary interventions, coronary artery bypass graft surgery, acute coronary syndromes, recent/on going atrial fibrillation, frequent atrial arrhythmias, frequent ventricular arrhythmias, or acute myocardial ischemia; (c) dose changes 2 months prior to enrollment or during the course of the study for antihypertensive and antidiabetes medications and medications that may affect the ANS; (d) pregnant or lactating females; (e) impaired renal function (i.e., creatinine >1.5 mg/dl), a history of dialysis, nephrotic syndrome or renovascular hypertension; and (f) potassium levels at screening that were within 0.5 mmol/l of the upper limit of normal.

Cardiovascular autonomic function tests

Autonomic function was performed after an overnight fast at baseline and follow-up. Participants were asked to refrain from taking any prescribed or nonprescription medications, to avoid consuming tobacco products, caffeine-containing or alcoholic beverages, and to refrain from engaging in any vigorous exercise 8-10 hours before testing.

There are several different assessment modalities used for the determination of cardiovascular autonomic function (i.e., HRV). Two widely used clinical methods for assessing HRV include: RR-variation during deep breathing and the Valsalva maneuver [1]. In this study, RR-variation during deep breathing and the Valsalva maneuver were assessed using the ANS2000 ECG Monitor and Respiration Pacer (DE Hokanson, Inc., Bellevue, WA, USA). These methods have been previously described [22]. Briefly, RR-variation during deep breathing, performed for six minutes, was measured by vector analysis (i.e., mean circular resultant (MCR)) and by the expiration/inspiration (E/I) ratio of the first six breath cycles. Using age normative values [23], we considered an individual to have an abnormal MCR if their average MCR was greater than 1 SD below the mean. An E/I ratio < 1.10 was considered abnormal [24]. Heart rate response to the Valsalva maneuver was determined by having participants expire into the mouthpiece of a manometer, maintaining a pressure of 40 mmHg for 15 seconds. The results for the Valsalva maneuver were incomplete for 2 participants.

Heart rate variability was also assessed by power spectral analysis with and without respiratory analysis (ANX 3.0, ANSAR Medical Technologies, Inc., Philadelphia, PA, USA). Details of these analyses were previously published [22]. Briefly, for power spectral analysis without respiratory analysis, high frequency (HF) power (marker of parasympathetic function) was computed as the area of the frequency spectrum in the range of 0.15-0.40 Hz while low frequency (LF) power (potentially influenced by both sympathetic and parasympathetic activity [25]) was computed as the area of the frequency spectrum in the frequency range of 0.04-0.15 Hz. The HF and LF components were also calculated in normalized units (nu) (i.e., normalized with respect to the total spectral power). It has been suggested that the LF/HF ratio (which is independent of normalization) may mirror sympathovagal balance or reflect sympathetic modulations [25]. Spectral analysis of HRV was also combined with spectral analysis of respiratory activity. Respiratory analysis identified vagal activity that produced respiratory sinus arrhythmia (i.e., respiration frequency area [RFA]). The RFA is found by locating the peak mode of the respiratory spectrum (i.e., fundamental respiratory frequency (FRF)). The FRF is transferred to the heart rate spectrum and the RFA is computed as the area under the heart rate spectral curve over a frequency range centered on the FRF. The remaining area under the curve in the LF range is then computed as the low frequency area (LFA). The LFA/RFA ratio was also examined.

Clinical measurements

Weight and height were measured using a stadiometer. Body mass index (BMI) was calculated as body weight divided by height squared (kg/m^2). Blood pressure was monitored electronically in the supine posture using an oscillometric automatic recorder. The average of four BP readings was used for analysis.

RAAS components and other biochemical markers

With the exception of HbA_{1c} , RAAS components and other blood and urine analytes were measured at baseline and at the end of the treatment period with blood samples being immediately centrifuged at 4°C and stored at -70°C until analysis. HbA_{1c} was measured by high performance liquid chromatography (HPLC) using a Tosoh G7 automated HPLC analyzer (Tosoh Bioscience, Inc., South San Francisco, CA, USA). Fructosamine levels were determined by a colorimetric rate reaction using a Roche Cobas c501 chemistry analyzer (Roche Diagnostics Corporation, Indianapolis, IN, USA). Urine samples were assayed for 8-iso-prostaglandin $\text{F}_{2\alpha}$ by an enzyme linked immunosorbent assay (ELISA) kit (Oxford Biomedical Research, Rochester Hills, MI, USA).

