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Combination therapy with exenatide decreases the dapagliflozin-induced changes in brain responses to anticipation and consumption of palatable food in patients with type 2 diabetes: A randomized controlled trial

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Abstract

Aims: Sodium-glucose cotransporter-2 inhibitors induce less weight loss than expected. This may be explained by sodium-glucose cotransporter-2 inhibitor-induced alterations in central reward- and satiety circuits, leading to increased appetite and food intake. Glucagon-like peptide-1 receptor agonists reduce appetite and body weight because of direct and indirect effects on the brain. We investigated the separate and combined effects of dapagliflozin and exenatide on the brain in response to the anticipation and consumption of food in people with obesity and type 2 diabetes.

Materials and Methods: As part of a larger study, this was a 16 week, double-blind, randomized, placebo-controlled trial. Subjects with obesity and type 2 diabetes were randomized (1:1:1:1) to dapagliflozin 10 mg with exenatide-matched placebo, exenatide twice-daily 10 μg with dapagliflozin-matched placebo, dapagliflozin plus exenatide, or double placebo. Using functional magnetic resonance imaging, the effects of treatments on brain responses to the anticipation of food and food receipt were assessed after 10 days and 16 weeks.

Results: After 10 days, dapagliflozin increased activation in right amygdala and right caudate nucleus in response to the anticipation of food, and tended to decrease activation in right amygdala in response to actual food receipt. After 16 weeks, no changes in brain activation were observed with dapagliflozin. Dapagliflozin plus exenatide reduced activation in right caudate nucleus and amygdala to the anticipation of food, and decreased activation in the right amygdala in response to food receipt after 16 weeks.

Conclusions: The dapagliflozin-induced changes in brain activation may contribute to the discrepancy between observed and expected weight loss with dapagliflozin.

Exenatide blunted the dapagliflozin-induced changes in brain activation, which may contribute to the additional weight loss with combined treatment.

KEYWORDS
central regulation of appetite, dapagliflozin, exenatide, functional neuroimaging, GLP-1 receptor agonist, obesity, SGLT2 inhibitor, type 2 diabetes

1 | INTRODUCTION

Two novel drug classes for the treatment of type 2 diabetes (T2D) and obesity are sodium-glucose cotransporter-2 inhibitors (SGLT2is) and glucagon-like peptide 1 receptor agonists (GLP-1RAs). Both classes improve glycaemic control, but additionally reduce body weight.[1,2]

SGLT2is improve glycaemic control by directly inhibiting glucose reabsorption in the renal proximal tubule, thereby enhancing urinary glucose excretion, which leads to body weight reduction.[3] Weight loss induced by SGLT2i is about 2-3 kg, which is consistently less than expected based on measured urinary glucose excretion.[4,5] As energy expenditure is unchanged with SGLT2i treatment[3] the discrepancy between observed and expected weight loss may reflect an increased energy intake.

Food intake is regulated by the complex interaction between nutrients, hormones and neuropeptides, and involves several different brain areas, implicated in homeostatic feeding and reward-related processing.[6] The brain's reward system is therefore crucial to understand the regulation of body weight. Altered brain reward responses to food intake and other food-related stimuli may promote excessive eating, thus making people prone to develop obesity and eventually T2D.[7-10] Using functional magnetic resonance imaging (fMRI), it has been shown that compared with subjects of normal weight, subjects with obesity with or without T2D have higher activity in reward-related areas such as the insula, amygdala and striatum when watching palatable food images or cues that predict palatable food receipt, which may be best explained as increased craving for food. In addition, individuals with obesity with and without T2D have less activation in these brain areas to actual receipt of palatable food, probably resulting in compensatory overconsumption.[11-13] These changes in brain activation predict less weight loss during weight loss programmes and future weight (re)gain.[14-16] The insula (taste perception, decision making and interoception), limbic-striatal system (reward motivation), and the prefrontal and orbitofrontal cortex (reward evaluation and decision making) are part of a complex interconnected reward circuitry, and these areas are consistently shown to be involved in the response to food cues[11,13,17-21]).

