Randomized Trial Comparing SGLT2 Inhibition and Hydrochlorothiazide on Sympathetic Traffic in Type 2 Diabetes

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Introduction: Reductions in sympathetic nervous system activity may contribute to beneficial effects of sodium glucose cotransporter 2 (SGLT2) inhibition on cardiovascular outcomes. Therefore, we tested the hypothesis that SGLT2 inhibition with empagliflozin (Empa) lowers muscle sympathetic nerve activity (MSNA) in patients with type 2 diabetes mellitus (T2DM) compared with hydrochlorothiazide (HCT) to discern SGLT2-specific actions from responses to increased natriuresis.

Methods: We randomized patients with T2DM on metformin monotherapy to either 25 mg/d Empa ($n = 20$) or 25 mg/d HCT ($n = 21$) for 6 weeks in a parallel, double-blind fashion. We assessed MSNA by peroneal microneurography, blood pressure, cardiovascular and metabolic biomarkers at baseline and at the end of treatment.

Results: Both drugs elicited volume depletion, as indicated by increased thoracic impedance. Compared with HCT, Empa caused 1.23 kg more body weight loss ($P = 0.011$) and improved glycemic control. Seated systolic blood pressure decreased with both treatments ($P < 0.002$). MSNA did not change significantly with either treatment; however, MSNA changes were negatively correlated with changes in body weight on Empa ($P = 0.042$) and on HCT($P = 0.001$). The relationship was shifted to lower MSNA on Empa compared with HCT ($P = 0.002$).

Conclusion: Increased renal sodium excretion eliciting body weight loss may promote sympathetic activation. However, sympathetic excitation in the face of increased sodium loss may be attenuated by SGLT2 inhibitor-specific actions.

S GLT2 inhibitors improve cardiovascular and renal outcomes in patients with T2DM[1,2] and in nondiabetic patients with heart failure.[3,4] The mechanisms behind these benefits are not fully understood. Actions on the sympathetic nervous system have been implicated.[5,6] Sympathetic overactivity indicated by raised MSNA, which is sympathetic vasoconstrictor outflow to skeletal muscle vasculature, has been described in patients with obesity, T2DM, hypertension, and heart failure.[7-10] Sympathetic overactivity heralds poor clinical outcomes.[11] Conversely,

treatments attenuating sympathetic actions on the cardiovascular system can improve outcomes as evidenced by beta-blockade in patients with heart failure. In animals, SGLT2 expressing neurons known to regulate sympathetic activity responded to pharmacological SGLT2 inhibition[12] and SGLT2 inhibition may attenuate sympathetic activity.[13] In patients, SGLT2 inhibition while lowering blood pressure does not increase heart rate[14-17] possibly indicating sympathetic inhibition. In a several days proof-of-concept study, MSNA did not increase on SGLT2 inhibition despite reductions in blood pressure and volume loss,[18] whereas an uncontrolled study showed reductions in MSNA on SGLT2 inhibition in patients with T2DM and more so with concomitant heart failure.[19] Moreover, renin-angiotensin system activity, which is affected through renal sympathetic

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efferents, did not increase significantly.[20] Therefore, we tested the hypothesis that SGLT2 inhibition with Empa lowers MSNA in patients with T2DM compared with HCT in a double-blind and randomized fashion. We chose HCT as comparator to discern SGLT2 specific actions on the sympathetic nervous system from nonspecific responses to increased renal sodium excretion.

METHODS

Patients

We included patients with T2DM aged between 50 and 80 years on stable metformin monotherapy for at least 12 weeks. At inclusion, patients had to have hemoglobin A1c values between 6.5 and 10.0%, body mass index between 25 and 40 kg/m^2, systolic blood pressure between 110 mm Hg and 160 mm Hg, and diastolic blood pressure >60 mm Hg. Key exclusion criteria comprised heart failure NYHA II to IV; estimated glomerular filtration rate <60 ml/min per 1.73 m^2; myocardial infarction, stroke, or vascular interventions in the previous 12 months; history of ketoacidosis; significant liver disease; and frequent genital or lower urinary tract infections. Patients with contraindications to one of the study drugs, and pregnant or lactating women were also excluded.

