



Empagliflozin Improves Insulin Sensitivity of the Hypothalamus in Humans With Prediabetes: A Randomized, Double-Blind, Placebo-Controlled, Phase 2 Trial

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Stephanie Kullmann,^{1,2} Julia Hummel,^{1,2}
Robert Wagner,^{1,2,3} Corinna Dannecker,^{1,2}
Andreas Vosseler,^{1,2,3} Louise Fritsche,^{1,2}
Ralf Veit,^{1,2} Konstantinos Kantartzis,^{1,2}
Jürgen Machann,^{1,2,4}
Andreas L. Birkenfeld,^{1,2,3}
Norbert Stefan,^{1,2,3}
Hans-Ulrich Häring,^{1,2,3}
Andreas Peter,^{1,2,5} Hubert Preissl,^{1,2,3,6,7}
Andreas Fritsche,^{1,2,3} and
Martin Heni^{1,2,3,5}

OBJECTIVE

Insulin action in the human brain reduces food intake, improves whole-body insulin sensitivity, and modulates body fat mass and its distribution. Obesity and type 2 diabetes are often associated with brain insulin resistance, resulting in impaired brain-derived modulation of peripheral metabolism. So far, no pharmacological treatment for brain insulin resistance has been established. Since sodium–glucose cotransporter 2 (SGLT2) inhibitors lower glucose levels and modulate energy metabolism, we hypothesized that SGLT2 inhibition may be a pharmacological approach to reverse brain insulin resistance.

RESEARCH DESIGN AND METHODS

In this randomized, double-blind, placebo-controlled clinical trial, 40 patients (mean ± SD; age 60 ± 9 years; BMI 31.5 ± 3.8 kg/m²) with prediabetes were randomized to receive 25 mg empagliflozin every day or placebo. Before and after 8 weeks of treatment, brain insulin sensitivity was assessed by functional MRI combined with intranasal administration of insulin to the brain.

RESULTS

We identified a significant interaction between time and treatment in the hypothalamic response to insulin. Post hoc analyses revealed that only empagliflozin-treated patients experienced increased hypothalamic insulin responsiveness. Hypothalamic insulin action significantly mediated the empagliflozin-induced decrease in fasting glucose and liver fat.

CONCLUSIONS

Our results corroborate insulin resistance of the hypothalamus in humans with prediabetes. Treatment with empagliflozin for 8 weeks was able to restore hypothalamic insulin sensitivity, a favorable response that could contribute to the beneficial effects of SGLT2 inhibitors. Our findings position SGLT2 inhibition as the first pharmacological approach to reverse brain insulin resistance, with potential benefits for adiposity and whole-body metabolism.

¹Institute for Diabetes Research and Metabolic Diseases of the Helmholtz Center Munich at the University of Tübingen, Tübingen, Germany

²German Center for Diabetes Research, Neuherberg, Germany

³Division of Diabetology, Endocrinology and Nephrology, Department of Internal Medicine, Eberhard Karls University Tübingen, Tübingen, Germany

⁴Department of Diagnostic and Interventional Radiology, Section of Experimental Radiology, Eberhard Karls University Tübingen, Tübingen, Germany

⁵Institute for Clinical Chemistry and Pathobiochemistry, Department for Diagnostic Laboratory Medicine, Eberhard Karls University Tübingen, Tübingen, Germany

⁶Institute of Pharmaceutical Sciences, Department of Pharmacy and Biochemistry, Interfaculty Center for Pharmacogenomics and Pharma Research at the Eberhard Karls University Tübingen, Tübingen, Germany

⁷Institute for Diabetes and Obesity, Helmholtz Diabetes Center, Helmholtz Zentrum München, German Research Center for Environmental Health (GmbH), Neuherberg, Germany

Corresponding author: Martin Heni, martin.heni@med.uni-tuebingen.de

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S.K. and J.H. contributed equally to this work.

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See accompanying article, p. XXX.

Over the last 15 years, the human brain has been identified as an important insulin-sensitive organ (1,2). Insulin action in the brain modulates eating behavior, body

weight, and body fat content (1). Moreover, it improves peripheral metabolism by enhancing whole-body insulin sensitivity (1,3), suppressing hepatic glucose production (3,4), and stimulating pancreatic insulin secretion (5). Pioneering studies in mice revealed that selective disruption of neural insulin receptors, particularly in the hypothalamus, induced an obese phenotype with increased body fat and peripheral insulin resistance (6). Restoring insulin action in the hypothalamus, in contrast, prevented diabetes (7). The tremendous significance of hypothalamic insulin action for peripheral metabolism was first shown in rodent models, in which insulin signaling in the hypothalamus was discovered to control hepatic glucose production (8). In line with experimental evidence from animals (2), insulin action in the human hypothalamus appears to be central for these brain-derived effects on the periphery (3,5). Many studies on insulin action in the human brain combined brain imaging with the administration of insulin as nasal spray. This route of administration enables insulin delivery into the brain, while only tiny amounts are absorbed into the bloodstream (9). Thus, intranasal insulin enables selective stimulation of brain insulin action. In this study, we used the nasal insulin dose that showed robust effects on regional brain activity in two previous dose-response trials (10,11). A reduction in hypothalamic activity in response to intranasal insulin indicates insulin sensitivity (12–14).

Of note, a substantial number of people display reduced, or even absent, brain responses to insulin, a condition termed brain insulin resistance (1). While obesity is the most prominent condition linked to insulin resistance of the brain, a number of additional associated risk factors have been identified (1,2). Impaired hypothalamic insulin action, based on failure to reduce hypothalamus activity in response to central insulin (12,14), hinders signals to the periphery (1,2,14) and thereby predisposes for whole-body insulin resistance (3) and insufficient postprandial insulin secretion (5). Furthermore, brain insulin resistance is linked to long-term weight gain and unhealthy body fat distribution (14).