Animal studies suggest that increased food intake (compensatory hyperphagia) may indeed explain the discrepancy between the observed and expected weight loss with SGLT2 inhibition.[22-24] Using fMRI in humans, we previously showed that SGLT2i increased brain activation in response to the viewing of food items,[25] which may be related to the predictive value of food consumption and craving for food. However, it is unknown if SGLT2i affects brain responsiveness to actual food consumption in humans. The viewing of food pictures is often used as a relatively simple and relevant tool in fMRI studies investigating the regulation of food intake; however, from a pathophysiological point of view, the central responses to actual food consumption may be an even more important aspect of central food evaluation. We therefore also performed an fMRI task to measure brain responses during actual food consumption and during anticipation of food consumption.

GLP-1RA substantially reduces body weight and food intake.[1,2] Via direct and indirect actions on the central nervous system (CNS), appetite signalling is suppressed and satiety increased, which results in a reduced food intake, and subsequently in body weight loss. Previously, we showed that acute administration of GLP-1RA and short-term treatment with liraglutide improves brain responses to the consumption of palatable food in people with obesity and T2DM.[12,26] Because of the effects of GLP-1RA, combination therapy with SGLT2i may lead to favourable effects on the brain, thereby resulting in reduced food intake and greater reduction in body weight. Supporting this hypothesis, the hyperphagic effect of intracerebroventricular tofogliflozin was counteracted by intraperitoneal liraglutide in rats.[23] Using fMRI, we previously showed that adding exenatide to dapagliflozin blunts the hyperactivation in appetite and reward-related brain regions after short-term treatment, in response to visual food cues in patients with T2D.[25] However, how the combination of exenatide and dapagliflozin affects brain responsiveness to actual food consumption in humans is unknown.

We hypothesized that SGLT2i alters regional brain responses to the anticipation and actual consumption of palatable food in obese patients with T2D, leading to increased appetite and food intake. In addition, we hypothesized that adding a GLP-1RA to an SGLT2i may blunt these alterations.

2 | MATERIALS AND METHODS

This prespecified secondary analysis of the DECREASE (Dapagliflozin Plus Exenatide on Central REgulation of Appetite in diabeteS typE 2) study (NCT03361098), was approved by the ethics review board of the Amsterdam University Medical Center, location VUmc and complied with the Declaration of Helsinki and Good Clinical Practice guidelines. The effects of dapagliflozin monotherapy and in combination with exenatide on brain responses to visual food cues have been described previously.[25]

2.1 | Participants

We included men and postmenopausal women, aged 18-75 years, with a stable body weight (<5% reported change during the previous 3 months), a body mass index >27 kg/m², and diagnosed with T2D.[25,27] For the current treatment of T2D, metformin with or without sulphonylurea derivatives was allowed (stable dose for ≥3 months). Glycated haemoglobin HbA1c levels for participants treated with metformin monotherapy were 7-10% (53-86 mmol/mol) and for metformin plus sulphonylurea 7.5-10% (58-86 mmol/mol). Exclusion criteria were a history of serious cardiovascular, renal or liver disease, malignancies (excluding basal cell carcinoma), uncontrolled thyroid disease, the use of any centrally acting agent or oral glucocorticoids, substance abuse, neurological or psychiatric disease, including eating disorders and depression, MRI contraindications, and the use of all antidiabetic medications other than metformin and sulphonylurea derivatives. Written informed consent was obtained from all participants before any trial-related activities.

2.2 | General experimental protocol

The design of this randomized double blind placebo controlled trial has been described in detail previously.[25,27] In summary, patients were randomized 1:1:1:1, performed by an independent trial pharmacist using computer-generated numbers, to dapagliflozin 10 mg with exenatide-matched placebo, exenatide twice daily 10 μg with dapagliflozin-matched placebo, dapagliflozin and exenatide (n = 16, or placebo dapagliflozin and placebo exenatide for 16 weeks. Exenatide (or placebo) was injected twice daily 15-30 min before breakfast and dinner, and was initiated at a dose of 5 μg, followed by a dose increase to 10 μg after 4 weeks, which was maintained until the end of the study. Dapagliflozin (or placebo) was taken once daily at 20:00 h during the 16-week treatment period. To maintain blinding throughout the study, participants were treated in a double-dummy design. There was no difference in appearance between exenatide and placebo injections or dapagliflozin or placebo tablets. All study medications were provided by AstraZeneca (Cambridge, UK).

