

Research Article

Systemic and tissue-specific effects of aliskiren on the RAAS and carbohydrate/lipid metabolism in obese patients with hypertension

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Abstract

Aliskiren penetrates adipose and skeletal muscle in hypertensive patients with abdominal obesity and reduces renin–angiotensin–aldosterone system activity. After discontinuation, blood pressure–lowering effects are observed possibly through drug–tissue binding. We performed microdialysis evaluation of adipose tissue and skeletal muscle before and during an insulin-modified frequently sampled intravenous glucose tolerance test (IM-FSIGT). Aliskiren 300 mg (n = 8) or amlodipine 5 mg (n = 8) once daily were administered during a 12-week randomized treatment period. Aliskiren elicited variable changes in median interstitial angiotensin II (Ang II) in adipose (2.60–1.30 fmol/mL) and skeletal muscle (2.23–0.68 fmol/mL); amlodipine tended to increase adipose and skeletal muscle Ang II ($P = .066$ for skeletal muscle treatment difference). Glucose/insulin increased median plasma Ang II 1 hour after glucose injection (1.04–2.50 fmol/mL; $P = .001$), which was markedly attenuated by aliskiren but not amlodipine. Aliskiren increased glucose disposition index ($P = .012$) and tended to increase acute insulin response to glucose ($P = .067$). Fasting adipose glycerol (–17%; $P = .064$) and fasting muscle glucose dialysate (–17%; $P = .025$) were decreased by aliskiren but not amlodipine. In summary, aliskiren decreased Ang II production in response to glucose/insulin stimulus and elicited metabolic effects in adipose and skeletal muscle suggestive of increased whole-body glucose utilization. *J Am Soc Hypertens* 2017;■(■):1–10. © 2017 American Society of Hypertension. All rights reserved.

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Introduction

All components of the renin–angiotensin–aldosterone system (RAAS) are expressed in human adipose tissue, with upregulation of genes encoding renin, angiotensin-converting enzyme, and AT1 receptors in obese patients with hypertension compared with lean or obese normotensive patients.¹ Weight loss decreased overexpression of these genes in adipose tissue and reduced circulating concentrations of these RAAS components.² Preclinical data suggest that the adipose tissue RAAS may control local renin concentrations independently of plasma concentrations.³ Adipose-generated angiotensin II (Ang II) may contribute to obesity-related hypertension and metabolic abnormalities. Of note, interstitial Ang II modulated adipose and skeletal muscle carbohydrate and lipid metabolism in a tissue-specific fashion in healthy subjects.⁴

We previously showed that the direct renin inhibitor, aliskiren, was distributed to adipose and skeletal muscle tissue at concentrations sufficient to reduce tissue RAAS activity in hypertensive patients with abdominal obesity.⁵ Here, we report part 2 of this study, which further explored systemic and tissue-specific effects of the RAAS in regulating lipid and carbohydrate metabolism in patients with hypertension and abdominal obesity. The main objectives were to: (1) assess the effects of aliskiren on Ang II concentrations in interstitial fluid of subcutaneous adipose tissue and skeletal muscle and on RAAS biomarkers in the plasma and (2) compare the effects of aliskiren and amlodipine on lipid and carbohydrate metabolism in subcutaneous adipose tissue and skeletal muscle before and during an insulin-modified frequently sampled intravenous glucose tolerance test (IM-FSIGT). Amlodipine was chosen as a comparator^{5,6} to control for the blood pressure–lowering effects of aliskiren.

Methods

Patients

The study population included men and women 18–65 years of age with essential hypertension and abdominal obesity (waist circumference ≥ 102 cm in men, ≥ 88 cm in women, and body mass index between 30 and 36 kg/m²). Blood pressure criteria for study entry included: $\geq 135/85$ mm Hg and $< 160/100$ mm Hg at screening, predose, and baseline for newly diagnosed (untreated) hypertension and at baseline only for patients with a history of treated hypertension. Patients had to be nonsmokers or light smokers (urine cotinine concentration < 500 ng/mL). The study design was approved by the Institutional Review Board of Hannover Medical School and was conducted in accordance with Good Clinical Practice and in compliance with the Declaration of Helsinki and applicable European Regulations. The study was registered with clinicaltrials.gov (identifier NCT00498433). The experiments were

conducted with the understanding and the consent of each participant. All patients provided written informed consent.

Key exclusion criteria included current treatment with ≥ 3 antihypertensive drugs; type 1 or 2 diabetes mellitus; history of autonomic dysfunction in the recent past; any history of hypertensive encephalopathy or stroke; history of major cardiovascular events (myocardial infarction, unstable angina) during the past 6 months; and any history of acute or chronic bronchospastic disease. Women of child-bearing potential had to be using highly effective contraception; postmenopausal women could not be using hormone replacement therapy.