For the treatment of brain insulin resistance, several approaches are discussed (1). Among the pharmacological candidates are sodium–glucose

cotransporter 2 (SGLT2) inhibitors. This class of substances has been developed for the treatment of diabetes, as inhibition of SGLT2 promotes glucose excretion through the urine, thereby lowering blood glucose (15). Large clinical trials with SGLT2 inhibitors demonstrated their ability to improve morbidity and reduce mortality (15), with benefits even in patients without diabetes (15). Among others, an increase of glucagon (16–18) or ketone body concentrations (16) in the circulation has been proposed as a potential mechanism explaining their beneficial effects (19,20).

Comparable to humans, brain insulin action is reduced in rodents with high-fat diet–induced obesity (2). Administration of the SGLT2 inhibitor dapagliflozin to high-fat diet–fed rats restored brain insulin signaling, improved whole-body metabolism, and prevented cognitive decline (21). One proposed pathomechanism for brain insulin resistance is hypothalamic subclinical inflammation. In fact, canagliflozin, another SGLT2 inhibitor, was able to revert inflammation in the hypothalamus of mice fed a high-fat diet (22). At least some of the beneficial effects of this class of substances on the entire body appear to rely on intact brain–periphery cross talk via the parasympathetic nervous system. Vagotomy in obese mice attenuated body weight reduction in response to the SGLT2 inhibitor tofogliflozin (23), indicating that the therapeutic effect at least partially depends on the intact brain–periphery communication via the vagus nerve. Thus, there is evidence that SGLT2 inhibition is able to suppress inflammation and revert insulin resistance in the hypothalamus of obese rodents. Furthermore, an intact communication between the brain and the periphery appears to be necessary for some of the beneficial effects of these substances. Though, the potential of SGLT2 inhibitors to treat brain insulin resistance in humans is still unknown.

In this randomized, controlled, phase 2 trial, we therefore tested effects of the potent SGLT2 inhibitor empagliflozin on brain insulin action in overweight and obese patients with prediabetes.

RESEARCH DESIGN AND METHODS

This randomized, placebo-controlled, double-blind, phase 2 trial was conducted at the University Hospital of Tübingen

between July 2017 and October 2019. The protocol was approved by the local ethics committee and conducted according to the Declaration of Helsinki and the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use Good Clinical Practice guidelines. This study was pre-registered at ClinicalTrials.gov under number NCT03227484 and at the European Union Clinical Trials Register under EudraCT2016–003477-18 (<https://eudract.ema.europa.eu/>).

Study Design and Patients

Forty-two patients were randomized to receive either 25 mg empagliflozin or placebo, once daily, for 8 weeks (Supplementary Fig. 1) using a block randomization with a block size of 4. Patient characteristics are presented in Tables 1 and 2. Power considerations and sample size determination are presented as Supplementary Material 6. Before enrollment, patients provided informed written consent. Prior to randomization, a medical history was obtained, and a physical examination as well as a 75-g oral glucose tolerance test (OGTT) were performed. Detailed inclusion and exclusion criteria are given in Supplementary Table 5. In short, patients between 30 and 75 years of age had to be overweight or obese ($\text{BMI} \geq 25$ and $\leq 40 \text{ kg/m}^2$) and show impaired fasting glucose and/or glucose tolerance (Table 2), according to the American Diabetes Association criteria (24). Detailed phenotyping was conducted before and after drug intake (for details, see Supplementary Fig. 2). On the days of study visits, patients took any concomitant medication (and the study drug at the end of study visit) only after completion of the respective examinations. Further, they were instructed to refrain from exercise and alcohol intake the day prior to each study visit and to refrain from smoking on the days of examinations. Depression was ruled out by Beck Depression Inventory II. Patients completed food diaries on 7 consecutive days before and at the end of treatment. Diet composition was estimated with a validated software using 4 days with complete data out of the 7-day food diary (DGE-PC 3.0; German Nutrition Society, Bonn, Germany). A questionnaire addressing a subjective feeling of hunger was assessed before nasal spray application