The fMRI session took place at baseline, after 10 days and after 16 weeks of treatment.

2.3 | Functional magnetic resonance imaging protocol

The current fMRI task, designed to investigate the CNS activation to actual consumption of palatable food, was used as described previously.[12,26,28] Participants were presented two images (an orange triangle or a blue star) that signalled possible delivery of 0.4 ml chocolate milk [Chocomel (FrieslandCampina); 86 kcal, 2.7 g fat, 11.8 g sugar per 100 ml] or a tasteless solution (consisting of 2.5 mM NaHCO₃ and 25 mM KCl), respectively while within the MRI scanner. Images were randomly presented for 2 s and were followed by 3 s of grey blank screen with a fixation cross (anticipation). On 40% of the chocolate and tasteless solution trials, the taste was not delivered as expected (unpaired trials) to decrease the possible impact of conditioning.

2.4 | Magnetic resonance imaging acquisition and analyses

MRI acquisition and analyses have been described in detail previously.[25] Imaging data were acquired using a 3.0 Tesla GE Signa HDxt scanner (General Electric, Boston, Massachusetts, US). Functional images were preprocessed with fMRIprep 1.2.3 as described previously.[25] T1-coregistered volumes were normalized to Montreal Neurological Institute space. Preprocessed data were analysed in the context of the general linear model with SPM12 (Wellcome Trust Centre for Neuroimaging, Londen, UK). At the first (single subject) level, the anticipation of chocolate milk, the anticipation of the tasteless solution, the receipt of chocolate milk and the receipt of the tasteless solution were modelled as events of interest. Next, two contrast images were computed at each time point (baseline, 10 days and 16 weeks) for each subject, i.e. 'anticipation of chocolate milk versus tasteless solution' (anticipatory reward), and 'chocolate milk receipt versus tasteless solution receipt' (actual reward). These first level contrast images were entered into a second level ANOVA. To test our hypotheses, dapagliflozin and dapagliflozin plus exenatide were compared with placebo. In an additional analysis, exenatide was compared with placebo.

A priori regions-of-interest (ROIs) were determined based on previous studies [i.e. insula (including adjacent opercular cortices), striatum (i.e. putamen and caudate nucleus), amygdala and orbitofrontal cortex], as these regions are consistently shown to be involved in responses to food cues and are part of the central reward circuits.[29] CNS activations were reported as significant when these survived family-wise error (FWE) (p_{FWE} < 0.05) correction for multiple comparisons on the voxel level using small volume correction within the predefined ROIs, using 5-mm (for amygdala) or 10-mm (for insula, putamen, caudate nucleus and orbitofrontal cortex) radius spheres as described previously.[20,21]

2.5 | Ad libitum lunch buffet

After the fMRI sessions, participants were presented a standardized choice buffet to assess energy intake.[20] Participants were instructed to eat as much as they liked and were not informed that their choices and intake were monitored. Total intake of energy (kcal), and percentages of kcal derived from fat, protein and carbohydrates were calculated. Nutritional analysis was performed using the Dutch Food Tables (NEVO; https://www.rivm.nl/nevo/).

2.6 | Statistical analyses

The sample size was calculated based on previous fMRI studies, addressing activity in comparable brain circuits involved in satiety and reward regulation. To detect differences in treatment effects between groups of 3% (SD 2.5%) in mean difference in BOLD fMRI signal

change, 12-16 participants per group were required for 85% power (after adjustment for multiple comparisons among the four groups).