Protocol

We conducted a prospective, randomized, active-controlled, 2-arm parallel, double-blind, double-dummy, matched, single-center phase IV clinical trial (Figure 1). After enrolment, which started in December 2017, patients were submitted to a 1-week run-in phase during which diuretics were discontinued. All other antihypertensive drugs were continued throughout the study. Following the run-in phase, patients underwent baseline autonomic cardiovascular profiling including microneurography. Then, we randomized patients 1:1 to either 25 mg/d Empa and HCT placebo or Empa placebo and 25 mg/d HCT. The double-dummy design was necessary because of the different appearance of Empa and HCT. The daily doses were chosen based on the maximum daily dose recommended for clinical use in patients with T2DM or hypertension, respectively. We aimed at building matched patient pairs to allow for paired statistical analysis. Matching criteria between patients have been relaxed after trial commencement by approved protocol amendment to facilitate matching. Final criteria included age within ±10 years, mean arterial pressure within ±10 mm Hg, body mass index within ±2.5 kg/m^2, and baseline MSNA within ±12 bursts/min. The treatment phase was 6 weeks long. At the end of the treatment phase, we repeated cardiovascular autonomic profiling. In the subsequent run-out
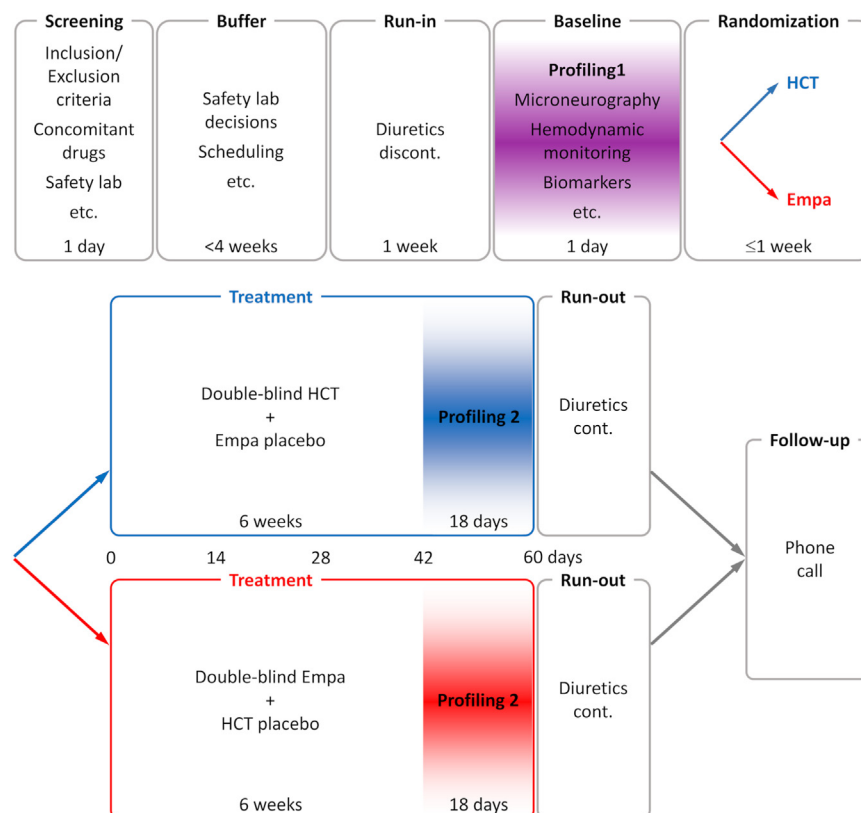

Figure 1. Study protocol.

phase, patients on diuretic therapy before the study were restarted on their diuretics. The trial ended regularly with the last follow-up visit in April 2020.

The study was designed, conducted, and reported in accordance with Good Clinical Practice and the Declaration of Helsinki. It was carried out at the Profil Institute for Clinical Research (Neuss, Germany). We obtained written informed consent from all participants before screening. The study protocol was approved by the North Rhine Medical Association (Ärztekammer Nordrhein, Germany; Protocol #2017267) and publicly registered at ClinicalTrials.gov, NCT03254849, and EudraCT, 2015-005733-44.

Cardiovascular and Autonomic Profiling
At screening, the arm with higher systolic blood pressure has been determined for subsequent visits. On visit days, we studied patients in a quiet room in the morning hours at a room temperature between 21 to 24 °C. After a 10-minunte rest, we measured office blood pressure thrice at the upper arm. Then, with the patient in supine position, we inserted an antecubital venous catheter and continuously recorded respiration, electrocardiogram , thoracic impedance (Cardioscreen, Medis GmbH), and beat-by-beat finger blood pressure (Finometer, FMS). We also determined median resting supine brachial blood pressure from 5 measurements with an automated oscillometric device (Dinamap, GE Medical Systems). We recorded postganglionic, multiunit MSNA from the peroneal nerve. Data were analog-to-digital converted and analyzed as described previously.[21] After 20 minutes of rest, we obtained baseline recordings for 5 minutes. Subsequently, we obtained venous blood sampling for plasma catecholamine, renin, and aldosterone determination. Then patients performed handgrip testing by pressing a rubber ball at 30% of maximal voluntary contraction for 3 minutes to evoke sympathoexcitatory metaboreflexes. We obtained estimates of heart rate and systolic blood pressure variability from the 5-minute supine rest recordings. Spontaneous cardiac baroreflex sensitivity was calculated as the slope of the linear regression between instantaneous systolic blood pressure and subsequent RR intervals (intervals between R-waves of the electrocardiogram) using the sequence method or by cross-spectral transfer function analysis in the low-frequency range (0.05–0.15 Hz).

Glucose Metabolism, Body Weight, and Safety Assessment
At baseline and at the end of therapy, we determined glucose, hemoglobin A1c, creatinine, sodium, potassium, hematocrit, transaminases, and urinary glucose excretion. During the study, urinary glucose measurements were not revealed to investigators in order to maintain blinding. We measured body height with a medical gauge with shoes off and body weight on a calibrated scale with the patient in underwear. We also determined waist circumference.