Table 1—Outcomes

	Placebo (n = 21)		Empagliflozin (n = 19)		<i>P</i> _(MANOVA)
	Before	After 8 weeks	Before	After 8 weeks	
Blood pressure					
Systolic (mmHg)	144 ± 12	145 ± 17	141 ± 17	137 ± 17	0.5
Diastolic (mmHg)	93 ± 10	91 ± 10	88 ± 10	88 ± 10	0.5
Heart rate (bpm)					
	67 ± 9	67 ± 11	76 ± 11	75 ± 8	0.6
Glycemia					
HbA _{1c} (%)	5.74 ± 0.30	5.70 ± 0.32	5.76 ± 0.28	5.69 ± 0.29	0.7
HbA _{1c} (mmol/mol)	39.3 ± 3.3	38.7 ± 3.4	39.4 ± 3.1	38.6 ± 3.3	0.7
Fasting glucose (mmol/L)	5.88 ± 0.66	5.64 ± 0.57	6.09 ± 0.43	5.60 ± 0.46	0.031
2-h glucose (mmol/L)	8.07 ± 1.68	7.85 ± 1.90	7.66 ± 1.35	7.77 ± 1.72	0.6
Area under the glucose curve (mmol/L)	18.49 ± 3.05	18.15 ± 3.23	18.63 ± 3.07	17.73 ± 2.83	0.4
HOMA-IR	5.1 ± 3.2	4.2 ± 2.6	5.1 ± 2.5	4.3 ± 2.3	0.8
Matsuda insulin sensitivity index (OGTT-derived)	6.7 ± 3.7	7.5 ± 4.4	6.8 ± 3.1	7.8 ± 3.9	0.8
NEFA insulin sensitivity index (OGTT-derived)	2.53 ± 1.02	2.75 ± 1.10	2.66 ± 1.05 ^a	2.72 ± 0.92	0.2
Body composition					
BMI (kg/m ²)	30.75 ± 3.32	30.61 ± 3.29	32.33 ± 4.15	31.90 ± 4.16	0.1
Total adipose tissue, MR-derived (L)	37.9 ± 9.3	38.1 ± 9.2	41.9 ± 10	41.2 ± 9.6	0.021
Subcutaneous adipose tissue, lower extremity, MR-derived (L)	13.2 ± 3.6	13.2 ± 3.4	14.5 ± 3.3	14.2 ± 3.2	0.1
Visceral adipose tissue, MR-derived (L)	6.1 ± 2.4	6.2 ± 2.4	5.6 ± 2.5	5.7 ± 2.5	0.5
Intrahepatic fat, MRS-derived (%)	9.9 ± 7.0	11.2 ± 7.6	9.7 ± 6.9 ^a	9.1 ± 6.6 ^a	0.005
Indirect calorimetry					
Resting energy expenditure (kcal)	1,862 ± 316 ^b	1,782 ± 291 ^b	1,834 ± 420	1,849 ± 413	0.3 ^c
RQ	0.94 ± 0.09 ^b	0.99 ± 0.15 ^b	0.95 ± 0.10	0.91 ± 0.07	0.026

Data are means ± SD. A repeated-measures ANOVA was performed to investigate treatment × time interactions. ^a*n* = 18. ^b*n* = 20. ^cAdjusted for sex.

(functional MRI measurement day). Subjective feeling of hunger was rated using a visual analog scale from 0 to 10 (0 = not hungry at all; 10 = very hungry).

Functional MRI

Data Acquisition

Brain imaging was conducted in a 3T whole-body Siemens scanner (MAGNETOM Prisma; Siemens Healthineers, Erlangen,

Germany) with a 20-channel head coil. The time course of this measurement is depicted in Supplementary Fig. 3. Whole-brain cerebral blood flow (CBF) was recorded at each visit before and 30 min after nasal insulin spray application. A total of 160 units of regular human insulin (Actrapid; Novo Nordisk, Bagsvaerd, Denmark) were administered as nasal spray (8 puffs containing 10 units in each

nostril over 4 min). In addition, high-resolution T1-weighted anatomical images were obtained.

To acquire CBF, a quantitative measure of brain activity, pulsed arterial spin labeling measurements, was obtained with a PICORE-Q2TIPS (proximal inversion with control for off-resonance effects—quantitative imaging of perfusion by using a single subtraction) sequence by using a frequency offset corrected inversion pulse and echo planar imaging readout for acquisition. A total of 16 axial slices with a slice thickness of 4.5 mm (0.90-mm gap) were acquired in ascending order. Each measurement consisted of 79 alternating tag and control images with the following imaging parameters: inversion time (TI), TI1 = 700 ms, TI2 = 1,800 ms; repetition time = 3,000 ms; echo time = 13 ms; in-plane resolution = 3 × 3 mm²; field of view = 192 mm; matrix size 64 × 64; and flip angle = 90°, resulting in a total scan time of 4:02 min. The first image of the series (M0) was measured before the preparation scans and used to estimate the equilibrium magnetization of the blood (MOB) for absolute CBF quantification.

Table 2—Patient characteristics

	Placebo (n = 21)	Empagliflozin (n = 19)
Sex		
Men	11	5
Women	10	14
Age (years)	62.5 ± 7.7	57.2 ± 9.9
BMI (kg/m ²)	30.8 ± 3.3	32.3 ± 4.2
Waist-to-hip ratio	0.95 ± 0.06	0.93 ± 0.06 ^a
Glycemic category (IFG/IGT/IFG+IGT)	5/6/10	10/1/8
Smoker (yes/no)	3/18	1/18
Antihypertensive drugs (yes/no)	14/7	9/10
Lipid-lowering drugs (yes/no)	3/18	3/16
BDI score	3.7 ± 2.3	6.6 ± 5.6

Data are means ± SD unless otherwise indicated. BDI, Beck Depression Inventory II; IFG, impaired fasting glucose; IGT, impaired glucose tolerance. ^a*n* = 18.

ASL Image Processing

Image preprocessing was performed by using the ASLtbx with SPM12 (Wellcome Trust Centre for Neuroimaging). Functional images were motion corrected, coregistered to the individual anatomical image, and smoothed (full width at half maximum: 6 mm). Perfusion images were generated by calculating the control-tag differences by using surround subtraction. For accurate CBF quantification ($\text{mL} \times 100 \text{ g}^{-1} \times \text{min}^{-1}$), we used a unique MO value extracted from a region of interest in the cerebrospinal fluid. For absolute perfusion quantification, the general kinetic model was applied. Possible outliers were cleaned using a slice-wise procedure based on priors. The high-resolution T1-weighted image was normalized in Montreal Neurological Institute space ($1 \times 1 \times 1 \text{ mm}$) using SPM12's unified segmentation normalization, and the resulting parameter file was used with the individual coregistered CBF maps in normalized space ($3 \times 3 \times 3 \text{ mm}$). A brain mask was used to exclude extracranial voxels in the normalized CBF images. Image quality was visually inspected; patients showing strong artifacts were excluded from the analyses ($n = 3$).

