Effect of Various Diuretic Treatments on Rosiglitazone-Induced Fluid Retention

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The efficacy of diuretics in the management of rosiglitazone (RSG)-induced fluid retention was evaluated in a multicenter, randomized, open-label, parallel-group, proof-of-concept study. Of 381 patients who had type 2 diabetes and were on treatment with sulfonylurea or sulfonylurea plus metformin, 260 (63% male, 37% female) showed evidence of volume expansion as defined by an absolute reduction in hematocrit (Hct) of $\geq 0.5\%$ after 12 wk of rosiglitazone 4 mg twice daily. They were randomly assigned to five treatments for 7 d: (1) Continuation of RSG (RSG-C), (2) RSG + furosemide (RSG+FRUS), (3) RSG + hydrochlorothiazide (RSG+HCTZ), (4) RSG + spironolactone (RSG+SPIRO), and (5) discontinuation of RSG. The primary end point was change in Hct at day 7 of diuretic treatment phase, powered to compare each diuretic group and the RSG discontinuation with the control group of RSG-C, with adjustments for multiple testing. After 12 wk on RSG, Hct fell by mean of 2.92% (95% confidence interval [CI] -3.10 to -2.63%; $P < 0.001$) and extracellular fluid volume increased by 0.62 L/1.73 m^2 (95% CI 0.26 to 0.90 L/1.73 m^2; $P < 0.001$). After treatment, the RSG+SPIRO group only showed a mean increase in Hct of 0.24%. The estimated mean difference in Hct reduction was significant: 1.14% (95% CI 0.29 to 1.98%) for RSG+SPIRO ($P = 0.004$) and 0.87% (95% CI 0.03 to 1.71%) for RSG+HCTZ ($P = 0.041$) only. In additional analyses of between-diuretic treatment effects SPIRO induced a greater Hct rescue at 0.88% (95% CI -0.12 to 1.87%; $P = 0.095$) and extracellular fluid volume reduction of -0.75 L/1.73 m^2 (95% CI -1.52 to 0.03 L/1.73 m^2; $P = 0.06$) compared with FRUS, suggesting superiority in the management of RSG-associated fluid retention. There were no significant differences between SPIRO and HCTZ. These findings are consistent with peroxisome proliferator–activated receptor-γ agonist activation of the epithelial sodium channel in the distal collecting duct, a site of action of SPIRO and a potential target for thiazide diuretics.

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Rosiglitazone (RSG), a member of the thiazolidinedione (TZD) class of insulin sensitizers, is used widely as monotherapy or in combination with other oral agents (sulfonylurea [SU] and metformin [MET]), or with insulin in the treatment of type 2 diabetes (1–4). However, RSG can cause or exacerbate fluid retention. In some patients, this is visible as peripheral edema. The frequency of peripheral edema is approximately 5% when these agents are used in monotherapy or combination oral therapy and approximately 15% when used with insulin (5–7). In extreme cases, TZD-induced volume overload may result in precipitation or exacerbation of pulmonary edema and congestive heart failure, a common complication of type 2 diabetes (8).

The underlying mechanisms of TZD-induced plasma volume expansion and edema remain unclear. The TZD molecular target, a nuclear transcription factor, peroxisome proliferator–activated receptor-γ (PPAR-γ) (9), is expressed diffusely in humans, including the cardiovascular and renal systems (10–13). This suggests a potential for TZD to elicit direct effects on these systems independent of their effects on glucose and lipid metabolism (14,15). Importantly, TZD do not influence erythropoiesis or the rate of red blood cell destruction (16). TZD seem to have a mild arterial vasodilatory action, which potentially could induce peripheral edema, but a clear connection between vasodilation and volume expansion has not been made. *In vitro* and animal data suggest that PPAR-γ agonists stimulate sodium reabsorption in the distal nephron by upregulating the expression and the translocation of the collecting duct epithelial sodium channel (ENaCα) (17,18). Selective deletion of the gene that encodes PPAR-γ in the collecting duct results in increased renal sodium excretion and prevents TZD-induced fluid retention, hemodilution, and weight gain in transgenic mice (19). Primary sodium retention by the kidney would lead to expanded plasma volume and, in susceptible patients, to edema and potentially heart failure.

The management of TZD-associated fluid retention remains contentious and no controlled trial to date has assessed the impact of various diuretic agents on this condition (20–24). We

therefore evaluated the management of RSG-related fluid retention by investigating the effect of three mechanistically distinct diuretics (furosemide [FRUS], thiazide, and spironolactone [SPIRO]) on plasma volume in patients who had type 2 diabetes and, after treatment for 12 wk with RSG, had evidence of volume expansion.

Materials and Methods

Patients

This was a multicenter, open-label, randomized, parallel-group, proof-of-concept study in patients with type 2 diabetes as defined by World Health Organization criteria (25). All patients were aged 35 to 80 yr at screening, and female patients had to be postmenopausal. Patients had to have stable fasting plasma glucose of ≥7.0 and ≤12.0 mmol/L with HbA_{1c} ≤10% on established SU or SU+MET treatment for at least 2 mo. Addition of RSG to treatments that include SU is more likely to cause plasma volume expansion (2,26).