Clinical data were analysed with the SPSS version 26 and are expressed as mean ± SEM (unless otherwise stated). To test treatment effects versus placebo, linear mixed models were used in the intention to treat population. The endpoint of interest was added as the dependent variable, and treatment allocation as the independent variable in dummy variables. Visit was added as fixed factor. The intervention-by-visit interaction and a random intercept were included in the model. In addition, the corresponding baseline values were included as the independent variable. Results were considered statistically significant at $p < .05$.

3 | RESULTS

3.1 | Baseline characteristics

Between September 2017 and March 2020, 68 of 106 people screened were included. During baseline testing, four patients experienced previously unknown claustrophobia regarding the MRI scanner; three patients were excluded and replaced, and one patient continued treatment but without fMRI measurements. Two patients dropped out, just before the last test visit, one because of personal reasons, and the other one because of ongoing nausea. In the dapagliflozin group, 16 patients were analysed, in the exenatide group 17 patients,

in the dapagliflozin plus exenatide group 15 patients, and in the placebo group 15 patients were analysed. Patient characteristics were well balanced between treatments (Table 1). For all participants, the medications used at baseline, remained unchanged during the study.

3.2 | Anthropometrics and glycaemic control

As previously published, dapagliflozin reduced body weight by −2.5 ± 0.5 kg ($p < .001$), exenatide by −1.4 ± 0.5 kg ($p < .01$), and the combination by −2.8 ± 0.5 kg ($p < .001$), compared with placebo after 16 weeks of treatment.[25] In addition, dapagliflozin reduced HbA1c by 0.5 ± 0.19% (5 mmol/mol, $p < .01$), exenatide by 0.8 ± 0.18% (8.4 mmol/mol, $p < .001$), and the combination by −1.2 ± 0.19% (11.9 mmol/mol, $p < .001$) after 16 weeks of treatment compared with placebo.[25]

3.3 | Treatment effects on brain responses to anticipation and consumption of palatable food

3.3.1 | Dapagliflozin

Anticipation
After 10 days of treatment, dapagliflozin compared with placebo increased brain activations in response to anticipation of chocolate milk

TABLE 1 Baseline characteristics

	Dapagliflozin (n = 16)	Exenatide (n = 17)	Dapagliflozin + exenatide (n = 15)	Placebo (n = 15)
Age (years)	64 ± 8.4	65 ± 5.8	64 ± 7.4	60.9 ± 7.2
Female, n (%)	4 (25)	6 (35.3)	4 (25)	4 (25.0)
Weight (kg)	97.8 ± 15.4	96.6 ± 13.3	93.6 ± 13.4	99.1 ± 21.9
BMI (kg/m^2)	31.7 ± 3.3	32.7 ± 5.1	30.9 ± 3.4	31.5 ± 5.9
Diabetes duration (years)	8.0 [5.5,13.5]	10.0 [6,18]	7.0 [5,12.8]	9.5 [7,10.5]
HbA1c (%)	7.8 ± 0.6	7.9 ± 0.8	8.0 ± 1.3	8.0 ± 0.95
(mmol/mol)	61.3 ± 6.1	65.0 ± 11.1	63.5 ± 14.5	64.7 ± 11.7
SBP (mmHg)	136.4 ± 10.7	132.1 ± 11.1	130.3 ± 10.8	132.6 ± 13.2
DBP (mmHg)	80.8 ± 5.8	81.0 ± 7.2	79.7 ± 6.8	81.3 ± 6.8
HR (bpm)	64.6 ± 11.1	71.0 ± 9.8	71.4 ± 7.9	68.5 ± 9.6
eGFR (ml/min/1.73m^2)	83.4 ± 14.6	83.2 ± 13.7	88.8 ± 10.6	87.8 ± 11.2
Use of, n (%)				
Metformin	16 (100)	17 (100)	15 (100)	15 (100)
SU derivative	5 (31.3)	6 (35.3)	3 (18.8)	8 (50.0)
Beta blocker	4 (25.0)	4 (23.5)	3 (18.8)	2 (12.5)
Statin	12 (75.0)	14 (82.4)	12 (75.0)	14 (87.5)
Anticoagulant	4 (25.0)	4 (23.5)	5 (31.3)	1 (6.3)
RAS inhibition	5 (31.3)	12 (70.6)	10 (66.7)	9 (56.3)
ACE inhibitor	2 (40.0)	8 (47.1)	7 (70.0)	6 (66.7)
ARB	3 (60.0)	4 (23.5)	3 (30.0)	3 (30.0)