High sensitivity PRA was measured by a quantitative radioimmunoassay (RIA) kit (DiaSorin, Stillwater, MN, USA) at the Clinical Reference Laboratory, Lenexa, KS, USA. Briefly, the assay is based on measuring the amount of angiotensin I generated by endogenous renin after timed incubation at 37°C . By increasing the generation time of angiotensin I from 1.5 hours to 18 hours, lower levels of PRA (below the assay's lower limit of quantitation) can be determined. For this study, all incubated fractions of the samples were assayed at both 1.5 and 18 hours. The value reported was dependent upon the angiotensin I amount generated in the sample. If the amount of angiotensin I in the sample at 1.5 hours was $0.5 \mu\text{g/l/hr}$ the 1.5 hour fraction was used to calculate the $\mu\text{g/l/h}$ concentration of PRA. If the 1.5 hour fraction was $<0.5 \mu\text{g/l/hr}$ then the 18 hour fraction was used in the calculation. The lower limit of reporting for PRA was $0.03 \mu\text{g/l/h}$. Serum aldosterone and plasma angiotensin II were measured by RIA at the Hypertension Core Laboratory, Wake Forest University, Winston-Salem, NC, USA. Total adiponectin and leptin levels were measured by RIA (Millipore Corporation, Billerica, MA, USA). High molecular weight adiponectin, nitrotyrosine, and interleukin-6 (IL-6) were measured by ELISA kits (Millipore Corporation, Billerica, MA, ALPCO Diagnostics, Salem, NH, and

R&D Systems, Minneapolis, MN, USA, respectively). Samples for leptin, adiponectin, nitrotyrosine, and IL-6 were analyzed at the Nemours Biomedical Research & Analysis Laboratory, Jacksonville, FL, USA. For data presented in conventional units, the following SI conversion factors may be used: to convert angiotensin II values to pmol/l, multiply by 0.957; and to convert aldosterone to nmol/l, multiply by 0.0277.

Statistical analyses

Comparisons of demographic and medication data between aliskiren and placebo groups were made with unpaired t-tests for continuous data and contingency table (chi-square) analysis for categorical variables. Change in cardiovascular autonomic function measurements, BP, and blood analytes were analyzed by a mixed effects model for repeated measures data. The interaction of intervention group and visit (before and after intervention) was included in all of the models to test whether the degree of change was the same for both groups. A natural logarithmic transformation was applied to highly skewed measurements for analysis.

Results

Demographic information with regard to the study cohort (Table 1) indicates that at baseline there were no statistical differences with regard to age, duration of diabetes, gender, BMI, glycemic control, type of diabetes, or antihypertensive medications between the two groups randomized to aliskiren or placebo. It should be noted that no participants used a combination of an ACE-inhibitor and an ARB. Approximately 23% of the study cohort had an abnormal MCR and/or E/I ratio. Table 2 presents the observed mean $\pm$ SD for both groups at baseline and follow-up for BP, weight, and blood analytes. As expected those on aliskiren had a significantly greater reduction in SBP, DBP, and PRA than placebo. There were, however, no other significant differences between baseline and follow-up for angiotensin II, aldosterone, leptin, adiponectin levels, IL-6, nitrotyrosine, or 8-iso-prostaglandin F_{2 α} for either group (Table 2).

Table 3 presents the mean values for measures of cardiovascular autonomic function. Improvement in the MCR and E/I ratio was shown for those taking aliskiren, but not for placebo, while the difference in improvement in the Valsalva ratio was borderline statistically significant. There were no significant changes seen for any of the measures of power spectral analysis in either group (Table 3). One individual in the placebo group appeared to be an outlier with regard to some measures of power spectral analysis. Statistical analyses for these parameters were reevaluated excluding this individual with no effect on the conclusion of the results.