Exclusion criteria were use of more than two concomitant oral antidiabetic agents or agents other than SU or SU+MET and current use of insulin. Patients who were currently receiving any diuretic medication and patients who had started within the previous month drugs that could affect sodium balance (e.g., diuretics, nonsteroidal anti-inflammatory drugs, Cox-2 inhibitors, β blockers, Ca^+ antagonists) also were excluded. Other exclusion criteria were current systemic glucocorticoid treatment, a fasting C-peptide level of <0.5 nmol/L, and previous exposure or hypersensitivity to a TZD. A systolic BP (SBP) >170 mmHg or diastolic BP (DBP) >100 mmHg, a history of ischemic heart disease or congestive heart failure (New York Heart Association class I through IV), a serum albumin ≥10% outside normal range, a hemoglobin (Hb) concentration <11 g/dl for men or <10 g/dl for women, and serum creatinine level >130 μmol/L were additional exclusion criteria.

Procedures

Hematocrit (Hct) was used as a surrogate marker to assess plasma volume changes (27,28). The magnitude of Hct reduction to detect plasma volume expansion was based on previous studies (29). Eligible patients continued on their established antidiabetic agents during a 2-wk run-in period, after which, open-label RSG 4 mg twice daily was added for 12 wk. On completion of RSG treatment, patients who achieved an absolute Hct reduction of 0.5% or more were randomly assigned to one of five treatment arms. In three treatment arms, RSG was continued in addition to one of three diuretics (40 mg/d FRUS, 25 mg/d hydrochlorothiazide [HCTZ], or 50 mg/d SPIRO) for 7 d; in the fourth arm, RSG was withdrawn (RSG W/D group), and in the fifth arm, RSG was continued without diuretic (RSG-C). The short duration of diuretic treatment and the prompt uptitration schedule (see the Acute Diuretic Treatment Phase section) were chosen in the context of a proof-of-concept study designed to identify an intervention that under certain clinical conditions would have to act promptly.

Randomization was stratified by gender and background antidiabetic medications to ensure minimal between-group differences. The nondiuretic arms were blinded to RSG treatment, and patients in the RSG W/D group were administered placebo. The primary end point was the effect of diuretic therapy or RSG withdrawal on Hct change.

Chronic RSG Treatment Phase

Patients were seen in the morning after an overnight fast (at least 8 h) before any medication was taken and after withdrawal of nicotine and coffee for at least 10 h. Weight was taken at every scheduled visit after patients voided urine and wore only a hospital gown. The same weighing equipment was used at every visit and was calibrated every 3 mo

for the duration of the study. Height was measured at study entry only. At baseline (day 0) and days 8, 29, 57, and 85, Hct (uncuffed sample), serum electrolytes, fasting plasma glucose, plasma albumin, full blood count, HbA_{1c}, fasting C-peptide, plasma atrial natriuretic peptide (ANP), and aldosterone (days 0 and 85 only) were measured. Baseline C-peptide values were used to assess patient eligibility for entry into the study. Total body water (TBW) and extracellular fluid (ECF) were assessed using a noninvasive technique of bioelectrical impedance with an Akern soft tissue analyzer (Akern, Florence, Italy) validated by several studies to measure change in body fluid compartments as distinct from measures of fat mass (30–32). In brief, a 800-μA and 50-kHz alternating sinusoidal current was applied using a standard tetrapolar technique, with electrodes placed at the wrist and the foot, and the resistance and reactance values that were obtained were used to determine a patient's hydration status by bioimpedance vector analysis (30).

Before measurements, patients were rested in the supine position for approximately 10 min to equalize fluid compartments. BP was measured in the seated position after a 5-min rest at each visit using an automated oscillometer. The average of three measurements was used for calculation. At baseline, day 57, and day 85, all patients performed a 24-h ambulatory BP measurement (Spacelabs, Redmond, WA). A standard 12-lead electrocardiogram was recorded for all patients at days 0 and 85.

Patients received advice to follow a weight-maintaining diet with stable salt and fluid intake (Na^+ intake approximately 130 mEq/d, fluid intake approximately 2 L/d) in accordance with their standard diabetic diet, which remained stable throughout the study. Compliance with RSG was monitored by tablet counting at each study visit.

Acute Diuretic Treatment Phase

Patients who showed an absolute Hct reduction of 0.5% or more at day 85 entered the diuretic treatment phase. They were randomly assigned to one of five treatment arms, as described under procedures, and admitted to a metabolic unit for prediuretic baseline measurements. During this phase, patients continued on their standard diabetic weight-maintaining diet with salt and fluid intake as indicated previously. At 8:00 a.m., fasting blood samples were taken for Hct, plasma glucose, full blood count, plasma albumin, and serum electrolytes. Patients had BP measured using the same procedure as per the chronic RSG treatment phase. Bioimpedance measurements of TBW and ECF were carried out. A 24-h urine collection was started and urine output was recorded. All assessments were completed by the next morning, when the first diuretic and placebo medication dose was dispensed. RSG treatment continued in all but the withdrawal arm, which received placebo. Patients then were observed for effects of acute volume depletion and potential adverse reactions for 4 h before discharge.