Whole-Body Metabolism

Before and after treatment, patients underwent a 75-g OGTT in a seated or lying position after an overnight fast (Accu-Check Dextrose O.G-T.; Roche Diagnostics Deutschland GmbH, Mannheim, Germany). Venous blood samples were obtained before as well as 30, 60, 90, and 120 min post-glucose ingestion. Plasma nonesterified fatty acid (NEFA) concentrations (enzymatic method; WAKO Chemicals, Neuss, Germany) as well as clinical chemical parameters were measured on the ADVIA Clinical Chemistry XPT System. Serum insulin and C-peptide were measured on the ADVIA Centaur and erythropoietin on the Immulite immunoassay systems (all from Siemens Healthineers). Glycated hemoglobin (HbA_{1c}) measurements were performed using the Tosoh A1c analyzer HLC-723G8 (Tosoh Bioscience GmbH, Griesheim, Germany). All of these measurements have been performed in an accredited diagnostic laboratory.

Glucagon was measured at all five time points of the OGTTs using a commercial immunoassay (Mercodia, Uppsala, Sweden). Fasting β -hydroxybutyric

acid was quantified using an enzymatic 3-hydroxybutyrate dehydrogenase-based Ketone Body Assay Kit (Sigma-Aldrich, St. Louis, MO).

Areas under the curve were calculated according to the trapezoid rule. Whole-body insulin sensitivity was assessed as HOMA of insulin resistance (HOMA-IR) in the fasting state and Matsuda index as well as NEFA index during the OGTT.

While we planned to address heart rate variability, technical recording errors prevented sufficient analyses of these data.

Investigators were blinded for the urine glucose excretion data.

Intrahepatic Fat and Body Fat Distribution

Intrahepatic fat content and body fat distribution were assessed by ^1H -MRS and MRI as described before (25). Briefly, intrahepatic fat content was quantified from a volume of interest of $3 \times 3 \times 2 \text{ cm}^3$, which was placed in the posterior part of segment seven in the liver with subjects being in expiration during data acquisition. Intrahepatic fat is given by the integral of methylene plus methyl resonances (fat) divided by water plus fat in percentage of total signal. Axial T1-weighted whole-body MRI was performed providing quantification of different adipose tissue compartments along the axis of the body.

Indirect Calorimetry

The exhaled air was analyzed for at least 15 min after bed rest prior to measurement with Vyntus CPX (CareFusion, Hoechberg, Germany) after an overnight fast to assess resting energy expenditure and the respiratory quotient (RQ). An RQ of 1 indicates preferential glucose metabolism, whereas an RQ of 0.7 reflects predominant lipid oxidation.

Statistical Analyses

All analyses were performed in the intention-to-treat population, including all patients, for whom baseline and 8-week data were available. Unless otherwise stated, data are presented as mean \pm SD. A P value of ≤ 0.05 was considered to indicate statistical significance.

Analysis of the Primary Outcome Brain Insulin Action Using CBF

The prespecified primary outcome was absolute CBF changes in response to intranasal insulin from before (pre-) to

after the 8-week intervention. To this end, CBF maps of each patient were corrected for baseline measurements to determine the effect of central insulin action before ($\Delta\text{CBF}_{\text{pre}} = \text{CBF}_{\text{fMRI-2}} - \text{CBF}_{\text{fMRI-1}}$) and after the intervention ($\Delta\text{CBF}_{\text{post-8-wk}} = \text{CBF}_{\text{fMRI-2}} - \text{CBF}_{\text{fMRI-1}}$) (see Supplementary Fig. 3). Whole-brain analyses were performed using a voxel-wise approach in SPM12. ΔCBF maps of each measurement day of each patient were entered into a flexible factorial design to determine the interaction of treatment \times time on central insulin action. A statistical threshold of $P < 0.001$ uncorrected and a $P < 0.05$ family-wise error (FWE) corrected for multiple comparisons at a cluster level was applied. Additionally, small volume correction was performed for the hypothalamus, the striatum, and the hippocampus, as they are a priori regions of interest. The masks were based on the *wfu* pick atlas (<https://fmri.wfubmc.edu/software/PickAtlas>).

Additionally, we extracted CBF values of significant clusters to perform post hoc analyses in SPSS (version 27; SPSS Inc).

Analyses of Secondary Outcomes

Statistical analyses of the secondary outcome data were conducted using JMP 14 (SAS Institute, Cary, NC). Treatment \times time interactions were tested by repeated-measures ANOVA. Correlations were assessed by multiple linear regression analyses.

Mediation analyses of the relationship among treatment group (binary variable), fasting glucose levels, intrahepatic fat, urinary glucose, and hypothalamus insulin response ($\Delta\text{CBF}_{\text{post-8-wk}}$) at follow-up were performed using PROCESS version 3.3 procedure in SPSS (www.afhayes.com). The relationship between a predictor (x) and an outcome variable (y) can be explained by their relationship to a third variable (the mediator [m]). The predictor (x) predicts the mediator (m) through the path denoted by path a . The mediator (m) predicts the outcome (y) through the outcome denoted by path b . The relationship between predictor and outcome controlling for the mediator in the model is denoted as direct effect (i.e., path c'). The total effect is the effect of the predictor on the outcome when the mediator is not present in the model (i.e., path c). The effect of

mediation is investigated through the indirect effect, which is a cross product of a and b . The mediation model included treatment group (binary variable) as the predictor (x), fasting glucose or urinary glucose or intrahepatic fat at follow-up as the outcome variable (y), and hypothalamus insulin response at follow-up as the mediator (m). The significance of the mediation analysis (i.e., indirect effect ab) was estimated based on a bias-corrected bootstrap CI (95% CI, 10,000 bootstrap samples). If the CI does not contain zero, then the “true effect” size is different from “no effect,” indicating a significant mediation.