Study Design

This was a randomized, double-blind, parallel-group, single-center study conducted at the Institute of Clinical Pharmacology, Hannover Medical School, Germany. The study consisted of a screening period of up to 21 days, followed by a washout period of five elimination half-lives of the longest acting drug (1–2 weeks), a 2-week single-blind, placebo run-in period (period 1), and a 12-week double-blind, active treatment period (period 2). Active treatment consisted of 1:1 randomization to once daily aliskiren 300 mg and amlodipine placebo or once daily amlodipine 5 mg and aliskiren placebo. Study drug was administered between 8 AM and 10 AM each day, except on visit days when medication was taken after all visit procedures were completed.

Randomization numbers were assigned in ascending, sequential order and generated in a manner to ensure that treatment assignment was unbiased and concealed from patients and investigator staff. A randomization list was produced by Novartis Drug Supply Management using a validated system that automated the random assignment of treatment arms to randomization numbers in the specified ratio. Patients, investigator staff, persons performing the assessments, and data analysts were blinded to treatment; the identity of treatments was concealed by the use of study drugs that were identical in packaging, labeling, schedule of administration, appearance, taste, and odor. A double-dummy design was used because the identity of the study drug (aliskiren tablet) and active control (amlodipine capsule) could not be disguised due to their different forms.

Pharmacodynamic assessments were performed at baseline (end of placebo run-in period, day 14) and at the end of the active treatment period (day 98). Office sitting blood pressure was measured at all study visits.

Adipose Tissue and Skeletal Muscle Microdialysis

Two microdialysis catheters each (CMA 60, CMA Microdialysis AB, Solna, Sweden) were inserted into the subcutaneous adipose tissue (at the level of the umbilicus)

and into skeletal muscle (*Vastus lateralis* muscle), as described previously.⁷ The ethanol dilution technique was used to monitor changes in tissue perfusion.⁷ Dialysate concentrations of marker metabolites for carbohydrate metabolism (glucose, lactate, pyruvate) and lipid metabolism (glycerol) were measured using an automated analyzer (CMA/600, CMA Microdialysis AB) based on combined enzymatic/colorimetric methods. Dialysate samples for hemodynamic/metabolic monitoring were obtained every 15 minutes at a flow rate of 2.0 $\mu\text{L}/\text{min}$. In situ recovery for dialysate metabolites was assessed by near-equilibrium dialysis.⁸ Recoveries of glucose, lactate, and pyruvate were about 30% in adipose tissue and 50% in muscle. Recovery for glycerol was 30% in adipose tissue and 80% in muscle. Dialysate samples for Ang II concentrations were obtained via separate catheters at a flow rate of 0.3 $\mu\text{L}/\text{min}$ prior to and throughout the IM-FSIGT and pooled for each patient both at baseline and posttreatment to obtain sufficient sample volumes for detection of Ang II. The lower limit of quantification (LLQ) for the Ang II assay was 0.8 fmol/mL. In the aliskiren group, Ang II results from the skeletal muscle of two patients and the adipose tissue of one patient were excluded for technical reasons. In the amlodipine group, Ang II results from the skeletal muscle of two patients and the adipose tissue of three patients were excluded for technical reasons.

IM-FSIGT and Blood Samples

Fasting blood samples were obtained for analysis of plasma renin activity (PRA), plasma renin concentration (PRC), and serum aldosterone, as described previously.^{9,10} Fasting plasma samples for Ang II were obtained by transferring the drawn blood immediately into precooled tubes containing enzyme inhibitors followed by centrifugation. Plasma samples were aliquoted and frozen immediately at -70°C after processing. Samples were shipped on dry ice in batches to external laboratories (Erasmus Medical Centre for PRA and PRC; University Hospital of Lausanne for Ang II; Medizinisches Labor Hannover for aldosterone). The IM-FSIGT is a well-validated method for determining insulin sensitivity following intravenous bolus administration of glucose followed later by insulin and fitting the measured plasma glucose and insulin values using mathematical modeling (minimal model approach). The model accounts for the effect of glucose to enhance its own disappearance independent of changes in insulin and for insulin-mediated glucose uptake. A detailed description of this method can be found elsewhere.¹¹ All plasma samples were analyzed for glucose and insulin by standard methods in a certified laboratory.

The MINMOD Millennium Software¹² was used to derive measures of glucose homeostasis including: glucose effectiveness (Sg), acute insulin response to glucose (AIRg), insulin sensitivity index (SI), and glucose disposition index (DI). To assess effects of the glucose/insulin bolus on plasma Ang

II, samples were obtained during the baseline period of the IM-FSIGT and 1 hour after the start of the IM-FSIGT test. In addition, the homeostasis model assessment insulin resistance index (HOMA-IR) was calculated from fasting glucose and insulin according to the following formula: $(\text{insulin } [\mu\text{U}/\text{mL}] \times \text{glucose } [\text{mmol/L}]/22.5)$.¹³

Blood Pressure

Sitting blood pressure was measured by using a calibrated device and appropriate cuff size, in accordance with American Heart Association guidelines.¹⁴ The arm that recorded the highest blood pressure measurement at the screening visit was used for blood pressure measurement throughout the study. After sitting for 5 minutes, three sitting blood pressure measurements were obtained at 1- to 2-minute intervals, and the average of these values was taken as the mean blood pressure for that visit.