Discussion

The results of the present study show that reduced activity of the RAAS via direct inhibition of renin had a favorable affect on parasympathetic function as measured by two cardiovascular autonomic reflex tests (i.e., MCR, E/I ratio). The pathogenic process leading to the development of diabetic neuropathy is complex. Experimental data provides evidence of vascular dysfunction (e.g., reduced endoneurial blood flow), driven by metabolic changes that are involved in the pathogenesis [16]. Thus, other risk factors besides glycemic control are likely involved. Previous studies have shown an association between hypertension and diabetic neuropathy [26-28]. The underlying mechanism, however, by which hypertension leads to nerve dysfunction is not clear. Potential mechanisms may include: hypertension reflecting a direct BP load or hemodynamic stress [26], hypertension potentiating the adverse effects of hyperglycemia, or the upregulation of the RAAS in the vasculature leading to vasoconstriction. As expected in this study where aliskiren catalyzed the first and

rate-limiting step of the RAAS, there was a significant decrease in PRA accompanied by a reduction in SBP and DBP, with no adverse events as a result of lower BP. Previous studies in animal models have shown that the overexpression of renin was associated with impaired vagal function [29]. Thus, it is possible that the improvement in parasympathetic function in the present study is a direct result of decreased PRA or indirectly associated with vasodilation or reduction in BP (e.g., improved nerve blood flow or decreased endoneurial hypoxia). We do not believe the improvement in vagal function was the result of improved glycemic control as fructosamine levels were not significantly different from baseline to follow-up.

Obesity increases the risk of hypertension. The SNS may play a role in obesity-related hypertension with increased sympathetic activity contributing to the elevation of BP and stimulation of the RAAS [30]. Components of the RAAS are expressed on adipocytes [5]. The adipocyte-derived peptide leptin increases activity of the SNS and elevates BP [30]. Moreover angiotensinogen, the precursor of angiotensin II, is positively correlated with leptin in normotensive, nonobese men [31]. Animal studies showed that aliskiren limited a gain in adiposity that was not due to caloric intake but may have been related to down-regulation of the RAAS accompanied by a reduction in leptin levels [32]. In the present study, the average BMI was obese for both groups. There was, however, no reduction in weight or leptin levels shown in either treatment group. Another adipocyte-derived protein potentially regulated by the SNS is adiponectin. In a group of individuals with type 2 diabetes, sympathovagal balance (i.e., 24-hour LF/HF ratio) favoring sympathetic activation was shown to be associated with low levels of adiponectin [33]. We examined both total and high molecular weight adiponectin levels and found no change in either group.

Additional biochemical perturbations as a result of hyperglycemia may contribute to the dysfunction of nerves. Previous investigations have suggested oxidative and nitrosative stress from free radicals [17, 18], activation of the polyol pathway [19], advanced glycation end products, elevated markers of inflammation (e.g., IL-6) [20], and decreased nerve growth factor may be involved. During metabolic activity when cells do not detoxify free radicals, oxidative stress can develop [17]. Both acute and chronic hyperglycemia can cause oxidative stress in nervous tissue. Angiotensin II also causes pro-oxidative effects on the vascular endothelium and can induce intraendothelial peroxynitrite formation [18]. Previous investigations, measuring serum antioxidant capacity, have shown that alpha-lipoic acid was effective in treating some forms of diabetic autonomic neuropathy [34]. We evaluated whether direct renin inhibition reduced oxidative stress (i.e., serum nitrotyrosine levels (an indirect marker of peroxynitrite) and urinary 8-iso-prostaglandin $F_{2\alpha}$) but found no effect in this study.

The expression of inflammatory cytokines (e.g., IL-6) is present in the nervous system, with sympathetic activation known to be pro-inflammatory. Previous cross-sectional studies have shown reduced HRV with increased levels of markers of inflammation [35, 36]. However, given the cross-sectional nature of these studies, it could not be determined whether autonomic dysfunction mediated the inflammatory process or if autonomic dysfunction was a result of inflammation. It should also be noted that IL-6 is a neurokine playing a role in neural development and has neurotrophic activity. In the present study of 6 weeks duration, there was no difference in IL-6 levels in either group during the follow-up period.