End Points
The primary end point of the study was the change in MSNA burst frequency from baseline to the end of therapy. Secondary endpoints were changes from baseline to end of therapy in office blood pressure, fasting glucose, hemoglobin A1c, body weight, fat mass, lean body mass, MSNA burst incidence [bursts/100 heart beats], MSNA burst area [arbitrary units/min], sympathetic baroreflex function, parasympathetic baroreflex function, and cardiac output.

Sample Size Justification
In a previous proof-of-concept study, MSNA burst frequency was similar before and after treatment with Empa.[18] In contrast, another study suggested that MSNA increased ≥5 bursts/min with spironolactone.[22] We expected an SD of therapy-baseline differences of 7 bursts/min.[23] Sample size calculation was conducted using the nQuery Advisor® 7.0 and calculation was performed using a paired t-test for mean difference regarding the matched structure of the data. The type I error was set to 2.5% (1-sided). Assuming further a clinically relevant difference of 5 bursts/min and conservatively an SD of 7 bursts/min, a sample size of 18 patients per treatment group is necessary to detect a difference between the groups with a power of 80%. Expecting a 10% drop-out rate, 2 additional matched pairs were planned to be included. This led to a total sample size of $n = 20$ pairs or $n = 40$ patients.

Randomization
We generated randomization codes using the computer program RANCODE Professional 3.6 (Datenanalyse und Versuchsplanung, Gauting/Munich, Germany). The 4-digit randomization numbers were a combination of 2 identifiers. The first 3 numbers were related to a matching pair. A subject's randomization number resulted from the pair number by appending 1 for partner A and 2 for partner B. Matched pairs were blocked on the matching list to avoid double matchings. The randomization/matching process and documentation was controlled and validated by an unblinded statistician who did not disclose the information. Allocation concealment has been achieved by having the study drug preparation, labeling, and accountability performed by an external pharmacy. Patients, study personnel, investigators assessing

outcomes, and sponsor representatives remained blind until database lock.

Statistics

As shown in Figure 2, the only reason for loss to follow-up was failure to obtain the prespecified primary readout, namely MSNA. In these 3 participants, baseline MSNA was above 30 bursts/min. Theoretically, MSNA could have been decreased by treatment to a degree that prevented its detection on follow-up. However, according to previous publications, this is very unlikely.[18,24] Therefore, we assume that these failures are not due to either treatment, and excluding these cases would not cause bias. On the other hand, their inclusion in intention-to-treat analysis might introduce bias through imputation methods such as, for example, last observation carried forward. Given the mechanism-oriented nature of the study and lack of a preferable approach for intention-to-treat analysis in the presence of missing outcome data,[25,26] we conducted the primary analysis on the modified per-protocol population, which included all patients with evaluable MSNA recordings at baseline and at the end of therapy who complied with the study protocol. Within the modified per-protocol analysis, we included patients without a matching partner using a mixed model analysis. The results of the per-protocol analysis (only including matched pairs) did not substantially differ from the results in the modified per-protocol population and are therefore not presented. We performed safety analyses in the full analysis set consisting of all randomized patients. We analyzed the primary end point (changes from baseline in MSNA burst frequency) by a mixed model with a Toeplitz covariance structure, including treatment and MSNA burst frequency at baseline as fixed effects and pair as random effect. Differences in MSNA change between Empa and HCT were considered significant when the upper bound of the mixed model-derived 2-sided 95% confidence intervals between treatments was below 0. Secondary endpoints were analyzed with the same mixed model as the primary analysis. To account for the consequences of body weight reduction on MSNA, which lumps together therapeutic effects on volume homeostasis and metabolism, the mixed model included change in body weight as covariate, treatment as fixed effect, and change in MSNA as response in an analysis of covariance.

RESULTS

Patients

Patient disposition is illustrated in Figure 2. Of 41 randomized patients, 20 were randomized to Empa and
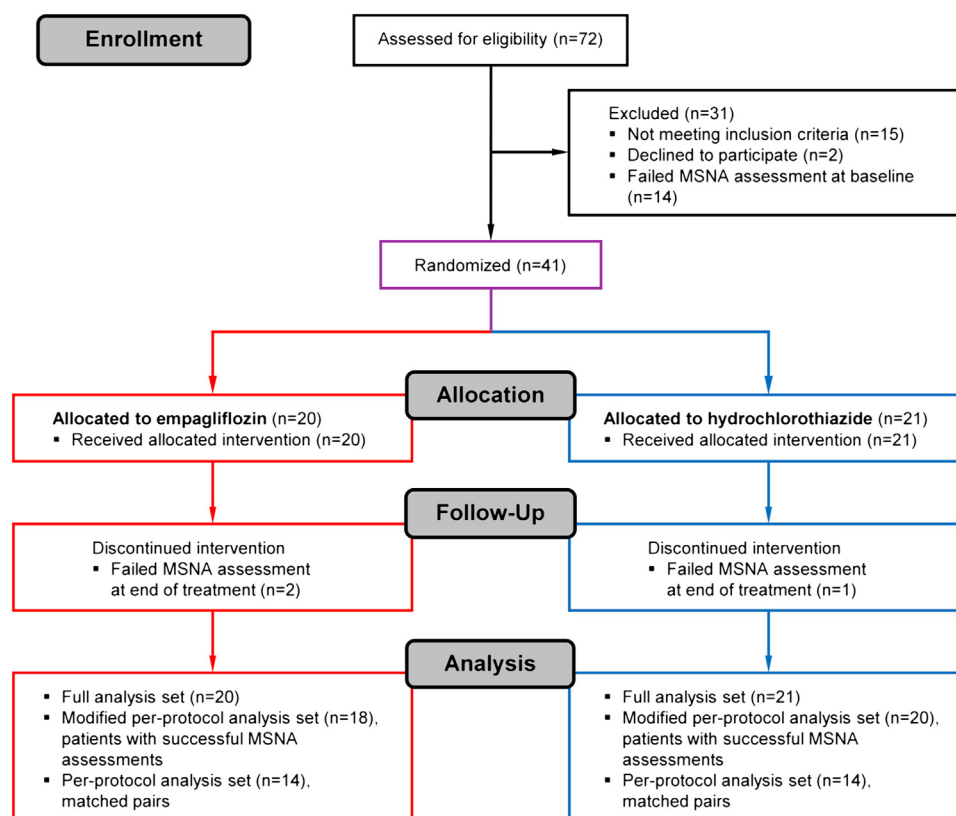