Twenty-four hours later, patients again were admitted to a metabolic ward and given the second dose of diuretic or placebo, and all baseline assessments including a second 24-h urine collection were carried out. Salt and fluid intake was standardized as per baseline. The volume of this urine collection was used to assess the need for uptitration of the diuretic. Patients in the diuretic arms who achieved a 24-h metabolic ward urine volume <130% of the prediuretic 24-h urine volume were regarded as potential nonresponders and had their dosage of diuretic doubled for the following 5 d unless there was weight loss of >1 kg, postural hypotension, tachycardia, or any other indication of clinically significant volume depletion.

Efficacy end points (as per baseline assessments) were measured at visit study end when the patient was seen again in the fasted state in the morning. Study drugs then were stopped, and patients returned to

their original antidiabetic therapy and were followed for safety assessments for another 2 to 4 wk.

The study was conducted according to Good Clinical Practice for clinical trials and was approved by the ethics committee of each local center. Patients gave their written informed consent to participate.

Laboratory Measurements

All measurements were carried out in a central laboratory (Quest Diagnostics, London, UK). Hct, full blood count, and Hb were determined by electronic sizing and counting/cytometry (Coulter GEN-S, Fullerton, CA). Plasma albumin and serum electrolytes were carried out by flame photometry (Olympus AU640/AU2700/AU5400; Shinjuku-ku, Tokyo, Japan), plasma ANP by RIA after cartridge extraction (Iso-Data 20/20; Global Medical Instrumentation, Ramsey, MN), and plasma aldosterone by solid-phase RIA (Genesys 6000; Lab Technologies, Maple Park, IL). Plasma glucose and HbA$_{1c}$ were determined by spectrometry (Olympus AU5200/AU5400) and ion exchange chromatography (BioRad, Hercules, CA), respectively. C-peptide (DPC Immulite, 2000, Los Angeles, CA) was measured by immunoassay.

Statistical Analyses

Sample size calculation was based on evidence from previous clinical trial data for RSG and Hct change, and simulations indicated that 170 assessable patients (30 in each of the treatment arms and 50 in control arm) at the end of diuretic treatment were needed to have 90% power to detect at least one significant difference at an overall 5% level for each comparison with the control group, with adjustment for multiple comparisons. Of the 381 patients who entered the chronic RSG treatment phase, 260 met the randomization criterion and were assigned to the five arms of the acute diuretic treatment phase in the same proportion as per original sample size calculation, thereby exceeding the original estimate for power. Descriptive statistics were used for the analysis of demographic and clinical features of the cohort. Paired t test was used to compare Hct, Hb, plasma albumin, serum electrolytes, plasma ANP, plasma aldosterone and TBW and ECF change during the chronic RSG treatment phase.

The primary analysis compared change in Hct for each diuretic arm and the RSG W/D arm with the RSG-C arm using analysis of covariance. Covariate adjustments were made for demographic and baseline characteristics (including body weight at randomization), and *P* values adjusted for multiple comparisons against the control group using the Edwards and Berry's simulation-based multiple comparisons method (33); therefore, all *P* values can be interpreted as multiplicity adjusted. Additional comparisons were performed to assess effects relative to the control group on ECF and TBW. Analyses also were made to test between-diuretic effects on Hct and ECF with multiplicity adjustment accounting for all pair-wise comparisons between the diuretic groups (34). Analyses were carried out using SAS version 8 (SAS Institute, Cary, NC).

Results

Chronic RSG Treatment Phase

After 12 wk of RSG therapy, 260 patients showed a decrease in Hct ≥0.5%. Baseline HbA$_{1c}$ was comparable in patients with (7.5 ± 0.9%) and those without volume expansion (7.3 ± 0.9%) and improved similarly by approximately 0.6% in both groups with RSG treatment (data not shown). The comparison of patients who did not have volume expansion with those who did are the subject of a separate report (35). This article deals

exclusively with the cohort of 260 patients who showed volume expansion and their response to diuretic treatment.

The demographic and clinical features of this cohort are shown in Table 1. Background antidiabetic medication was combination therapy of SU plus MET in 74% and SU alone in 26%. Sixteen percent were on lipid-lowering therapy, and 53% had hypertension; of these 67% were receiving inhibitors of the renin-angiotensin-aldosterone system (RAAS).

Table 2 shows the change in Hct, Hb, albumin, TBW, ECF, and weight after 12 wk of RSG treatment. The fall in Hct was accompanied by a statistically significant reduction in total Hb and plasma albumin concentrations. The reduction in Hct was significantly related with the fall in Hb concentration ($r = 0.82$, $P < 0.001$), and this was inversely significantly associated with the changes in ECF volume ($r = -0.25$, $P < 0.01$). TBW and ECF increased significantly. These findings all are consistent with plasma volume expansion. Weight increased by an average of 1.78 kg. Mean (SD) plasma aldosterone concentration fell significantly from 287.5 pmol/L (183.0) to 243.9 pmol/L (166.8; $P < 0.001$), and plasma ANP concentration rose from 70.4 ng/L (50.5) to 80.3 ng/L (51.5; $P = 0.001$). Serum sodium rose from 139.9 mmol/L (2.2) to 140.3 mmol/L (2.1; $P = 0.003$), but serum potassium showed no change. The patients who did not show volume expansion had a mean (SD) increase in Hct of 0.5% (2.5) and no significant changes in total Hb concentration and plasma electrolytes. Plasma aldosterone levels remained unchanged, and plasma ANP tended to fall. TBW and ECF volume (measured in 69 patients only) were unaltered.