Statistics

The primary objective of this analysis was to compare the effects of aliskiren and amlodipine on Ang II in the interstitial fluid of subcutaneous fat and skeletal muscle and on RAAS biomarkers in plasma from hypertensive patients with abdominal obesity. Planned enrollment was 50 patients in order to have 40 patients complete the study resulting in a power of 80% in detecting significance between treatment groups. A total of 36 patients were enrolled before the study was terminated early, subsequently to early termination of the ALTITUDE study.¹⁵ The early termination of our study was not related to any safety concerns in the 16 randomized patients. Due to premature termination and high interpatient variability, the study was underpowered to detect statistically significant differences between treatments for the primary objective. Therefore, biomarker and pharmacodynamic analyses were evaluated based on numerical observations of group summary statistics, and trends were examined for statistical significance. A mixed-effects linear model with treatment, visit, and the two-way interaction of treatment \times visit as fixed effects and subject as random effect was performed on raw microdialysis data for each tissue (adipose tissue or skeletal muscle), plasma RAAS biomarkers, and IM-FSIGT data including HOMA-IR; appropriate contrasts were used to estimate day 98 versus day 14 differences for aliskiren and amlodipine treatments and between treatment groups for each tissue. Changes in dialysate metabolites in response to IM-FSIGT were analyzed per time point with a mixed-effects linear model with treatment, visit, and the two-way interaction of treatment \times visit as fixed effects and subject as random effect for each tissue (adipose tissue or skeletal muscle) and time point; appropriate contrasts were used to estimate day 98 versus day 14 differences for aliskiren and amlodipine treatments and between

treatment groups for each tissue and time point. Due to the high variability and nonnormal distribution in plasma and dialysate Ang II values, nonparametric analyses were performed using the Wilcoxon signed rank test and Wilcoxon 2-sample test to determine treatment effects and differences. For cases in which biomarker data were below the LLQ, a value of half the LLQ was used when calculating summary statistics or analyzing the data. The significance level was set at $<.05$ for all analyses.

Results

Patients

At the end of the placebo run-in (period 1), 15 patients were excluded for abnormal test procedures (Figure 1). Specifically, five discontinued due to blood pressure measurements below the inclusion criterion, nine discontinued due to blood pressure measurements above the inclusion criterion, and one discontinued due to a body mass index below the inclusion criterion. A total of 16 patients (8 per treatment group) were randomized according to the protocol (Figure 1). Baseline demographics were similar across the aliskiren and amlodipine treatment groups. Mean age was 47.9 years (range: 26–64), all participants were white, 69.4% were men, and mean body mass index was 32.7 kg/m^2 .

Office Blood Pressure

Mean blood pressure was reduced from $145.6/93.0 \text{ mm Hg}$ at baseline to $134.3/84.5 \text{ mm Hg}$ following aliskiren treatment and from $145.0/93.9 \text{ mm Hg}$ to $138.5/90.8 \text{ mm Hg}$ following amlodipine treatment. We observed no treatment differences.

Plasma RAAS Biomarker Concentrations

Mean PRA was reduced ($P = .001$) and mean aldosterone concentrations tended to be reduced ($P = .097$) following treatment with aliskiren (Figure 2). In contrast,

both biomarkers were increased following treatment with amlodipine ($P = .004$ and $P = .016$, respectively; Figure 2). Mean PRC increased following treatment with aliskiren ($P < .001$) but not with amlodipine ($P = .175$). All treatment differences were significant ($P \leq .007$). Analysis of median values showed a similar pattern of change in PRA, PRC, and aldosterone (data not shown).

Aliskiren treatment tended to reduce fasting plasma Ang II concentrations (from 0.86 to 0.26 fmol/mL ; $P = .313$), whereas amlodipine increased fasting plasma Ang II levels (1.50 – 3.65 fmol/mL ; $P = .039$). Treatment differences were significant ($P = .015$). The glucose/insulin intervention significantly increased median plasma Ang II concentrations at baseline in the aliskiren group (0.86 – 2.65 fmol/mL ; $P = .008$) and tended to increase median plasma Ang II concentrations at baseline in the amlodipine group (1.50 – 2.40 fmol/mL ; $P = .102$; Figure 3A). Aliskiren treatment attenuated the glucose/insulin-stimulated increase in median plasma Ang II concentrations (0.26 fmol/mL before and 0.29 fmol/mL 1 hour after IM-FSIGT; $P = .109$) compared to baseline. Amlodipine treatment in contrast had no effect on postglucose/insulin plasma Ang II concentrations.