Figure 2. Patient disposition (CONSORT diagram).

21 to HCT treatment. Of those, 18 in the Empa group and 20 in the HCT group completed the study. Recruitment was stopped when the study medication expired although 20 matched pairs had not been reached at the time. Nevertheless, among completers, we could build 14 pairs matched for age, body mass index, mean arterial pressure, and baseline MSNA (per-protocol population). The remaining patients could not be paired but were included in the modified per-protocol analysis.

Patient demographics and baseline characteristics, including glycemic control and background medication are summarized in Table 1 and were comparable between groups. At screening, 6 patients in the Empa group and 5 patients in the HCT group were treated with thiazide diuretics. These were discontinued 1 week before baseline measurements and continued in the run-out phase. Patients' previous medical events are listed in Table 2. Both study treatments were well-tolerated and we did not observe any serious adverse event or any adverse event leading to drug discontinuation.

Body Weight, Glucose Metabolism, and Thoracic Volume Status

Body weight (Figure 3), waist circumference, metabolic and thoracic impedance responses to Empa and HCT are provided in Supplementary Table S1. Body weight decreased more on Empa compared with HCT illustrated by a mean difference of −1.23 kg

Table 1. Patients' baseline characteristics

Parameter	Empa	HCT	Total
Gender (F/M)	7/13	9/12	16/25
Age, y	64.6 ± 6.6	63.0 ± 6.1	63.7 ± 6.4
Weight, kg	92.8 ± 12.1	91.5 ± 17.1	92.2 ± 14.7
BMI, kg/m^2	31.2 ± 2.9	31.5 ± 4.0	31.3 ± 3.5
Diabetes duration, y	11.7 ± 5.7	12.6 ± 5.9	12.1 ± 5.8
HbA1c (DCCT), %	7.8 ± 0.8	7.9 ± 0.8	7.9 ± 0.8
HbA1c (IFCC), mmol/mol	62 ± 9	63 ± 9	63 ± 9
ALT, U/l	33.1 ± 16.2	34.4 ± 21.5	33.8 ± 18.9
AST, U/l	25.2 ± 8.1	29.8 ± 16.6	27.5 ± 13.2
Creatinine, μmol/l	74.3 ± 12.3	70.9 ± 14.3	72.5 ± 13.3
eGFR, ml/min per 1.73 m^2	86.9 ± 11.6	89.2 ± 9.6	88.1 ± 10.6
Proteinuria, dipstick	13 neg/7 pos	15 neg/6 pos	28 neg/13 pos
Antihypertensive treatment			
Ramipril/HCT	2	1	3
ARBs/HCT	3	2	5
Beta-Blockers/HCT	1	1	2
HCT	0	1	1
Antidiabetic treatment			
Metformin	20	21	41
DPP-4 Inhibitor	0	1	1

ALT, alanine transaminase; ARBs, angiotensin II receptor blockers; AST, aspartate transaminase; BMI, body mass index; DCCT, Diabetes Control and Complications Trial; DPP-4, dipeptidyl peptidase-4; eGFR, estimated glomerular filtration rate; Empa, empagliflozin; F, female; HCT, hydrochlorothiazide; IFCC, International Federation of Clinical Chemistry and Laboratory Medicine; M, male; neg, negative; pos, positive. Data are mean ± SD or numbers. All subjects were White.

Table 2. Medical history

Medical events	Empa	HCT	Total
Cardiac disorders	1	1	2
	Myocardial infarction	Supraventricular extrasystoles	
Musculoskeletal disorders	2	1	3
	Tendon rupture, back pain	Exostosis	
Benign neoplasms (lipoma)	1	0	1
Depression	1	0	1
Knee arthroplasty	1	0	1
Cerebrovascular events	1	1	2
Nasopharyngitis	0	1	1

Empa, empagliflozin; HCT, hydrochlorothiazide.