Data and Resource Availability

The data generated during the current study are not publicly available due to the data containing information that could compromise research participant privacy/consent.

Results

Of the 42 randomized patients, 40 completed the trial with 2 dropouts in the empagliflozin group (Supplementary Fig. 1). Retrospective measurement of urinary glucose concentrations ensured drug adherence in patients randomized to empagliflozin (Supplementary Fig. 4). Among the routine clinical chemistry measurements, only differences in alkaline phosphatase, CRP, magnesium, and uric acid were detected (Supplementary Table 4). A number of adverse events were recorded in both treatment groups (empagliflozin, $n = 18$; placebo, $n = 12$) (Supplementary Table 1). No severe adverse events occurred during the conduct of this trial.

For the prespecified primary outcome, we analyzed the impact of empagliflozin versus placebo on insulin responsiveness of the brain by combining functional MRI with nasal insulin. Whole-brain analysis revealed a significant time \times treatment interaction solely in the hypothalamus [$F(1,35) = 13.18$; $P < 0.001$, uncorrected; $P_{FWE} = 0.03$, small-volume corrected] (Fig. 1A and B). This remained significant after adjustment for only BMI as well as for BMI, sex, and age. Post hoc analyses detected no differences between groups prior to treatment ($P > 0.05$), though the insulin response was different between the two treatment arms after 8 weeks of treatment [$T(32) = 2.46$; $P =$

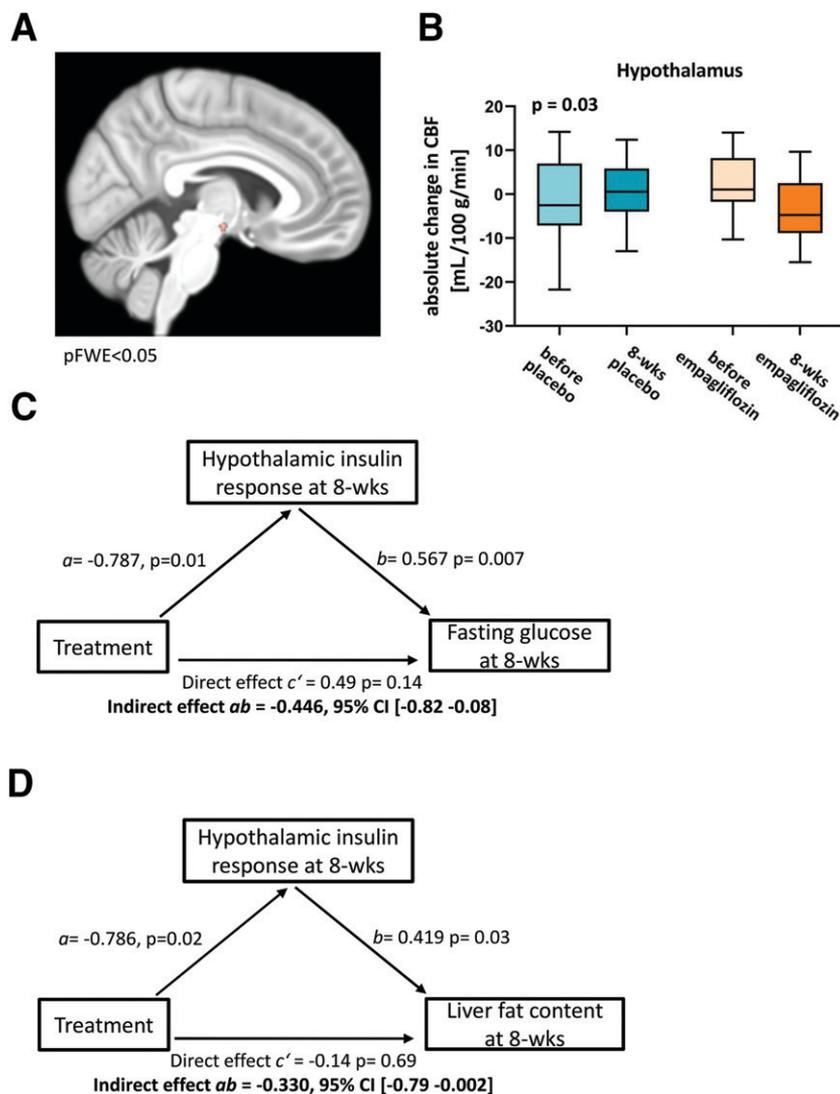


Figure 1—Primary outcome: impact of empagliflozin treatment on insulin action in the brain. A: Interaction of treatment \times time on insulin responsiveness of the brain. Whole-brain analysis revealed a significant cluster in the hypothalamus, as indicated by the color-coded F map ($P_{FWE} < 0.05$, small-volume corrected). B: Insulin response in regional CBF of the hypothalamus was extracted. Presented are box plots with whiskers indicating 1.5 interquartile range. P value is for treatment \times time interaction that was tested by repeated-measures ANOVA. Model of treatment group (empagliflozin vs. placebo) as a predictor of fasting glucose (C) and liver fat content (D) after 8 weeks of treatment mediated by improved hypothalamic insulin responsiveness. Path coefficients and corresponding P values are shown next to arrows; path a indicates the relationship between treatment and hypothalamic insulin action after treatment; path b indicates the relationship between the hypothalamic insulin response and fasting glucose levels or liver fat content at the end of treatment; path ab indicates the indirect effect of treatment on fasting glucose or liver fat via hypothalamic insulin response; and path c' indicates the direct effect of treatment on fasting glucose or liver fat.