Adipose and Skeletal Muscle Interstitial Ang II Concentrations

Baseline interstitial Ang II concentrations ranged from 0.44 to 20.00 fmol/mL in adipose tissue and 0.44 to 14.00 fmol/mL in skeletal muscle. Individual patient responses to aliskiren and amlodipine treatment were highly variable in both the direction and magnitude of responses in either tissue (Figure 3B, 3C). Changes observed for Ang II concentrations with aliskiren in adipose tissue (2.60 – 1.30 fmol/mL ; $P = 1.0$) and skeletal muscle (2.23 – 0.68 fmol/mL ; $P = .219$) were not significant. Changes observed for Ang II concentrations with amlodipine in adipose tissue (0.44 – 4.60 fmol/mL ; $P = .06$) and skeletal muscle (0.80 – 3.95 fmol/mL ; $P = .313$) were also not significant. No treatment effects were found in adipose tissue ($P = .27$; Wilcoxon sign rank test; $P = .40$, linear mixed-effect model) or skeletal muscle ($P = .065$; Wilcoxon sign rank test; $P = .066$, linear mixed-effect model).

HOMA-IR and Glucose Homeostasis During IM-FSIGT

HOMA-IR was calculated from fasting insulin and glucose concentrations. Neither aliskiren (day 14: 2.08 ± 1.19 ; day 98: 1.94 ± 0.91 ; $P = .444$) nor amlodipine (day 14: 1.61 ± 0.86 ; day 98: 1.80 ± 1.02 ; $P = .301$) had an influence on HOMA-IR, and no treatment effect was observed ($P = .209$). With the injection of glucose and insulin, several indices of glucose handling and insulin activity can be calculated. Aliskiren treatment increased mean glucose DI from 845.5 at baseline to 1183.2 ($P = .012$)

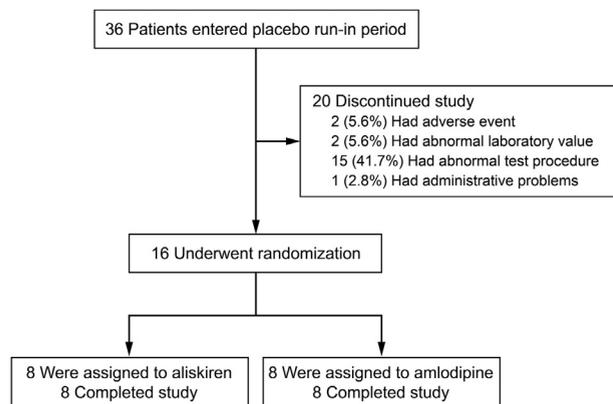


Figure 1. Patient disposition.

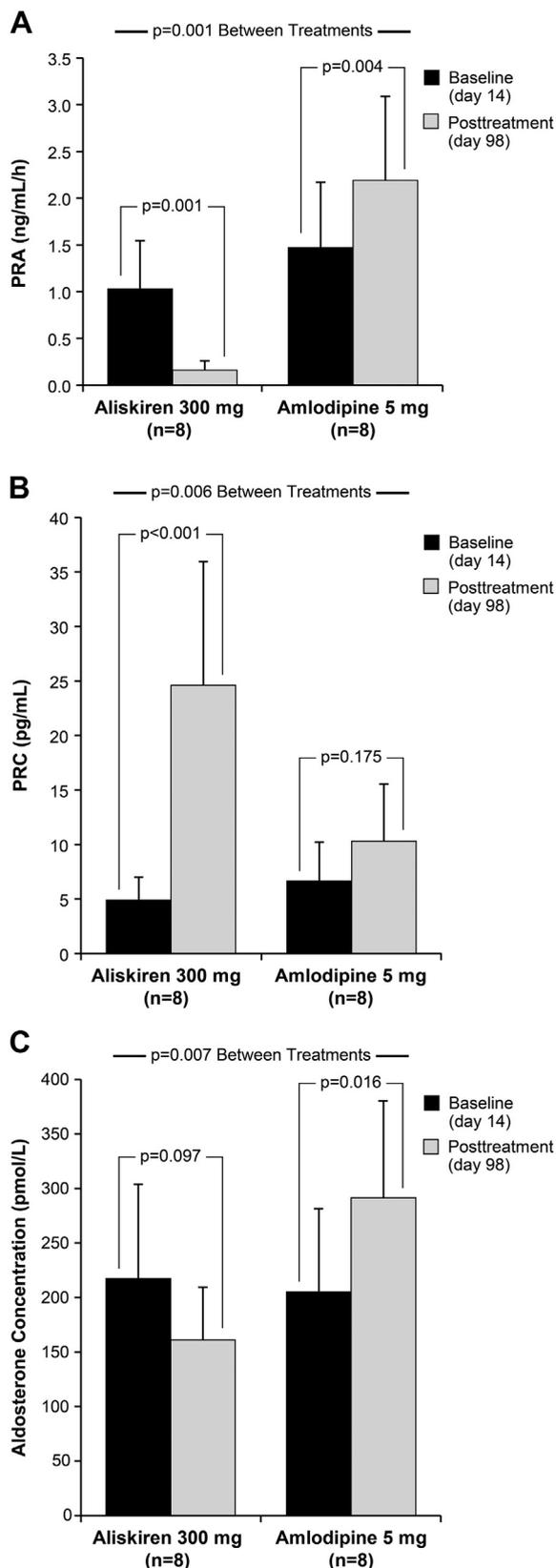


Figure 2. Effects of aliskiren and amlodipine treatment on (A) plasma renin activity (PRA), (B) plasma renin concentration (PRC), and (C) aldosterone concentration. Values are means

and tended to increase the mean AIRg from 898.6 to 1128.6 ($P = .067$). Amlodipine in contrast had no effect on mean DI (510.0–513.4, $P = .977$) or mean AIRg (349.5–289.9, $P = .615$). Treatment differences were not observed ($P = .062$ for DI, $P = .099$ for AIRg). There were no significant treatment effects or differences for SI or Sg.