Note: Baseline characteristics of the per protocol population. Data are means ± SD or median [interquartile range] for continuous metrics, and n (%) for categorical characteristics.
Abbreviations: ACE, angiotensin converting enzyme; ARB, angiotensin-II receptor blocker; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; HbA1c, glycated haemoglobin; HR, heart rate; RAS, renin-angiotensin system; SBP, systolic blood pressure; SU, sulphonylurea.
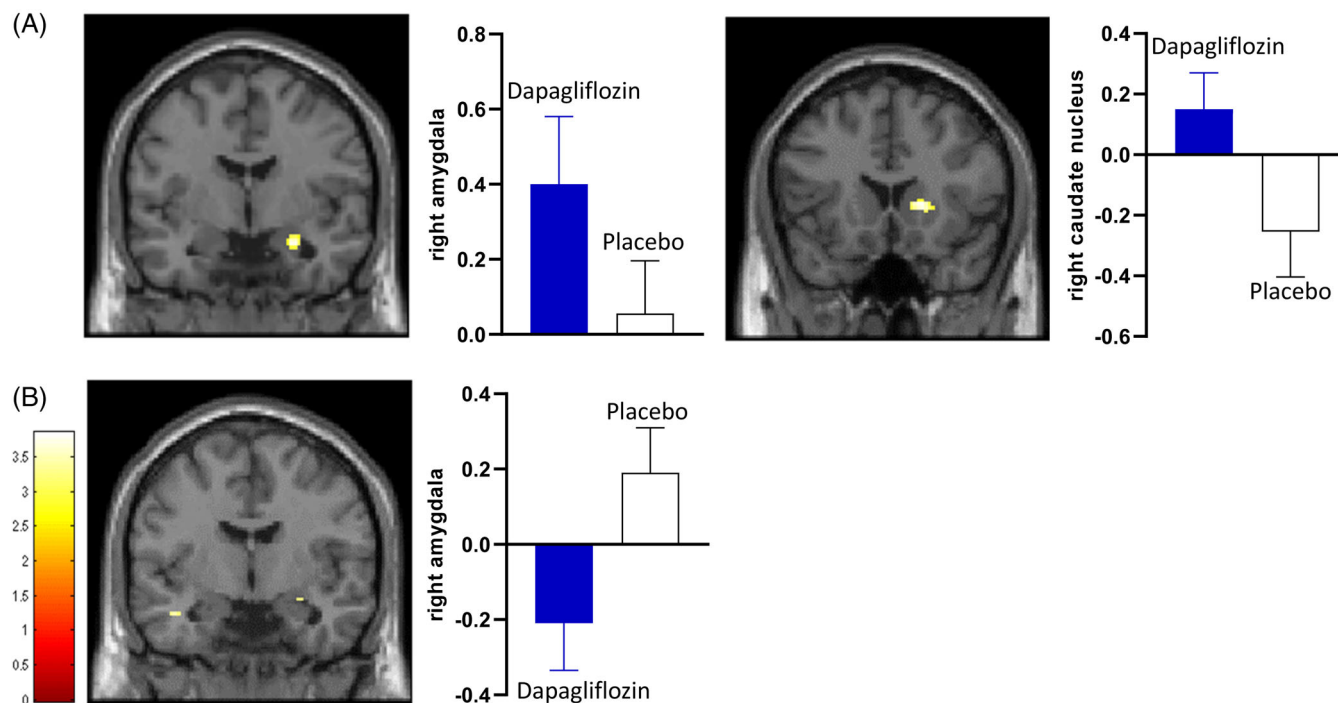
FIGURE 1 Differences in central nervous system activation after 10 days of treatment. A, Axial slices showing average differences between dapagliflozin and placebo in right amygdala (p_{FWE} < 0.01, t = 3.7) (left panel) and right caudate nucleus (p_{FWE} < 0.05, t = 3.8) (right panel) in response to the anticipation of chocolate milk versus tasteless solution. B, Axial slices showing average differences between dapagliflozin and placebo (p_{FWE} < 0.05, t = 3.4) in response to the receipt of chocolate milk versus tasteless solution. Left side of the axial slices is the left side of the brain. Colour scale reflects the t value of the functional activity. Results are presented at the threshold of p < .05, familywise error corrected on cluster extent. In the graphs, blood oxygen level-dependent signal intensity (effect size) is plotted (arbitrary units), mean and SEM