0.019]. Within-group comparisons revealed a significant change in hypothalamic insulin response in the empagliflozin group [$T(14) = 2.2$; $P = 0.04$], while no significant change was observed in the placebo group ($P > 0.05$) (Fig. 1B).

Subjective feeling of hunger in the fasted state changed significantly different between the two treatment groups [time \times treatment interaction: $F(1,35) = 6.4$; $P = 0.016$]. This remained significant

after adjustment for BMI, sex, and age. Within-group comparisons revealed a significant decrease in hunger rating in the empagliflozin group [$T(17) = 2.1$; $P = 0.05$], while no significant change was observed in the placebo group ($P > 0.05$) (Supplementary Fig. 5).

By contrast, estimates for whole-body insulin sensitivity did not change differently between groups, neither in the fasting state (HOMA-IR, $P = 0.8$) (Table 1) nor

during the OGTT (Matsuda index, $P = 0.8$; NEFA index, $P = 0.2$) (Table 1). Empagliflozin significantly reduced fasting blood glucose by 0.49 ± 0.39 mmol/L [$F(1,38) = 0.13$; $P = 0.03$] (Fig. 2A and Table 1). Glucose during the OGTT and 2-h after the OGTT and HbA_{1c} did not change differently between groups ($P \geq 0.7$) (Fig. 2B and Table 1).

Fasting glucagon, glucagon kinetics during the OGTT, erythropoietin, as well as fasting β -hydroxybutyric acid did not significantly differ between treatments ($P \geq 0.1$) (Supplementary Table 3).

Body weight and BMI did not significantly change ($P \geq 0.1$) (Fig. 2C and Table 1), though there was a decrease in the total amount of adipose tissue mass upon empagliflozin treatment [$F(1,38) = 0.16$; $P = 0.02$] (Fig. 2D and Table 1). Neither visceral nor subcutaneous fat content changed differently between groups ($P \geq 0.1$) (Table 1). While energy expenditure was unaltered ($P = 0.3$, adjusted sex) (Table 1), RQ decreased in the patients who received empagliflozin, indicating preferred lipid oxidation [$F(1,37) = 0.15$; $P = 0.026$] (Table 1). Total caloric intake as well as intake of the major macronutrients remained unaltered during treatment ($P \geq 0.06$) (Fig. 2F and Supplementary Table 2).

The majority of our patients (25 of 40) had fatty liver disease with a liver fat content $>5.56\%$. There was a time \times treatment interaction on intrahepatic lipids [$F(1,36) = 0.25$; $P = 0.005$] (Fig. 2E and Table 1) with an absolute $1.0 \pm 2.5\%$ reduction in liver fat content in patients treated with empagliflozin and an absolute $1.2 \pm 2.1\%$ increase in intrahepatic lipids in the placebo group. This difference between groups remained significant after adjustment for baseline BMI [$F(1,35) = 0.25$; $P = 0.0053$] or change in BMI [$F(1,35) = 0.16$; $P = 0.0222$].

Mediation analyses were performed to test whether hypothalamic insulin sensitivity served as a mediator between treatment (independent variable) and fasting glucose or liver fat (dependent variable). This analysis revealed a significant negative indirect effect of empagliflozin treatment on fasting glucose and liver fat content in the follow-up visit via hypothalamic insulin response (standardized indirect effect on fasting glucose $ab = -0.446$,