($P = 0.010$). There was no change in waist circumference ($P = 0.986$ and $P = 0.463$, respectively) and no difference between treatments ($P = 0.588$). Urinary glucose excretion increased by 45.6 mmol/l with Empa and 1.3 mmol/l with HCT ($P < 0.001$ between treatments). Fasting plasma glucose decreased with Empa ($P < 0.001$) but did not change with HCT ($P = 0.968$). Overall fasting plasma glucose decreased 1.92 mmol/l more on Empa than on HCT ($P < 0.001$). Hemoglobin A1c decreased 0.58% more on Empa than on HCT ($P < 0.001$). Basal thoracic impedance, which is an established surrogate for thoracic volume status, increased with both treatments ($P = 0.028$ with HCT and $P = 0.056$ with Empa). The response is consistent with reduction in thoracic blood volume. The change in thoracic impedance though numerically larger with HCT did not differ significantly between the 2 interventions ($P = 0.559$).

Hemodynamic Responses

Hemodynamic responses are summarized in Supplementary Table S2. Compared with before treatment, office systolic blood pressures were lower at the end of treatment with Empa ($P = 0.001$) and HCT
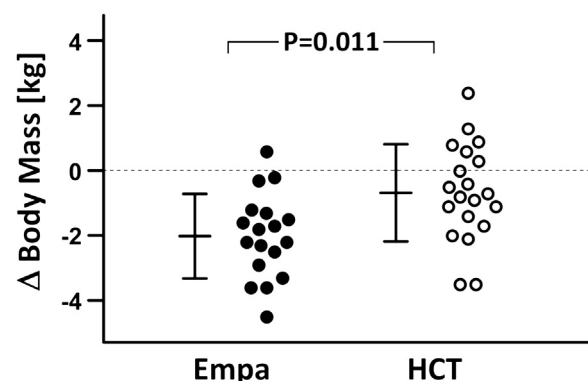

Figure 3. Treatment effects on body mass. Individual changes in body mass from baseline to the end of treatment with empagliflozin (Empa) or hydrochlorothiazide (HCT).
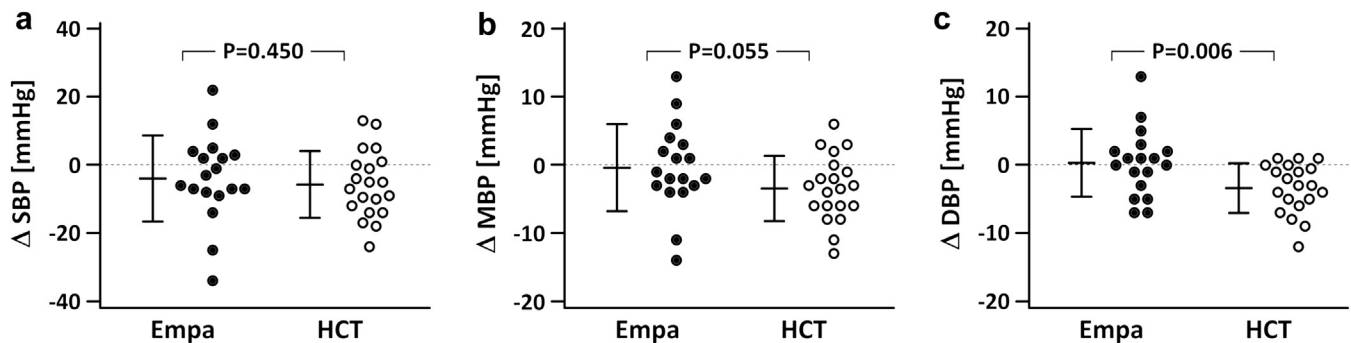
Figure 4. Treatment effects on blood pressure. Individual changes from baseline in supine blood pressure by treatment. (a) systolic blood pressure, SBP; (b) mean blood pressure, MBP; (c) diastolic blood pressure, DBP. Bars show mean values and SDs. Empa, empagliflozin; HCT, hydrochlorothiazide.

($P = 0.002$) with no difference between treatments ($P = 0.730$). Office diastolic blood pressure did not change significantly with either intervention ($P = 0.153$ and $P = 0.707$, respectively). Changes in supine blood pressure values, assessed at rest during microneurographic recordings, are shown in Figure 4. Supine systolic, mean, and diastolic blood pressures decreased with HCT treatment ($P = 0.008$, $P = 0.003$, $P < 0.001$, respectively). Supine diastolic blood pressure decreased 3.3 mmHg more with HCT compared with Empa ($P = 0.006$). The reduction in cardiac output was significant in the HCT arm ($P = 0.044$) but not significantly different from the change using Empa treatment ($P = 0.250$). There was a small drop in heart rate with Empa ($P = 0.028$), which was not statistically different from the change using HCT ($P = 0.192$). The pressor response to handgrip testing did not differ between interventions ($P = 0.491$).