versus tasteless solution in the right amygdala (t = 3.7, p = .008) and left caudate nucleus (t = 3.8, p = .027), and tended to increase brain activation in the right putamen (t = 3.5, p = .062) and left caudate nucleus (t = 3.2, p = .11) (Figure 1, Table 2). After 16 weeks of treatment, there were no significant differences between dapagliflozin and placebo in response to anticipation of chocolate milk versus tasteless solution.

Food receipt

After 10 days, dapagliflozin compared with placebo tended to decrease brain responses to receipt of chocolate milk versus receipt of saliva in right amygdala (t = 2.8, p = .055). After 16 weeks, no differences between dapagliflozin and placebo in brain activations to receipt of chocolate milk versus tasteless solution were observed.

3.3.2 | Dapagliflozin plus exenatide

Anticipation

After 10 days, the combination of dapagliflozin and exenatide compared with placebo, was not associated with increases in brain responses to anticipation of chocolate milk versus tasteless solution. In addition, after 16 weeks, no increase in brain responses was observed with the combination. Dapagliflozin plus exenatide compared with placebo resulted in decreased brain activations in the right caudate nucleus (t = 4.3, p = .007), and numerically decreased activation in the right amygdala (t = 2.8, p = .061) and the bilateral insula (right, t = 3.1, p = .12; left, t = 3.4,

p = .063) in response to anticipation of chocolate milk versus tasteless solution, after 16 weeks (Figure 2, Table 2).

Food receipt

After 10 days of treatment with dapagliflozin plus exenatide compared with placebo, no decrease in brain activation in response to the receipt of chocolate milk versus tasteless solution was observed. After 16 weeks of treatment with dapagliflozin plus exenatide, there was also no decrease in brain activation in response to the receipt of chocolate milk versus tasteless solution compared with placebo. The combination increased brain activation in the right amygdala (t = 3.2, p = .022) in response to receipt of chocolate milk versus tasteless solution (Figure 2, Table 2).

3.3.3 | Exploratory analysis: dapagliflozin plus exenatide versus dapagliflozin

To explore further the effect of combining exenatide with dapagliflozin, dapagliflozin plus exenatide was compared with dapagliflozin.

Anticipation

After 10 days of treatment dapagliflozin plus exenatide compared with dapagliflozin decreased activity in anticipation of chocolate milk versus tasteless solution in the right amygdala (t = 4.5, p < .001), and tended to decrease activation in the left putamen (t = 3.4, p = .073).

After 16 weeks, dapagliflozin plus exenatide reduced activation in the left amygdala (t = 3.1, p = .028).

Food receipt

After 10 days, there were no differences between dapagliflozin plus exenatide compared with dapagliflozin in response to receipt of chocolate milk versus tasteless solution. After 16 weeks, there was an increase in right amygdala (t = 2.9, p = .043) when dapagliflozin plus exenatide was compared with dapagliflozin.

3.3.4 | Exenatide

To confirm the GLP-1RA-induced decrease in brain activation in response to anticipation of food, and increased brain activation to food receipt, exenatide was compared with placebo.