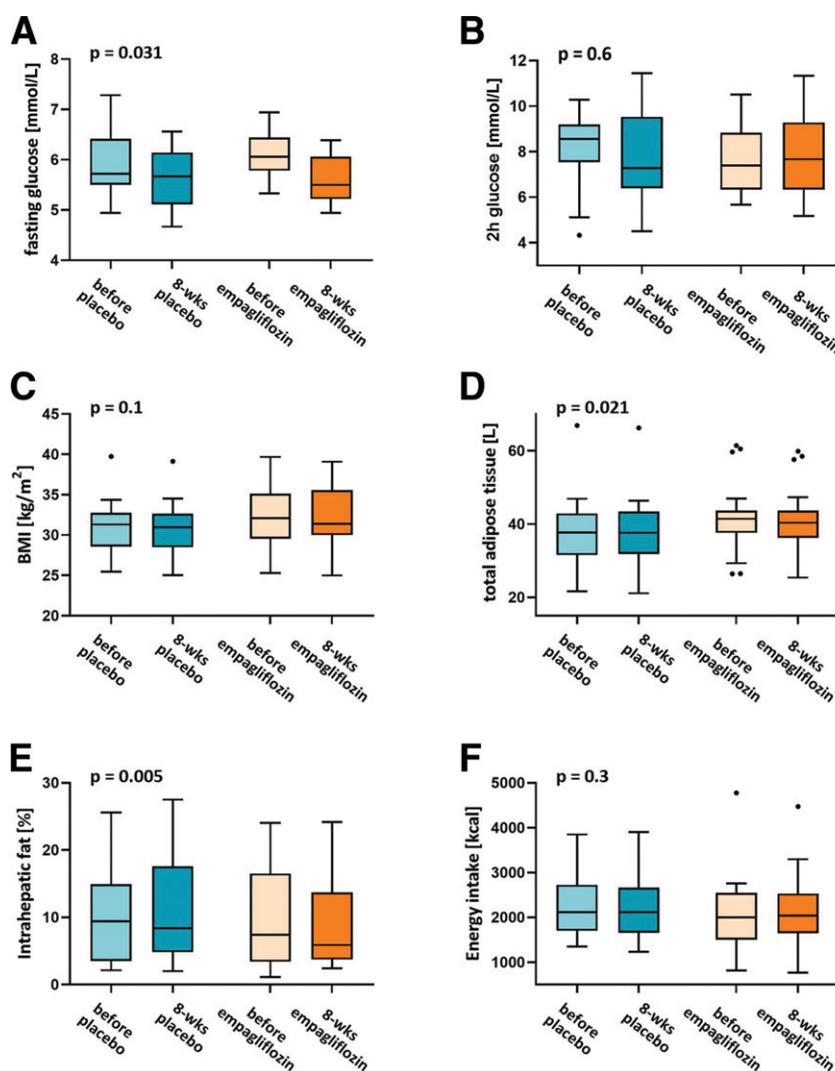


Figure 2—Impact of empagliflozin vs. placebo on key secondary outcomes. A: The course of fasting plasma glucose was different between the two treatment groups. No significant interaction between treatment \times time was detected for 2 h glucose during the OGTT (B) or BMI (C). The course of total adipose tissue content (D) and liver fat content (E) was significantly different between treatment groups. Caloric energy intake did not change during the study (F). Presented are box plots with whiskers indicating 1.5 interquartile range. P values are for treatment \times time interactions that were tested by repeated-measures ANOVA.

95% bootstrap CI -0.829 to -0.096 ; standardized indirect effect on liver fat $ab = -0.330$, 95% bootstrap CI -0.797 to -0.008) (Fig. 1C and D). Alternative models using glucose and liver fat content as mediators did not result in a significant mediation effect between treatment and hypothalamic insulin response. Thus, the increase in insulin action in the hypothalamus in response to empagliflozin significantly mediated the decreases in liver fat content and fasting glucose levels in the empagliflozin group. No such mediation was observed in the baseline visit. Further alternative models included urinary glucose as a possible mediator between

treatment (independent variable) and fasting glucose or liver fat (dependent variable). No significant indirect effect was observed.

Conclusions

We investigated the effect of SGLT2 inhibition on brain insulin sensitivity in patients with prediabetes. While central insulin application had no effect on hypothalamic activity prior to treatment, empagliflozin treatment significantly enhanced hypothalamic insulin action. In contrast, no effects on estimates of whole-body insulin sensitivity were detected. Reduction in fasting glucose and liver fat content

in response to empagliflozin treatment was mediated via improved hypothalamic insulin responsiveness. Despite having no effect on body weight, empagliflozin reduced whole-body fat content without changing food intake.

As predicted, patients with prediabetes do not adequately respond to intranasal insulin application, failing to reduce regional blood flow in the hypothalamus. This corroborates insulin resistance of the human hypothalamus in people with prediabetes, a condition previously described in normal glucose-tolerant individuals with visceral obesity (1,12,14).

Whole-brain analysis detected significant changes in insulin action in the brain in response to empagliflozin in one specific brain area: the hypothalamus. Empagliflozin introduced hypothalamic insulin responsiveness similarly, as previously detected in healthy lean people (12,14). Hence, our results demonstrate that SGLT2 inhibition is able to improve insulin responsiveness of the hypothalamus in humans with prediabetes and obesity. Our data are strengthened by previous findings in rodents, which reported improved brain insulin sensitivity upon SGLT2 inhibition (21). Our results support the hypothesis that insulin resistance of the human brain is not a fixed trait but represents a treatable condition with potential benefits for a number of diseases well beyond metabolism (1,26). Of notice, the observed improvement in insulin sensitivity was likely specific to the brain, as we did not detect effects on estimates for peripheral insulin sensitivity that were reached by other antidiabetic drugs in even shorter periods (27). Our data are well in line with previous trials with SGLT2 inhibitors in similar patients, in whom no improvement in whole-body insulin sensitivity was detected (28,29). More important, the brain response to insulin after empagliflozin treatment was independent of body weight, which by itself is closely linked to insulin sensitivity of at least some brain areas (1).

The capability of empagliflozin to reduce excessive fat accumulation in the liver has been reported before (28,30), making this substance and other SGLT2 inhibitors with comparable effects promising candidates for the treatment of fatty liver disease. Most of the patients in our study fulfilled the diagnostic

criteria for fatty liver disease, and we confirm a clinically relevant reduction of liver fat content upon empagliflozin treatment. Of note, this response was most likely not due to weight loss, but was mediated via improved hypothalamic insulin action, suggesting a potential neural contribution. Indeed, insulin action in the hypothalamus regulates liver metabolism in rodents via the vagus nerve (2). In humans, intranasal insulin was reported to improve hepatic energy metabolism with a rapid reduction in liver fat content (31). This response was blunted in type 2 diabetes (31), presumably due to brain insulin resistance (1). Our results suggest that the reversal of hypothalamic insulin resistance by empagliflozin might restore brain-derived regulation of liver metabolism with a subsequent reduction of liver fat content.