Neurohumoral Regulation

Data related to neurohumoral regulation are shown in Supplementary Table S3. The original MSNA recordings from 2 patients during baseline and at the end of treatment with either Empa or HCT are shown in Figure 5. Individual MSNA changes during treatment are presented in Figure 6. MSNA burst frequencies did not significantly change with either medication ($P = 0.827$ and $P = 0.270$, respectively). The difference in MSNA burst frequency change between Empa and HCT treatment, the primary end point of the study, was -1.95 bursts/min ($P = 0.541$). Similarly, we did not observe a significant difference among matched pairs (per-protocol analysis) with a mean difference of -3.73 bursts/min between Empa and HCT treatment ($P = 0.320$). In addition, MSNA burst incidence and burst area responses did not differ between treatments ($P = 0.469$ and $P = 0.270$, respectively). In Figure 7, we show that MSNA treatment responses are not mediated by the arterial baroreflex (Empa: $P = 0.120$, HCT: $P = 0.700$). In Figure 8, we relate changes in MSNA burst

frequency to changes in body weight with Empa and HCT treatment. Weight changes include 2 factors with opposite effects on MSNA, namely diuretic volume loss and body fat reduction. The diuretic effect is represented by the significantly negative slopes of the regression lines ($P = 0.042$ and $P = 0.001$, respectively). Exploratory analysis of covariance suggests that the diuretic sympathoexcitation takes place at a 10.5 bursts/min lower level on Empa than on HCT (95% confidence interval: -17.1 to -4.0; $P = 0.002$). We observed similar treatment differences for MSNA burst incidence ($P = 0.011$) and burst area ($P = 0.031$). There were no correlations between treatment responses in sympathetic activity and thoracic impedance.

Baroreflex heart rate control determined by cross-spectral and sequence analysis did not differ between Empa and HCT-treated patients ($P > 0.200$ for all parameters). Plasma norepinephrine, epinephrine, and dopamine did not change significantly with either treatment ($P > 0.800$ for all parameters). Plasma renin concentration numerically increased on both
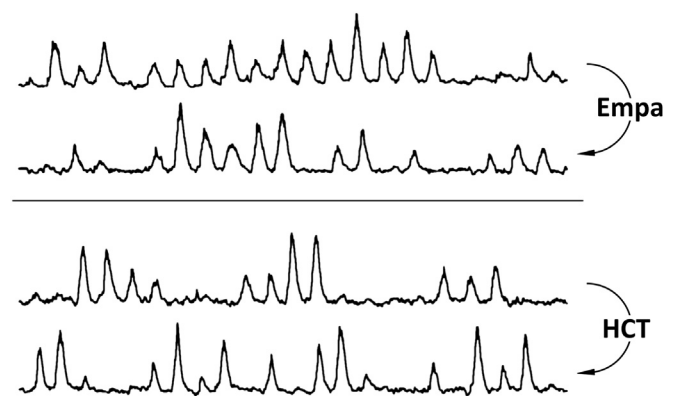

Figure 5. Original muscle sympathetic nerve activity recordings. Twenty seconds recordings of integrated muscle sympathetic nerve activity in 2 resting subjects during baseline and at the end of treatment with Empa or HCT. Empa, empagliflozin; HCT, Hydrochlorothiazide.
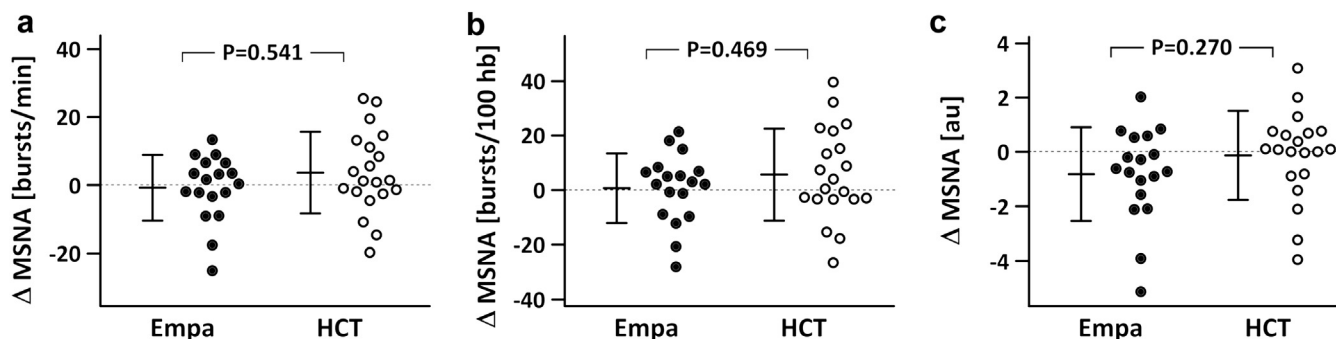
Figure 6. Treatment effects on sympathetic activity. Individual changes from baseline in sympathetic activity by treatment. (a) MSNA burst frequency; (b) MSNA burst incidence; (c) MSNA burst area. Bars show mean values and SDs. MSNA, muscle sympathetic nerve activity; Empa, empagliflozin; HCT, hydrochlorothiazide.

treatments; however, this did not reach statistical significance and did not differ between treatments ($P = 0.439$). Plasma aldosterone increased on Empa and not on HCT, however, the response did not differ significantly between treatments ($P = 0.173$).