Tissue Perfusion and Metabolism in the Fasting State

Fasting values for the ethanol ratio were lower in skeletal muscle (range: 0.1–0.4) compared with adipose tissue (range: 0.22–0.80) demonstrating larger resting tissue perfusion in muscle. In adipose tissue, mean ethanol ratio was slightly changed from 0.55 at baseline to 0.43 following treatment with aliskiren ($P = .162$), whereas no change was observed following treatment with amlodipine (0.51 at both time points, $P = .887$; $P = .365$ for treatment comparison). In skeletal muscle, ethanol ratio values were not affected by either treatment. Figure 4A, 4B summarize the relevant microdialysis data in adipose tissue and skeletal muscle.

Fasting dialysate glucose, lactate, and pyruvate concentrations were higher in skeletal muscle (range: 1.1–2.9 mmol/L, 0.6–2.8 mmol/L, and 11.2–107.8 $\mu\text{mol/L}$, respectively) compared with adipose tissue (range: 0.4–2.1 mmol/L, 0.2–1.4 mmol/L, and 7.3–53.5 $\mu\text{mol/L}$, respectively). Fasting dialysate glycerol concentrations were higher in adipose tissue (range: 58.2–203.1 $\mu\text{mol/L}$) compared with skeletal muscle (range: 20.4–167.7 $\mu\text{mol/L}$). Aliskiren treatment tended to reduce mean interstitial glycerol concentrations in adipose tissue (126.4–105.4 $\mu\text{mol/L}$; $P = .064$; Figure 4A), whereas amlodipine treatment did not (100.4–98.4 $\mu\text{mol/L}$; $P = .780$). In skeletal muscle, amlodipine treatment reduced mean dialysate glycerol concentrations (66.1–49.7 $\mu\text{mol/L}$; $P = .032$), whereas aliskiren treatment did not (52.2–52.0 $\mu\text{mol/L}$; $P = .957$; data not shown).

Mean dialysate glucose concentrations in skeletal muscle (Figure 4B) was reduced from 1.8 mmol/L at baseline to 1.5 mmol/L following treatment with aliskiren ($P = .025$), whereas it was unchanged following treatment with amlodipine (2.1–2.0 mmol/L; $P = .356$; $P = .312$ for treatment comparison).

Tissue-Specific Metabolic Effects of Glucose/Insulin Bolus During IM-FSIGT

In adipose (Figure 4A) and skeletal muscle tissue (Figure 4B), the time course plots for mean glucose concentrations before and during IM-FSIGT on day 14 were

and standard deviations. Mixed-effects linear model with treatment, visit, and the two-way interaction of treatment \times visit (fixed effects) and subject (random effect).