Our current analyses indicate that brain-derived mechanism could contribute to the isolated reduction in fasting blood glucose that we observed in patients treated with empagliflozin and that was previously reported in other trials in prediabetes and recent-onset diabetes (28,29). Hepatic endogenous glucose production (EGP) is the major determinant of fasting glucose concentrations (32). Reduction of liver fat content in response to SGLT2 inhibition was reported to be accompanied by lower EGP in some (33), but not all previous trials (28,34). Though, some trials even reported increased EGP in the fasting state upon SGLT2 inhibitor treatment as a compensatory mechanism in the face of increased glucose excretion (16,18,35). This was, however, attenuated after a few weeks (19). Of note, brain insulin action was shown to modulate EGP, both in rodents (2) and humans (3,4). Yet, this is thought to be most important in the postprandial state when systemic insulin concentrations are high, while no major effects were detected in the fasting state (36). The duration of treatment in our study might not have been long enough to restore all postprandial brain-derived mechanisms that modulate postprandial metabolism in the periphery (1), as we did not detect significant improvements in glucose tolerance or HbA_{1c}.

One interesting observation is the reduction of total adipose tissue mass, without changes in body weight or

body fat distribution. For empagliflozin, this has not been reported before. It is unlikely that brain insulin's regulation of eating behavior (1) is involved, as our patients reported no changes in total energy intake or macronutrient composition of their diet. It might either be due to the limited sample size and the short duration of treatment that could have hindered detection of smaller effects or due to a preferential breakdown of adipose tissue. The latter hypothesis is supported by our analysis of whole-body substrate oxidation. In line with previous results (16,35), our data indicate a shift from glucose toward lipid oxidation in the empagliflozin-treated group. This effect of empagliflozin is of major clinical importance, as it likely contributes to the ketoacidosis risk associated with SGLT2 inhibitors (37). Though, the underlying mechanism is still not fully clear. While basic studies indicate that brain insulin action regulates lipolysis (2,38), the relevance of this for humans is under debate (1). Our current analyses do not support a major contribution of improved hypothalamic insulin responsiveness to the shift in substrate oxidation in the current study.

As we detected improved hypothalamic insulin responsiveness, the underlying mechanism is of great interest. While animal studies uncovered structural and functional changes in this area upon SGLT2 inhibitor treatment (21,22), the detailed mechanisms are still unclear. Of note, SGLT2 is not only expressed in the kidney (19), but the protein is also detectable in the human brain (39). Some findings in animals suggest direct effects in the brain (40). Hence, inhibition of this transporter could reduce glucotoxicity in brain cells. This might subsequently diminish proinflammatory signals and restore insulin responsiveness (41,42). However, there is no proof that empagliflozin is transported across the blood-brain barrier (40), making direct effects of the drug unlikely.

SGLT2 inhibitors have previously been reported to increase glucagon (16–18,35) and ketone body (16,35) concentrations in the circulation. As both substances have known effects in the hypothalamus (43,44), we measured both. However, neither fasting glucagon, nor glucagon during the OGTT, nor β -hydroxybutyric

acid significantly changed upon treatment. They are, therefore, most likely not involved in the beneficial effects of empagliflozin in the hypothalamus. As most previous results have been obtained in patients with overt diabetes (17,18), a difference in the glycemic status might explain unaltered levels in our patients. Indeed, a previous study in subjects with prediabetes also did not detect changes in glucagon (29). Furthermore, studies that reported increasing glucagon levels used assays with higher potential cross-reactivity with other hormones, while our and other studies with more specific measurements could not detect this response (45).

We hypothesize that the autonomic nervous system is a major metabolic regulator. SGLT2 inhibitors were reported to modulate activity of the autonomic nervous system with a shift from sympathetic toward parasympathetic tone (20). The hypothalamus receives autonomic inputs, integrates them with further information, and generates outputs toward the autonomic nervous system. Indeed, realignments in the central nervous system are believed to underlie changes in autonomic tone in response to empagliflozin treatment (46). Hence, empagliflozin might exert its effects in the hypothalamus via neuronal pathways rather than the bloodstream. Further studies are needed to uncover the detailed mechanisms by which SGLT2 inhibitors improve insulin sensitivity of the hypothalamus.

A limitation of our study is the uneven distribution of sexes among treatment groups, as we did not randomize in a sex-stratified manner. Thus, we cannot analyze sex-specific effects that have been reported for brain insulin (1) or exclude an influence on our results. However, the lack of major sex differences in the safety or efficiency of SGLT2 inhibitors (47) argues against major sex differences in the response to empagliflozin. While we included overweight and obese persons with prediabetes to ensure brain insulin resistance in our patients, our findings must not necessarily be translatable to other patient populations. β -Hydroxybutyric acid was measured in the morning when drug effects were potentially weakened. Thus, we cannot exclude effects during other times of the day. Furthermore, limited statistical power might have hindered detection of smaller treatment

effects (e.g., in peripheral insulin sensitivity that was also not quantified by hyperinsulinemic-euglycemic glucose clamp). While our mediation analyses suggest a central role of insulin action in the hypothalamus for peripheral effects of empagliflozin, this does not exclude major direct peripheral effects. The complex interplay between insulin action directly in peripheral organs and insulin-induced signals from the brain (and possible effects of pharmacological treatment on this relationship) clearly needs further study.

In summary, we detected restored hypothalamic insulin sensitivity upon treatment with empagliflozin in people with prediabetes. Mediation analyses indicate that this effect could contribute to the observed reduction in liver fat content and fasting blood glucose, which are major risk factors for diabetes and cardiovascular complications. Therefore, improved brain insulin responsiveness might have contributed to the beneficial effects of empagliflozin in large clinical trials that demonstrated a relevant reduction of morbidity and mortality (15). Our current findings reveal that brain insulin resistance is a condition that is treatable by pharmacological interventions with potential benefits for adiposity and whole-body metabolism.

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