DISCUSSION

To the best of our knowledge, this is the largest randomized controlled clinical trial using microneurography – the gold standard to measure sympathetic activity – in patients with T2DM. The important finding is that 6 weeks' treatment with the SGLT2 inhibitor Empa or with HCT affect sympathetic nerve traffic in a complex fashion. The change in resting MSNA, which was the primary end point of the study, did not differ significantly between treatments. However, we observed significant negative correlations between changes in body weight and changes in MSNA with HCT and Empa. Sympathoexcitation with body weight loss likely results from reductions in central blood volume through diuretic actions.
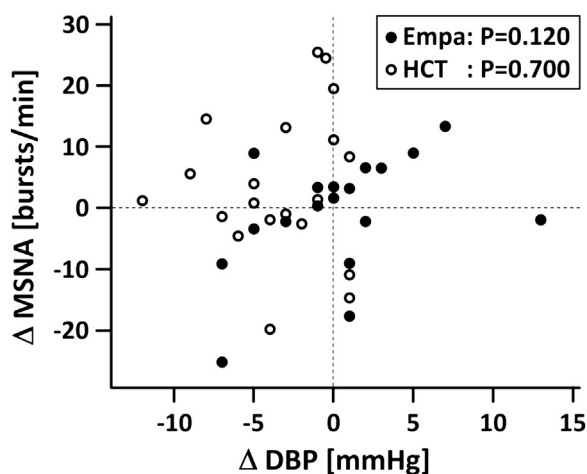

Figure 7. Sympathetic arterial baroreflex involvement in treatment effects. Relationship between changes in sympathetic activity (MSNA) and diastolic blood pressure (DBP) by treatment. Empa, empagliflozin; HCT, hydrochlorothiazide.

Remarkably, the relationship was shifted toward lower sympathetic activity on Empa.

In mice, single dose SGLT2 inhibition with dapagliflozin elicited c-Fos expression in brain areas governing sympathetic activity.[12] In rabbits with alloxan-induced diabetes, 1-week Empa treatment increased arterial norepinephrine and total norepinephrine spillover. Renal sympathetic nerve activity was unchanged and increased less during baroreflex unloading.[27] In mice fed with high fat chow, dapagliflozin improved blood pressure and weight gain while attenuating renal sympathetic innervation and norepinephrine content.[13] Conversely, pharmacological SGLT2 inhibition attenuated weight gain through sympathetically-mediated lipolysis and energy expenditure in another mouse model.[28] In patients with heart failure, heart rate variability, particularly the ratio between low frequency and high frequency RR interval oscillations, improved on Empa but not on placebo.[29] Finally, MSNA was reduced after 12 weeks' treatment with the SGLT2 inhibitor dapagliflozin compared with baseline measurements in a single-arm, open label study in patients with T2DM with or without heart failure.[19]

We applied biochemical and physiological profiling, including MSNA measurements through microneurography[10] in a rigorously conducted clinical trial comparing Empa and HCT. Both drugs increase renal sodium excretion and lower blood pressure. In a smaller study, a diuretic-induced volume loss elicited baroreflex-mediated sympathetic activation of approximately 5 bursts/min.[22] However, the relationship between sodium excretion, volume depletion, hemodynamics, and sympathetic activity on diuretics is complex. Early observations suggested that thiazide diuretics produce transient plasma volume reductions,[30] such that the stimulus raising sympathetic activity may abate over time. Plasma volume reductions may be more sustained with SGLT2 inhibition.[31] However, in our study, thoracic fluid volume
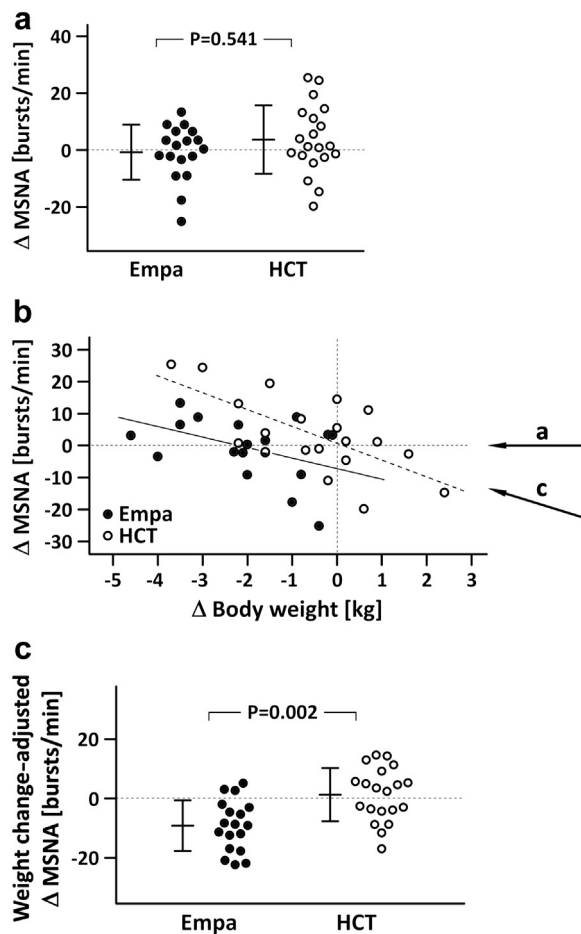
Figure 8. Method to unmask different treatment effects on muscle sympathetic nerve activity (MSNA). (a) Treatment effects on MSNA without consideration of weight changes (copy of Figure 6a). (b) Relationship between changes in body weight and MSNA from baseline to end of therapy with empagliflozin (closed circles and solid regression line) and hydrochlorothiazide (open circles and dashed regression line). Weight changes include 2 components with distinct effects on sympathetic vasoconstrictor tone. First, body fat reduction is known to lower MSNA. Second, diuretic volume loss increases MSNA which is reflected by the negative slopes of the regression lines ($P = 0.042$ and $P = 0.001$, respectively). The slopes are not different ($P = 0.338$, pooled slope: 4.42 (bursts/min)/kg). Looking at the data in Panel b along arrow 'a' reproduces the data in Panel a with large in-group data variance (min/max, Empa: −25.2/+13.3, HCT: −19.8/+25.4 bursts/min). The variance may obscure different medication effects on MSNA. Analysis of covariance with body weight change as covariate reveals that the weight loss-dependent sympathoexcitation takes place at a 10.5 bursts/min lower level with empagliflozin ($P = 0.002$). (c) Analysis of covariance with body weight change as covariate controls for weight changes. The approach can be envisioned by looking at the data in Panel b along arrow "c." Reduced in-group data variance (min/max, Empa: −22.6/+5.0, HCT: −17.2/+14.5 bursts/min) facilitates treatment group separation.