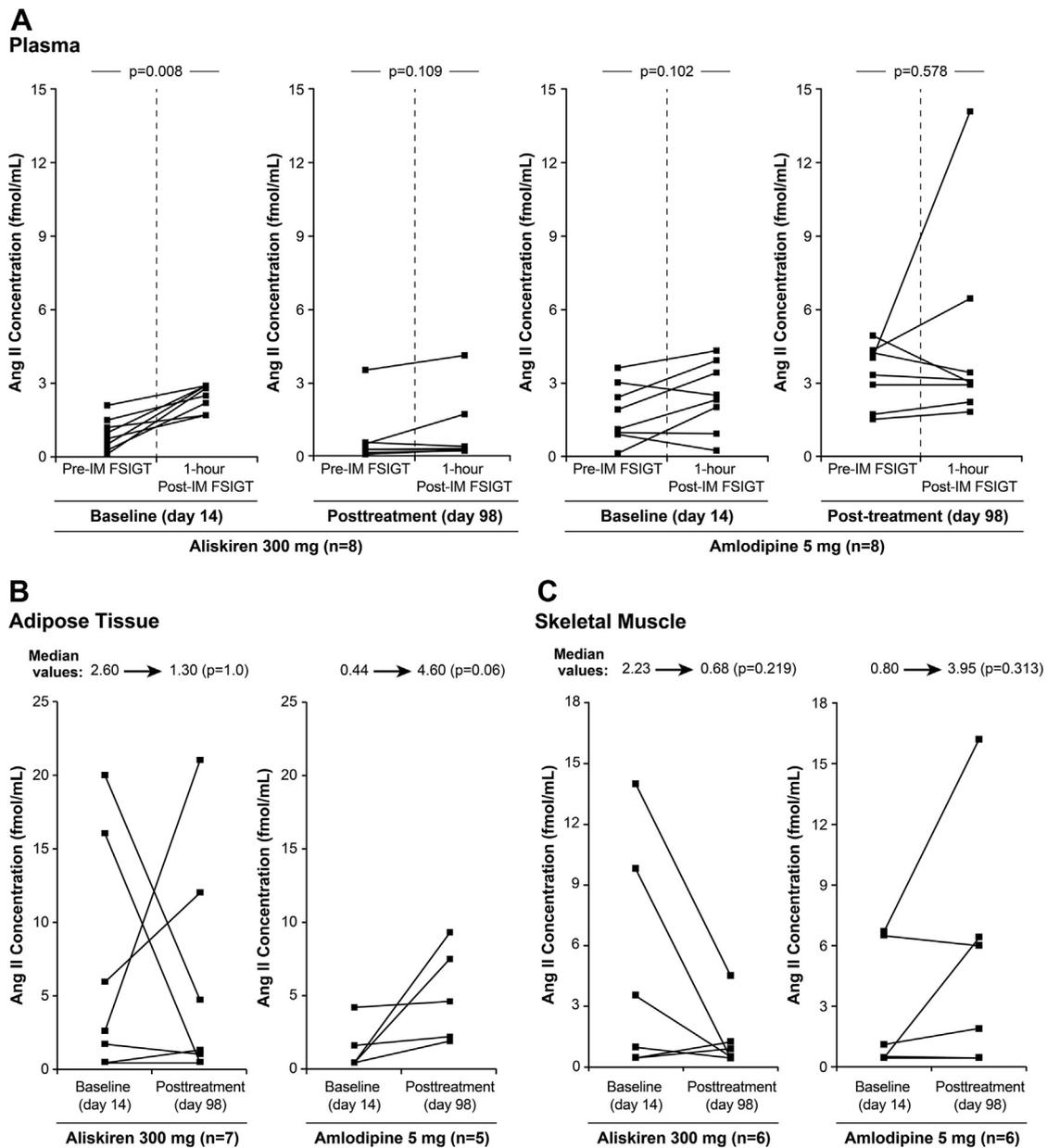


Figure 3. Effect of aliskiren, amlodipine, and insulin-modified frequently sampled intravenous glucose tolerance test (IM-FSIGT) on angiotensin II (Ang II). (A) Plasma Ang II at baseline and end of treatment (day 98) for both treatment arms. Also shown is the change of plasma Ang II 1 hour after glucose/insulin administration. (B) and (C) Effect of aliskiren and amlodipine on Ang II interstitial concentration in adipose tissue and skeletal muscle.

similar for both treatment groups. Following glucose bolus at 0 minutes and intravenous insulin at 20 minutes, interstitial glucose concentrations increased approximately 2- to 2.5-fold, reaching a maximum by 30 minutes and gradually returning to baseline levels at about 90–100 minutes. Neither aliskiren nor amlodipine changed the time course of interstitial glucose concentrations in either tissue on day 98, but interstitial glucose concentrations tended to be increased in adipose tissue with aliskiren treatment between minutes 15 and 45 (Figure 4A) and in skeletal

muscle with amlodipine between minutes 15 and 90 (Figure 4B).

In adipose tissue, the time course plots for mean glycerol concentrations before and during IM-FSIGT on day 14 demonstrated that the glucose/insulin intervention reduced interstitial glycerol concentration between 0 and 90 minutes (Figure 4A). Treatment with aliskiren tended to decrease dialysate glycerol concentration in adipose tissue compared with placebo on day 98. The effect appeared most prominent between 90 and 150 minutes post-IM-FSIGT and