During 12 wk of treatment with RSG, mean (SD) office SBP and DBP fell from 139.0 mmHg (16.8) to 136.2 mmHg (16.3; $P < 0.004$) and from 81.9 mmHg (9.7) to 79.7 mmHg (10.0; $P <$

Table 1. Baseline characteristics of 260 patients who had type 2 diabetes and showed Hct reduction ≥0.5% after 12 wk of treatment with RSG 4 mg twice daily in addition to background sulfonylurea or sulfonylurea plus metformin therapy[a]

Patients	Variable
N (gender)	260 (163 M, 97 F)
Age (yr)	60.6 (35 to 80)
White (n)	254
Black (n)	5
Asian (n)	1
Duration of diabetes (yr)	9.9 ± 6.3
Height (cm)	169 ± 12
Weight (kg)	84.7 ± 16.7
BMI (kg/m^2)	30.0 ± 7.9
Waist-to-hip ratio	0.96 ± 0.1
SBP (mmHg)	139.0 ± 16.8
DBP (mmHg)	81.9 ± 9.7
HbA$_{1c}$ (%)	7.5 ± 0.9
FPG (mmol/L)	9.3 ± 1.7

[a]Data are means ± SD or (range). BMI, body mass index; DBP, diastolic BP; FPG, fasting plasma glucose; Hct, hematocrit; RSG, rosiglitazone; SBP, systolic BP.

Table 2. Changes in Hct, Hb, plasma albumin, ECF, TBW, and body weight in 260 patients with type 2 diabetes after 12 wk of treatment with RSG 4 mg twice daily in addition to background sulfonylurea or sulfonylurea plus metformin therapy[a]

Variable	Baseline	End of Chronic RSG Treatment	Absolute Change	P
Hct (%)	42.1 ± 3.6	39.2 ± 4.0	−2.92 (−3.10 to −2.63)	<0.001
Hb (g/dl)	14.1 ± 1.2	13.0 ± 1.4	−1.03 (−1.09 to −0.93)	<0.001
Plasma albumin (g/L)	45.0 ± 2.4	44.6 ± 2.6	−0.46 (−0.77 to −0.22)	<0.001
ECF (L/1.73 m^2)	16.9 ± 2.5	17.5 ± 2.4	0.62 (0.26 to 0.90)	<0.001
TBW (L/1.73 m^2)	38.7 ± 4.4	39.5 ± 4.2	0.79 (0.52 to 1.08)	<0.001
Body weight (kg)	84.7 ± 16.7	86.5 ± 17.0	1.78 (1.51 to 2.06)	<0.001

[a]Data are means ± SD or means (95% CI) absolute change. CI, confidence interval; ECF, extracellular fluid; Hb, hemoglobin; Hct, hematocrit; TBW, total body water.

0.001), respectively. This change was confirmed by mean 24-h ambulatory BP recordings, in which SBP fell from 130.5 mmHg (12.4) to 128.1 mmHg (11.0) and DBP fell from 75.6 mmHg (7.9) to 72.8 mmHg (7.50; $P < 0.005$ for both). Patients who did not show volume expansion had a similar fall in mean 24-h ambulatory BP recordings.

Acute Diuretic Treatment Phase

The five groups that entered the diuretic treatment phase did not differ significantly in age, duration of diabetes, weight, body mass index, BP, metabolic control, and Hct (Table 3). Table 4 reports for the selected variables of weight, ECF, and TBW the baseline values before chronic RSG treatment in these five groups. It shows that changes after RSG treatment were consistent across groups. Use of concomitant medications (including inhibitors of the RAAS) was similar between the groups. Diuretic uptitration was required in 33% of patients in the RSG+FRUS group, 36% in the RSG+HCTZ group, and 38% in the RSG+SPIRO group. These differences were NS. Average daily dosage of each agent used was 53 mg of FRUS, 34 mg of HCTZ, and 69 mg of SPIRO.