The expected hormone reduction in angiotensin II was not observed in this study. These results are puzzling. A previous study in 18 healthy volunteers showed a significant suppression of angiotensin II for up to 6 hours with 640 mg (double the amount used in this study) of aliskiren [37]. At 10 and 24 hours post dose, angiotensin II levels had risen and were no longer statistically different from placebo [37]. It is also known that PRA is not

completely suppressed by aliskiren therapy [38]. Therefore it is possible that active renin can still generate angiotensin I [38] and be converted to angiotensin II. Furthermore, there are alternative pathways for angiotensin II generation by non-ACE synthetic pathways (e.g., serine proteases such as chymase, tissue plasminogen activator) [39]. These alternative pathways contribute to blood angiotensin II levels. We also failed to show a reduction in aldosterone levels. Similarly other investigators using 150 mg of aliskiren failed to show a reduction in aldosterone after 6 weeks of aliskiren therapy [40].

No changes in autonomic function measured via power spectral analysis were found in this study. Given that there was improvement in RR-variation with deep breathing as assessed by the MCR and E/I ratio (i.e., markers of parasympathetic function), we expected power spectral measures of vagal function (e.g., HF) to also reflect improvement. It should be noted, however, that the measures of vagal function via power spectral analysis relied on spontaneous heart rate rhythms with normal breathing while the MCR and E/I ratio assessments were performed with deep breathing. Other studies have also shown discordant results between power spectral analysis and other measures of autonomic function [36].

There are potential limitations to our study. First, despite the fact that there were no statistical differences between the two randomized groups, there were fewer females in the placebo group and this group was slightly older. Second, individuals were on concomitant medications (e.g., glucose-lowering agents, other antihypertensive medications) which may have masked changes. Nonetheless other than the need of an antibiotic during the 6 week study, participants did not have medication changes two months prior to enrolling in the study nor during the course of the study. Third, the length of the study was 6 weeks and long-term follow-up data was not obtained. It is possible that additional measures of ANS function and/or other biochemical parameters may have shown changes with further down-regulation of the RAAS.

In summary, parasympathetic function as measured by cardiovascular autonomic reflex tests was enhanced by down-regulation of the RAAS seen in this study as a reduction in PRA. We have demonstrated a previously undescribed association between the ANS and the RAAS that involves the PNS for individuals with diabetes. Prior studies have demonstrated interactions between the RAAS and ANS that were mediated through the SNS. Given the complexity of the RAAS and ANS, it seems likely that there are further undefined interactions. There are several clinically significant manifestations of CAN (e.g., exercise intolerance, painless myocardial ischemia, increased risk of mortality) [2]. Thus, it is important to search for ways in which to improve autonomic function. Whether improvement in autonomic function results in reduced incidence and/or progression of clinically significant sequelae requires further study.

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Table 1

Participant demographics (n=60)

	Aliskiren (n=30)	Placebo (n=30)	p-value
Age (year)	49±12	53±12	0.183
Duration of diabetes (year)	16±13	17±11	0.745
Diabetes type (n): type 1/ type 2	15/15	16/14	0.796
Gender (n): males/females	14/16	19/11	0.194
Body mass index (kg/m ²)	31.7±6.3	30.9±6.7	0.643
HbA _{1c} (%)	7.6±1.3	7.6±0.9	0.788
HbA _{1c} (mmol/mol)	60±14	59±10	0.788
Antihypertensive medications			
ACE-inhibitors (Yes/No)	17/13	17/13	1.000
ARBs (Yes/No)	2/28	4/26	0.389
Diuretics (Yes/No)	2/28	6/24	0.129
Calcium-channel blockers (Yes/No)	2/28	1/29	1.000
Beta blockers (Yes/No)	6/24	1/29	0.103
Abnormal MCR and/or E/I ratio (Yes/No)	7/23	7/23	1.000

Mean±SD

ACE-inhibitors angiotensin-converting enzyme inhibitors*ARBs* angiotensin receptor blockers*MCR* mean circular resultant*E/I* expiration/inspiration ratio