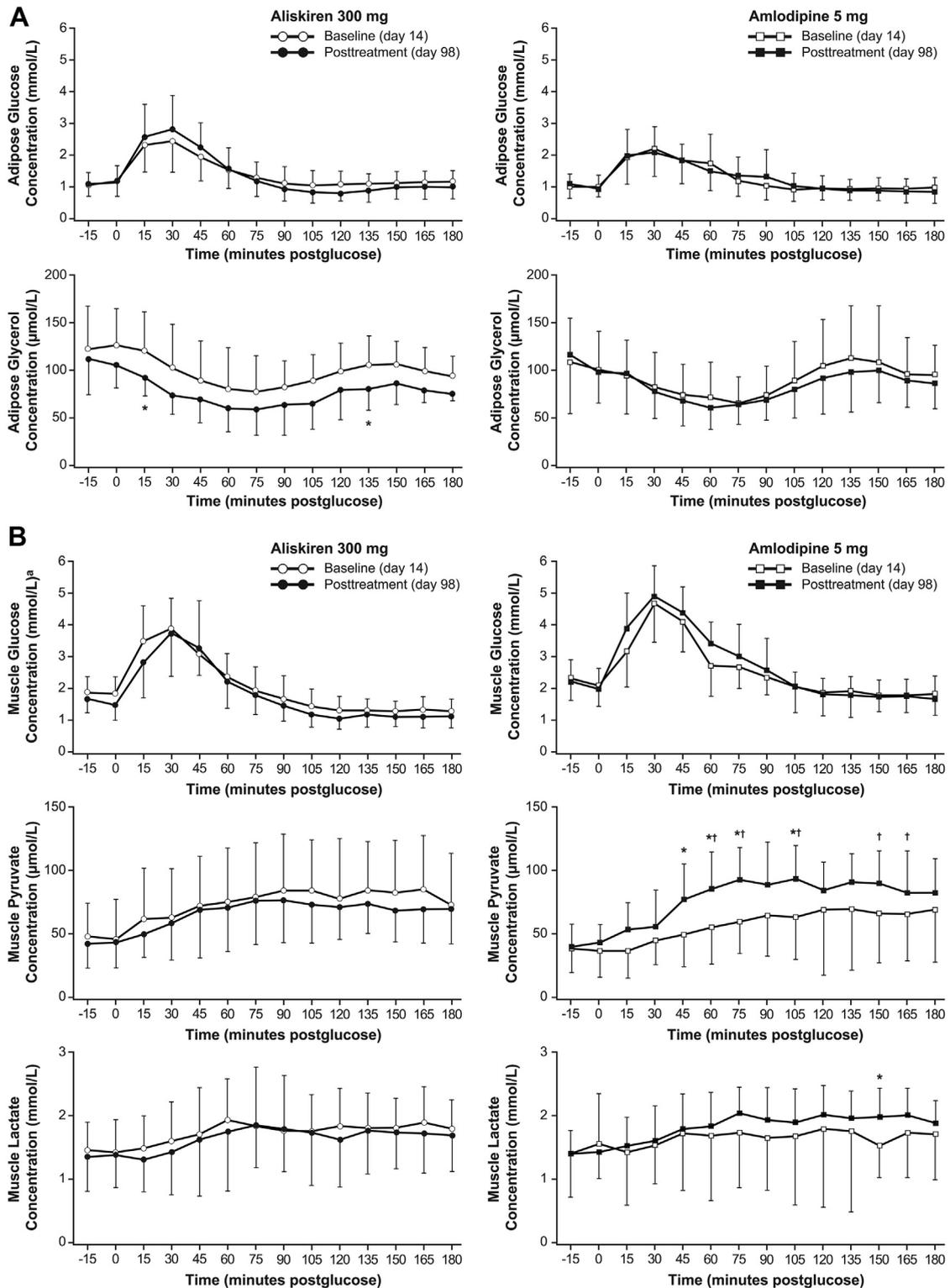


Figure 4. Time course of dialysate (A) glucose and glycerol concentrations in adipose tissue and (B) glucose, pyruvate, and lactate concentrations in skeletal muscle during insulin-modified frequently sampled intravenous glucose tolerance test (IM-FSIGT) at baseline (day 14) and following active treatment (day 98). Values are means and standard deviations. ^aWith aliskiren 300 mg, a significant treatment effect was observed with mean interstitial glucose concentrations slightly reduced from 1.83 mmol/L at baseline to 1.46 mmol/L after treatment ($P = .025$). * $P < .05$ for within-treatment group change (days 98–14). † $P < .05$ for difference between treatment groups (days 98–14).

reached statistical significance at 15 and 135 minutes ($P < .05$). In contrast, amlodipine did not change the glycerol response to glucose/insulin.

In skeletal muscle, pyruvate concentrations increased during the course of the IM-FSIGT in both treatment groups (Figure 4B). Pyruvate responses to glucose/insulin were increased on day 98 with amlodipine but not aliskiren treatment, reaching statistical significance at 45, 60, 75, and 105 minutes ($P < .05$). Differences between treatment groups were observed at 60, 75, 105, 150, and 165 minutes ($P < .05$). Interstitial muscle lactate concentrations in both treatment groups showed an increase starting at about 15 minutes and reaching a plateau by 60 minutes. No significant differences were observed for lactate concentrations during active treatment with both drugs (day 98) or compared with placebo (day 14).

Discussion

We tested influences of direct renin inhibition with aliskiren on insulin sensitivity and carbohydrate and lipid metabolism in adipose and skeletal muscle tissue in hypertensive patients with abdominal obesity at risk for type 2 diabetes mellitus. Calcium channel blockade with amlodipine served as control intervention. We previously showed that aliskiren attains adipose tissue and skeletal muscle concentrations sufficient to inhibit renin.⁵ In the second part of this study, aliskiren reduced PRA 6-fold and tended to decrease fasting plasma Ang II and aldosterone by 3-fold and 1.3-fold, respectively. Amlodipine increased each of these parameters. We identified a metabolic–RAAS interaction with the finding that exogenous intravenous glucose/insulin administration raised plasma Ang II concentrations. This response was markedly attenuated by aliskiren but not amlodipine. In previous studies, acute hyperinsulinemia also increased plasma Ang II concentrations in healthy volunteers¹⁶ and angiotensinogen protein expression in human subcutaneous adipocytes.^{17,18} Given the large variability and the small sample size, our findings on dialysate Ang II concentrations in adipose tissue and skeletal muscle are not conclusive. The tendency to reduce Ang II concentration by 1.3 fmol/mL in adipose tissue and by 1.6 fmol/mL in skeletal muscle, albeit not significant, suggests that aliskiren is able to reduce tissue RAAS activity.