In the RSG-C group, Hct continued to fall from randomization baseline to day 7 (−0.89%). Mean absolute reductions,

although smaller, also were observed for the RSG W/D (−0.12%), RSG+FRUS (−0.70%), and RSG+HCTZ (−0.02%) groups; a mean increase of 0.24% was observed in the RSG+SPIRO group. Mean (95% confidence interval [CI]) values for adjusted differences at day 7 relative to the RSG- C group were 0.77% (−0.05 to 1.59; $P = 0.073$) for the RSG W/D group, 0.19% (−0.66 to 1.03; $P = 0.961$) for the RSG+FRUS group, 0.87% (0.03 to 1.71; $P = 0.041$) for the RSG+HCTZ group, and 1.14% (0.29 to 1.98; $P = 0.004$) for the RSG+SPIRO group (Figure 1). The changes in total Hb and plasma albumin concentration during the 7-d acute diuretic treatment phase were concordant with the Hct changes (data not shown), and, consistently, ECF showed the greatest reduction in the RSG+SPIRO group (Figure 2A) by an adjusted mean (95% CI) treatment difference of −1.00 L/1.73 m^2 (−1.81 to −0.19; $P = 0.010$), compared with control. Mean (95% CI) effects on ECF for the other groups compared with control were −0.21 L/1.73 m^2 (−1.01 to 0.59; $P = 0.929$) for RSG withdrawal, −0.14 L/1.73 m^2 (−0.95 to 0.67; $P = 0.982$) for RSG+FRUS, and −0.47 L/1.73 m^2 (−1.29 to 0.36; $P = 0.463$) for RSG+HCTZ. TBW was reduced in all three diuretic groups (Figure 2B).

Multiplicity-controlled between-diuretic comparisons for Hct

Table 3. Characteristics of the five groups of patients with type 2 diabetes and evidence of volume expansion on chronic RSG therapy at randomization to the acute diuretic treatment phase[a]

Characteristic	RSG-C	RSG W/D	RSG+FRUS	RSG+HCTZ	RSG+SPIRO
n (gender)	78 (49 M, 29 F)	47 (30 M, 17 F)	45 (28 M, 17 F)	44 (28 M, 16 F)	46 (28 M, 18 F)
Age (yr)	59.9 ± 7.1	60.8 ± 9.2	60.9 ± 8.5	58.2 ± 9.5	63.5 ± 8.9
Duration of diabetes (yr)	9.4 ± 5.4	9.1 ± 6.3	10.3 ± 7.1	9.2 ± 5.7	11.7 ± 7.3
Weight (kg)	87.3 ± 17.3	84.9 ± 18.6	87.0 ± 15.4	89.9 ± 17.8	81.5 ± 14.3
BMI (kg/m^2)	29.7 ± 4.4	29.3 ± 4.7	32.5 ± 4.3	30.5 ± 5.4	28.4 ± 3.9
SBP (mmHg)	141.1 ± 16.8	142.1 ± 16.9	134.6 ± 17.4	138.8 ± 15.7	136.8 ± 17.0
DBP (mmHg)	83.0 ± 9.3	82.7 ± 7.8	80.3 ± 11.8	82.9 ± 9.8	80.0 ± 9.5
HbA$_{1c}$ (%)	6.9 ± 0.7	7.0 ± 0.8	6.9 ± 0.7	6.8 ± 0.8	6.9 ± 0.8
FPG (mmol/L)	7.3 ± 1.6	7.5 ± 1.7	7.2 ± 1.9	7.3 ± 1.5	7.7 ± 1.9
Hct (%)	39.1 ± 4.0	39.4 ± 4.5	39.1 ± 4.2	39.8 ± 3.9	38.5 ± 5.0

[a]Data are mean ± SD. FRUS, furosemide; HCTZ, hydrochlorothiazide; SPIRO, spironolactone; RSG-C, rosiglitazone control; RSG W/D, rosiglitazone withdrawal.

Table 4. Weight, ECF, and TBW at baseline and after 12 wk of treatment with RSG 4 mg twice daily in addition to background sulfonylurea or sulfonylurea plus metformin in the five subgroups of 260 patients who had type 2 diabetes and evidence of RSG-induced fluid retention and were randomly assigned to the acute diuretic treatment phase[a]

Characteristics after RSG	RSG-C	RSG W/D	RSG+FRUS	RSG+HCTZ	RSG+SPIRO
n	78	47	45	44	46
Weight (kg)					
baseline	85.6 ± 15.9	83.1 ± 17.4	85.1 ± 14.6	88.1 ± 16.6	79.6 ± 14.0
after RSG	87.3 ± 17.3	84.9 ± 18.6	87.0 ± 15.4	89.9 ± 17.8	81.5 ± 14.3
ECF (L/1.73 m^2)					
baseline	17.0 ± 2.5	16.8 ± 2.6	16.7 ± 1.8	16.5 ± 2.3	17.2 ± 3.0
after RSG	17.7 ± 2.4	17.6 ± 3.1	17.3 ± 2.9	17.4 ± 2.1	17.7 ± 2.3
TBW (L/1.73 m^2)					
baseline	39.0 ± 4.8	38.3 ± 4.6	38.9 ± 6.5	39.2 ± 3.7	38.1 ± 4.3
after RSG	39.9 ± 4.9	39.4 ± 4.2	40.1 ± 6.2	39.9 ± 3.1	39.1 ± 4.2