estimated from impedance measurements decreased with Empa and with HCT. The negative correlations between changes in MSNA and in body weight could indicate involvement of the cardiopulmonary baroreflex in individual treatment responses, whereas arterial baroreflex-mediated sympathoexcitation seems to play no role given the lack of a negative correlation between changes in MSNA and in diastolic blood pressure. However, changes in sodium balance may affect sympathetic activity through volume-independent actions. In addition, SGLT2 inhibition reduces tissue sodium storage in the skin in human beings.[32] Animal studies suggest that changes in tissue sodium in the brain could affect sympathetic activity.[33] Finally, intrarenal mechanisms regulating afferent renal nerve traffic could modulate sympathetic activity. Afferent renal nerves are important in the regulation of efferent sympathetic activity on high sodium intake.[34]

Unlike HCT, Empa improves glucose metabolism and produces a negative energy balance promoting significant weight loss.[14-17] These metabolic actions, particularly weight loss,[35-39] usually lower sympathetic activity. In fact, we observed a larger reduction in body weight with Empa whereas thoracic volume responded similarly to both drugs. The differential effect on energy balance may explain at least in part why the relationship between body weight changes and sympathetic activation was shifted toward lower sympathetic activity on Empa compared with HCT. The idea is supported by our previous observations that weight loss after short-term Empa treatment, which primarily resulted from volume loss, did not elicit sympathetic activation.[18]

Study Limitations

The main limitation of our study is that the sample size was limited. To improve statistical power, we minimized variabilities in several ways: in healthy subjects, it has been demonstrated, that repeat MSNA assessments within subjects is remarkably reproducible in contrast to the large inter-subject variability.[40] For instance, in a previous study, a 4.3 bursts/min difference in MSNA was statistically detectable in recordings from 22 healthy subjects.[41] In the present study, we compared MSNA changes over treatment instead of MSNA levels at the end of interventions only. Furthermore, we tried to alleviate variability by including matched pairs. However, despite major recruitment efforts we could pair 14 of the 18 to 20 completing patients in each treatment group. Microneurography studies are difficult to conduct, particularly in older patients with T2DM. Failure rates in finding sympathetic activity occurred in 60% or more of patients with diabetes.[42,43] We could not obtain stable nerve recording positions in 14 of 55 (25%) baseline measurements; 3 more failures occurred during follow-up (Figure 2).

Nevertheless, to our knowledge, this is the largest interventional microneurography study in patients with T2DM. Finally, by diminishing weight-change related variance we could detect treatment differences.

Another potential limitation is that we treated patients only for 6 weeks. Influences of SGLT2 inhibition on renal function, which could in turn affect sympathetic activity, may have a slower onset. Moreover, we were not able to accurately differentiate between the contributions of diuresis and body fat to weight changes. Therefore, we suggest that future studies testing influences of drugs with diuretic actions on sympathetic activity should utilize more detailed total blood volume measurements. Finally, because we excluded patients with heart failure, our findings cannot be simply extrapolated to this population.

CONCLUSION

In conclusion, our mechanistic profiling study provides insight into pharmacological actions of SGLT2 inhibitors on human cardiovascular autonomic control, which may have relevance for cardiovascular outcomes. Moreover, our study may help explaining discrepancies in the literature regarding changes in sympathetic activity on drugs with diuretic actions. For example, in smaller previous studies, sympathetic burst incidence increased ($n = 7$)[44] or remained unchanged ($n = 12$)[24] on HCT. Possibly, interindividual differences in volume loss on thiazides contribute to the variability in sympathetic nervous system responses. Given the important role of the sympathetic nervous system in the progression of cardiovascular disease, mechanisms attenuating sympathetic activity in the face of increased sodium excretion deserve further attention.

DISCLOSURE

Data Availability

The data underlying this article will be shared on reasonable request to the corresponding author.

SUPPLEMENTARY MATERIAL

Supplementary File (PDF)
Table S1. Glucose metabolism, body composition, and volume status.
Table S2. Hemodynamic responses.
Table S3. Neurohumoral responses.
CONSORT Checklist.

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