Table 2

Blood pressure, weight and blood analytes before and after treatment

	Aliskiren (n=30)*	Placebo (n=30)*	** p-value
Systolic blood pressure (mmHg)			
Baseline	121.0±11.3	124.1±13.8	
Follow-up	112.1±12.1	121.5±15.2	0.048
Diastolic blood pressure (mmHg)			
Baseline	66.1±7.0	68.2±8.3	
Follow-up	61.5±6.5	66.7±9.3	0.050
Weight (kg)			
Baseline	91.4±17.0	92.3±24.4	
Follow-up	90.3±17.3 (n=29)	92.0±24.0	0.686
Fructosamine (mcmol/l)			
Baseline	297.2±67.1	292.0±45.9	
Follow-up	300.5±74.5	290.4±48.5	0.469
Renin (µg/l/h)			
Baseline	2.4±3.8	3.1±6.0	
Follow-up	0.5±0.4	2.6±4.2	<0.001
Angiotensin II (pg/ml)			
Baseline	30.5±13.3	30.5±12.3	
Follow-up	32.0±15.8	32.2±11.5	0.674
Aldosterone (ng/dl)			
Baseline	5.6±4.2	5.7±4.0	
Follow-up	5.6±4.7	4.9±3.0	0.808
Leptin (µg/l)			
Baseline	16.7±11.3 (n=29)	11.1±6.6	
Follow-up	17.3±12.3	12.0±7.2	0.328
Adiponectin (mg/l)			
Baseline	12.7±9.2	14.1±12.7	
Follow-up	12.3±9.2	13.6±11.4	0.384
High molecular weight adiponectin (mg/l)			
Baseline	5.5±4.7	7.3±9.4	
Follow-up	5.9±5.3	7.3±8.8	0.814
Interleukin-6 (pg/ml)			
Baseline	2.2±1.3	1.8±1.4	
Follow-up	2.3±1.7	1.9±1.5	0.577
Nitrotyrosine (nmol/l)			
Baseline	1077.0±4024.9 (n=28)	746.8±1531.1 (n=29)	
Follow-up	1121.0±3207.4	790.4±1791.0	0.095
Urinary 8-iso-prostaglandin F _{2α} (ng/mg creatinine)			
Baseline	11.1±10.8	9.6±8.4	

	Aliskiren (n=30)*	Placebo (n=30)*	**p-value
Follow-up	10.1±12.7	7.8±8.8	0.982

Mean±SD

* sample size = 30 unless otherwise noted

** p-value is that of the interaction term: difference in change from baseline to follow-up between aliskiren and placebo.

Table 3

Measures of cardiovascular autonomic function before and after treatment

	Aliskiren (n=30)*	Placebo (n=30)*	** p-value
<i>Cardiovascular Autonomic Reflex Tests</i>			
Mean circular resultant			
Baseline	41.8±19.7	38.2±23.6	
Follow-up	50.8±26.1	37.5±24.1	0.003
Expiration/inspiration ratio			
Baseline	1.22±0.12	1.21±0.14	
Follow-up	1.28±0.15	1.20±0.14	0.003
Valsalva ratio			
Baseline	1.64±0.29	1.62±0.30 (n=29)	
Follow-up	1.72±0.28	1.62±0.30 (n=28)	0.057
<i>Power Spectral Analysis</i>			
Low frequency (ms ²)			
Baseline	338±358	674±1889	
Follow-up	332±383	542±1309	0.319
High frequency (ms ²)			
Baseline	351±622	886±3931	
Follow-up	326±679	753±3308	0.149
Low frequency/high frequency ratio			
Baseline	3.0±4.5	2.0±1.7	
Follow-up	3.6±4.6	2.6±2.1	0.649
Low frequency (nu)			
Baseline	54±22	56±17	
Follow-up	61±23	61±16	0.723
High frequency (nu)			
Baseline	46±22	44±17	
Follow-up	40±23	39±16	0.723
Total spectral power (ms ²)			
Baseline	701±902	1586±5764	
Follow-up	666±907	1310±4570	0.206
<i>Power Spectral Analysis Coupled with Analysis of Respiratory Activity</i>			
Respiration frequency area [RFA] (bpm ²)			
Baseline	2.1±2.8	3.3±12.7	
Follow-up	1.9±2.4	1.8±4.7	0.314
Low frequency area [LFA] (bpm ²)			
Baseline	1.2±1.2	1.8±4.7	
Follow-up	1.6±2.1	1.4±1.7	0.405
LFA/RFA ratio			
Baseline	1.7±3.5	1.6±1.4	

	Aliskiren (n=30)*	Placebo (n=30)*	** p-value
Follow-up	2.0±2.5	2.0±1.8	0.984

Mean±SD

* sample size = 30 unless otherwise noted

**
p-value is that of the interaction term: difference in change from baseline to follow-up between aliskiren and placebo.