[a]Data are mean ± SD.
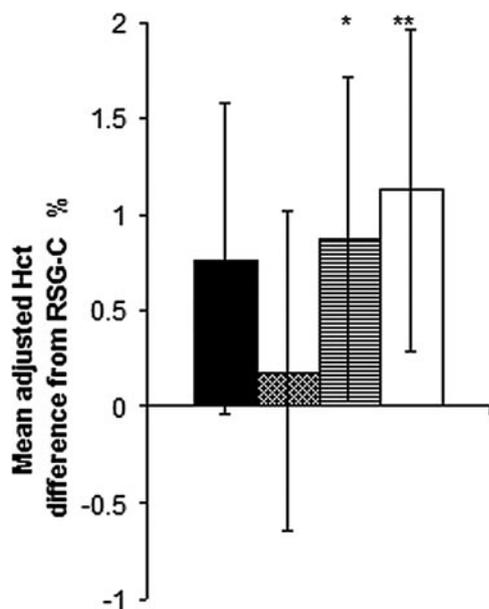
Figure 1. Adjusted mean hematocrit (Hct) differences after 7-d treatment relative to continuation of RSG (RSG-C; taken as = 0) in the three diuretic treatment groups—RSG plus furosemide (RSG+FRUS; ▨), RSG plus hydrochlorothiazide (RSG+HCTZ; ▤), and RSG plus spironolactone (RSG+SPIRO; ▢)—and RSG withdrawal (RSG-W/D) group ■ in patients with type 2 diabetes and evidence of volume expansion. Error bars indicate 95% confidence interval (CI). Adjustments were made for baseline Hct, change in Hct during chronic RSG treatment phase, gender, weight, season/location, and background antidiabetic medications. *P* values and CI also were adjusted for multiple comparisons with a common control. *$P = 0.041$; **$P = 0.004$.

showed a difference between RSG+SPIRO and RSG+FRUS of 0.88% (95% CI −0.12 to 1.87%; $P = 0.095$), between RSG+FRUS and RSG+HCTZ of 0.74% (95% CI −0.28 to 1.75%; $P = 0.205$), and between RSG+SPIRO and RSG+HCTZ of 0.14% (95% CI −0.90 to 1.19%; $P = 0.943$; Figure 3A). For ECF changes, the

differences among diuretic groups were for RSG+SPIRO *versus* RSG+FRUS of −0.75 L/1.73 m^2 (95% CI −1.52 to 0.03; $P = 0.06$), for RSG+HCTZ *versus* RSG+FRUS of −0.41 L/1.73 m^2 (95% CI −1.20 to 0.38; $P = 0.433$) and for RSG+SPIRO *versus* RSG+HCTZ of −0.33 L/1.73 m^2 (95% CI −1.15 to 0.48; $P = 0.596$; Figure 3B). These secondary analyses of between-diuretic group comparisons showing multiplicity-controlled $P = 0.095$ for Hct and 0.06 for ECF for the difference between RSG+SPIRO and RSG+FRUS suggest a genuine distinct treatment effect between these two diuretics.

Body weight after randomization to diuretic treatment had risen by an average of 0.32 kg in the RSG-C group. Mean reductions were observed in all of the other groups, and the adjusted mean (95% CI) treatment differences at day 7 relative to RSG-C were as follows: RSG+SPIRO −1.09 kg (−1.59 to −0.59; $P < 0.001$), RSG+HCTZ −1.00 kg (−1.50 to −0.50; $P < 0.001$), RSG+FRUS −0.83 kg (−1.33 to −0.32; $P < 0.001$), and RSG W/D −0.48 kg (−0.97 to 0.01; $P = 0.057$).

Urine flow was similar across all groups at baseline, and the median rise after diuretic treatment of between 220 to 480 ml/24 h did not differ among groups. There were no significant changes in BP, glycemic control, or serum sodium and serum potassium concentrations during the acute diuretic treatment phase in any of the groups.

Drug Compliance and Adverse Effects

Compliance with RSG treatment was consistently >86%. Of the patients who were randomly assigned to the diuretic treatment phase, one in the RSG+SPIRO group, two in the RSG+HCTZ group, and one in the RSG+FRUS group withdrew because of infection, dizziness, loss to follow-up, and protocol violation, respectively.