Changes in plasma RAAS biomarkers and office blood pressure induced by aliskiren and amlodipine were consistent with previous findings,^{19–22} including studies in obese hypertensive patients.^{5,23–25} The small difference in blood pressure reduction between aliskiren and amlodipine treatment groups was not statistically significant, and our study was not powered to detect such small differences. Hypertensive patients are typically affected by obesity, along with insulin resistance and adipose tissue dysfunction. Because Ang II plays a role in mediating adipose tissue dysfunction, any therapeutic approach that not only reduces

blood pressure but also improves insulin action and adipose tissue dysfunction is of potential clinical importance for the treatment of hypertensive patients.

Aliskiren tended to increase the AIRg and increased the glucose DI. In contrast, amlodipine had no effect on either parameter. Possibly, aliskiren, but not amlodipine, increases pancreatic insulin secretion in response to rising glucose concentrations and also increases glucose flux to peripheral tissues. In a previous study in rats overexpressing mouse renin, aliskiren treatment improved pancreatic islet morphology and insulin sensitivity.²⁶ In addition, angiotensin-receptor blockade with losartan did not alter peripheral insulin sensitivity but tended to improve pancreatic β -cell function in obese hypertensive patients with impaired fasting glucose.²⁷

The standard fasting parameter of insulin resistance, HOMA-IR, was not influenced by either aliskiren or amlodipine. It should be noted that the patients included in this study showed only mild insulin resistance at baseline. This finding is not inconsistent with the IM-FSIGT results because dynamic tests such as IM-FSIGT are considered to be more sensitive for detecting early changes in insulin resistance than tests that rely on fasting parameters.

We applied adipose tissue and skeletal muscle microdialysis to obtain more direct insight regarding tissue metabolism. Because aliskiren increased skeletal muscle glucose uptake and utilization through ameliorated mitochondrial function in mice fed a high-fat diet,²⁸ we were particularly interested in treatment effects on muscle metabolism. Aliskiren could influence glucose metabolism directly through changes in cellular glucose uptake or indirectly through changes in tissue perfusion determining glucose delivery.

Similar tissue perfusion and dialysate glucose concentrations before and after treatment suggest that aliskiren did not produce major changes in glucose delivery and uptake in skeletal muscle. The finding is supported by unchanged muscle lactate and pyruvate concentrations. In contrast, amlodipine tended to increase interstitial glucose concentrations in skeletal muscle during the IM-FSIGT, which may be partly due to the observed small increase in muscle tissue perfusion. Increased muscle interstitial glucose concentration with amlodipine was associated with increased lactate and pyruvate production suggestive of increased glucose utilization.

In adipose tissue, we observed a tendency to decreased ethanol ratio with aliskiren treatment suggesting increased adipose tissue blood flow. This change was not observed with amlodipine treatment. The slightly increased glucose concentrations in adipose tissue between minutes 15 and 45 may be explained by increased glucose delivery. These data are consistent with our finding that glucose/insulin activated the RAAS as evidenced by the observed increase in plasma Ang II. Increased Ang II could decrease adipose tissue blood flow. This possible mechanism may be counterbalanced by aliskiren through prevention of glucose/insulin-mediated

RAAS activation at the tissue level. Furthermore, aliskiren but not amlodipine tended to decrease dialysate glycerol concentrations in adipose tissue, indicating tissue-specific inhibition of lipolysis or increased glycerol removal by increased blood flow. These effects cannot be differentiated by the microdialysis technique. An earlier study showed that angiotensin-receptor blockade with valsartan increased adipose tissue perfusion in patients with impaired glucose metabolism.²⁹ Valsartan treatment also reduced abdominal subcutaneous adipocyte size and adipose tissue macrophage infiltration. Chronic aliskiren treatment diminished weight gain during high-fat and low-fat feeding periods in mice.³⁰ The authors attributed decreased Ang II concentrations in adipose tissue because Ang II promotes murine adipocyte growth.³¹ Improved adipose tissue function may be a mechanism by which RAAS inhibitors lower the risk of developing type 2 diabetes mellitus.^{32,33}

Our study has several limitations: The small number of patients in our study, due to early termination of the study, does limit interpretation of the findings and the translation of the findings into clinical relevance. However, the observed trends were consistent with the known mechanisms of action of the study drugs and previous findings. Due to the low concentrations of Ang II in tissue and the very small sample volumes obtained during microdialysis, we were not able to differentiate between fasting and post-glucose/insulin Ang II concentrations, as shown for circulating Ang II. Instead, we had to pool the dialysates taken before and during IM-FSIGT to increase sample volume. The effects of glucose on interstitial Ang II concentrations could therefore not be determined.

Based on our findings, RAAS blockade monotherapy with aliskiren is a suitable treatment for obese patients with hypertension and other patients with the metabolic syndrome to lower blood pressure and at the same time improve carbohydrate and lipid metabolism.

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