Discussion

Evidence of fluid retention on RSG therapy, as indicated by a fall in Hct of at least 0.5%, occurred in 68% of this cohort of patients with type 2 diabetes. The concomitant fall in total
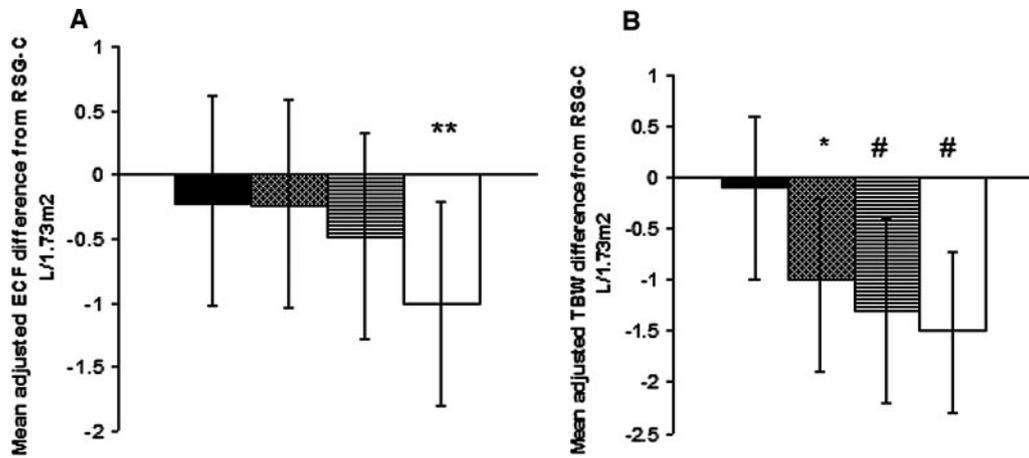
Figure 2. Adjusted mean extracellular fluid (ECF; A) and total body water (TBW; B) differences after 7-d treatment relative to RSG-C (taken as = 0) in the three diuretic treatment groups—RSG+FRUS (▨), RSG+HCTZ (▤), and RSG+SPIRO (□)—and RSG W/D group (■) in patients with type 2 diabetes and evidence of volume expansion. Error bars indicate 95% CI. Same adjustments as in Figure 1. *P = 0.025; **P = 0.009; #P < 0.001.
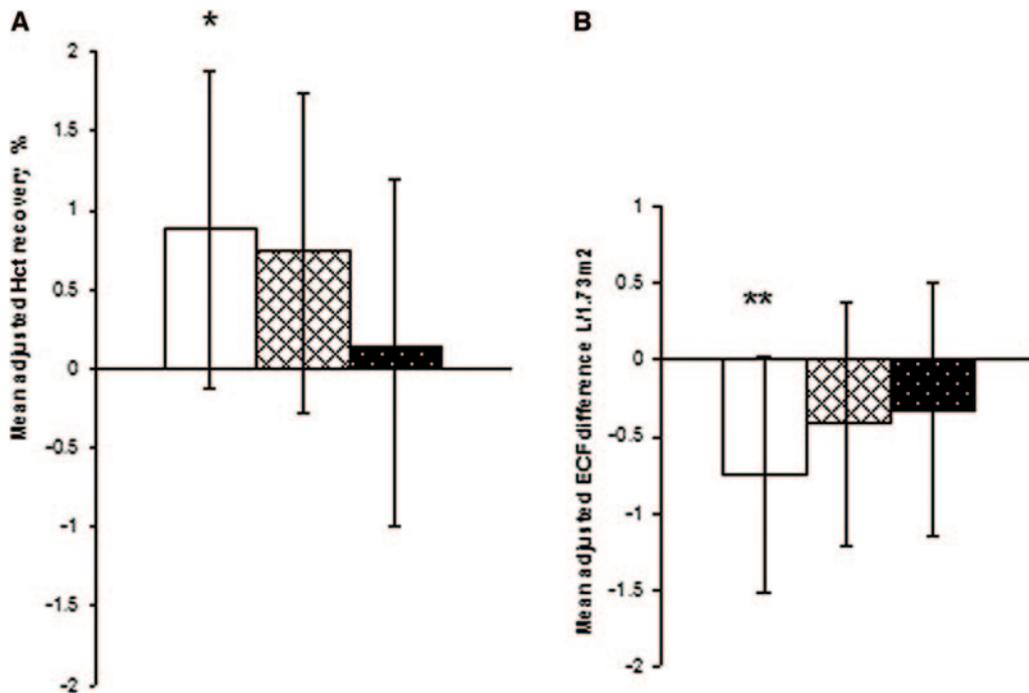

Figure 3. Adjusted mean Hct (A) and ECF (B) between diuretic group differences after 7-d treatment in patients with type 2 diabetes and evidence of volume expansion. Error bars indicate 95% CI. Adjustments were made for baseline Hct, change in Hct during chronic RSG treatment phase, gender, weight, season/location, and background antidiabetic medications. P values and CI are adjusted for multiplicity for all pair-wise between-group comparisons. *P = 0.095; **P = 0.060. Refer to the main text for other P values. □, RSG+SPIRO *versus* RSG+FRUS; ▨, RSG+HCTZ *versus* RSG+FRUS; ▨, RSG+SPIRO *versus* RSG+HCTZ.

Hb and plasma albumin concentrations and the significant increase in measured ECF and TBW confirm volume expansion. Furthermore, the reductions in plasma aldosterone concentrations and increases in plasma ANP levels were physiologically consistent with increased sodium reabsorption and central venous volume expansion. Salt and water balance therefore was reset and maintained as indicated by the slight but significant increase in serum sodium. The obser-

vation that in the group with no Hct changes these variables remained, by and large, unmodified provides further support for the use of Hct as a surrogate marker of volume expansion (28,29). Volume expansion, as indicated by ECF volume changes, was significant, although moderate, in our uncomplicated patients. However, in susceptible patients, chronic volume overload, which initially is compensated for by cardiac hypertrophy, would result, if the condition is not

corrected, in the cardiomyopathy of overload with the development of edema and heart failure (36).

The allocation of these patients with clear evidence of fluid retention to withdrawal of RSG treatment or different types of diuretic therapy for 7 d produced disparate results. Compared with a time-control group in whom RSG therapy was continued, patients who received SPIRO showed numerically the greatest reversal in fluid retention as indicated by a significant increase in Hct and fall in ECF volume, suggesting resolution of fluid retention in this group. This effect of SPIRO was of interest given that the magnitude of urine output obtained and the percentage of patients who needed uptitration after the first dose of diuretic both were similar to those of the other treatments. SPIRO, under ordinary circumstances and at the dosages used in this study, is notoriously slow acting and less potent than other diuretics. HCTZ treatment also was effective in inducing resolution of the fluid retention, whereas FRUS had a limited effect. Of interest, withdrawal of RSG treatment had an NS effect on reversal of fluid retention during a 7-d period. This suggests that there is a delayed and persistent action of the drug and, importantly, indicates that in situations of need, withdrawal of therapy alone would not be a reliable means of rapid correction of RSG-induced volume expansion. The secondary analyses, which compared the effect on Hct and ECF changes among the three diuretic groups strongly suggested that SPIRO was superior to FRUS in reversing RSG-induced fluid retention. FRUS also seemed to be less effective than HCTZ, but the difference between these two diuretics was far from significant. There was no indication of a significant difference between SPIRO and HCTZ. Studies with a larger sample size would be required to address these questions further.

Our results also demonstrated that at least part of the weight gain that accompanies RSG treatment was due to salt and water retention. Indeed, weight fell to the largest extent in the SPIRO group. The observed effects may find an explanation in the specific sodium and water transport systems that were affected by the various drugs. PPAR-γ is expressed in the human renal cortical collecting duct, a segment of the nephron that regulates sodium and water homeostasis *via* action of the ENaC (12). The activity of this channel is stimulated by aldosterone and insulin but also is induced by PPAR-γ agonists, such as RSG, *via* the serum glucocorticoid regulated kinase 1 (17). Recent work indicates that the probable target gene for the action of PPAR-γ agonists in the collecting duct also regulates the expression of the γ subunit of ENaC (19). Taken together, these observations suggest that RSG affects sodium reabsorption in the distal collecting duct *via* stimulation of ENaC activity, help in the understanding of why fluid retention is more pronounced by the simultaneous use of RSG and insulin, and, in the context of our study, explain why an aldosterone antagonist seems to be an effective means to correct RSG-induced fluid retention. HCTZ also significantly improved fluid retention. The major site of action of thiazide diuretics is the early distal convoluted tubule, where they block Na^+/Cl^- co-transport (37,38). However, they also can inhibit salt and water reabsorption from the medullary collecting duct (39), a site of expression of PPAR-γ in the nephron (12). It therefore is conceivable that thiazide di-

uretics may antagonize PPAR-γ agonist action at this site. By contrast, loop diuretics, which had no significant effect on Hct and ECF, exclusively act on the thick ascending limb of the loop of Henle, where they interfere with the Na^+-K^+-Cl^- co-transporter 2 (40,41). This segment of the nephron shows no expression of PPAR-γ. Moreover, hormones such as ANP, which showed a rise in these patients with diabetes and volume expansion, can reduce the response to loop diuretics *via* inhibition of NaCl reabsorption in the cortical thick ascending limb (42).

There are limitations to our study. We addressed the treatment of RSG-induced fluid retention in uncomplicated patients who had type 2 diabetes and showed moderate volume expansion, and, in this proof-of-concept study, we used an intensive diuretic regimen to prove efficacy. In clinical practice, patients with symptomatic edema and, more rarely, heart failure commonly would be treated. Diuretic regimens also may be less aggressive. There is no reason to believe, however, that response to treatment would be any different. We did not apply low-sodium intake to patients with fluid retention. Although this may be a recommendation for chronic therapy, it was believed that it would be inadequate to deal with the acute setting that the study was designed to simulate. Our study evaluated the effect of diuretic or RSG W/D for only 7 d and did not determine the long-term value and safety of continued therapy. All of our patients had normal renal function and normal electrolytes at baseline. We cannot exclude that the chronic use of SPIRO in patients who have renal impairment and receive concomitant treatment with inhibitors of the RAAS may increase the risk for hyperkalemia (43). Amiloride, a diuretic that, like SPIRO, also blocks sodium reabsorption in the collecting duct, may equally be effective. Indeed, in a recent study, amiloride prevented TZD-induced increase in TBW and weight gain in mice (19). We did not evaluate any changes in body fat distribution, which may contribute to weight gain and weight changes, but this was outside the scope of this work. Concomitant therapy with antihypertensive agents may have influenced the response to diuretic; however, use of inhibitors of the RAAS was similar across all randomized groups. The effect of these agents would lead, if anything, to an underestimation of the true benefits of aldosterone antagonism in treating RSG-induced fluid retention.

Conclusion

Our study suggests that diuretic agents, such as SPIRO and HCTZ, that interfere with the signaling of the PPAR-γ in the renal distal collecting duct are effective means to reverse RSG-induced fluid retention.

Acknowledgments

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