Anticipation

After 10 days, exenatide tended to decrease brain activations in response to anticipation of chocolate milk versus tasteless solution (anticipatory food reward) in the right insula (t = 3.3, p = .097). Exenatide decreased brain activation in response to the anticipation of chocolate milk versus tasteless solution in the right insula (t = 4.2, p = .009), and non-significantly in the left insula (t = 3.3, p = .096) after 16 weeks of treatment (Table 2).

Food receipt

After 10 days, exenatide tended to increase brain responses in the bilateral insula in response to the receipt of chocolate milk versus tasteless solution (right, t = 3.3, p = .075; left, t = 3.2, p = .068). After 16 weeks of treatment, exenatide increased CNS activation in response to the receipt of chocolate milk versus tasteless solution in the left caudate nucleus (t = 3.5, p = .049) (Table 2).

TABLE 2 Effects of dapagliflozin, exenatide and the combination of dapagliflozin and exenatide to the anticipation and receipt of chocolate milk versus saliva

Treatment group	Time point	Contrast chocolate milk versus saliva	Region	Side	Cluster	t	p_{FWE}	MNI coordinates (x, y, z)
Dapagliflozin	10 days	Anticipation	Amygdala	R	32	3.7	0.008	28, −4, −20
			Caudate	R	49	3.8	0.027	20, 18, 4
			Putamen	*R*	*22*	*3.5*	*0.062*	*30, 10, 0*
			Caudate	*L*	*7*	*3.2*	*0.110*	*−20, 18, 0*
			Insula	*R*	*6*	*2.9*	*0.188*	*36, 20, −14*
	10 days	Receipt	*Amygdala*	*R*	*2*	*2.8*	*0.055*	*28, −6, −14*
	16 weeks	Anticipation	NS	NS	NS	NS	NS	NS
	16 weeks	Receipt	NS	NS	NS	NS	NS	NS
Exenatide	10 days	Anticipation	*Insula*	*R*	*15*	*3.3*	*0.097*	*38, 28, 2*
	10 days	Receipt	*Insula*	*R*	*32*	*3.3*	*0.075*	*38, 30, 2*
			Insula	*L*	*15*	*3.3*	*0.068*	*−42, 30, 4*
	16 weeks	Anticipation	Insula	R	71	4.2	0.009	44, 6, −16
			Insula	*L*	*4*	*3.3*	*0.096*	*−26, 12, 14*
	16 weeks	Receipt	Caudate	L	6	3.5	0.049	−12, 2, 24
Combination	10 days	Anticipation	—	NS	NS	NS	NS	NS
	10 days	Receipt	*Caudate*	*L*	*4*	*3.0*	*0.141*	*−6, 20, 6*
	16 weeks	Anticipation	Caudate	R	26	4.3	0.007	10, 10, 16
			Amygdala	*R*	*2*	*2.8*	*0.061*	*26, 0, −18*
			Insula	*R*	*10*	*3.1*	*0.122*	*44, 8, −16*
			Insula	*L*	*14*	*3.4*	*0.063*	*−36, −16, 0*
			Caudate	*L*	*4*	*3.0*	*0.173*	*−10, 10, 20*
	16 weeks	Receipt	Amygdala	R	9	3.2	0.022	34, −6, −22

Note: Values are trends and not significant (in italic). Areas in which significant differences in activations were observed with dapagliflozin, exenatide and the combination of dapagliflozin plus exenatide compared with placebo treatment. For each comparison, the two contrasts (activation during the anticipation of food and actual food receipt) are presented. Areas with significant differences are listed, including the cluster size of this effect, the t value and the FWE corrected p-value after a small volume correction. Last column describes the coordinates of the peak voxel of the observed difference in MNI space. For completeness non-significant results in ROIs are showed in grey. Combination, dapagliflozin plus exenatide; L, left; MNI, Montreal Neurological Institute coordinates in mm, which represents the exact three dimensional location [x = horizontal, y = horizontal, z = vertical axis in mm distance from the origin (which is the intersection of the three axes)] in the brain of the activation peak; NS, indicating that there were no statistical significant results for this comparison; p_{FWE}, p-value family-wise error corrected for multiple comparisons based on the cluster extent (small volume correction); R, right; t, t-value.
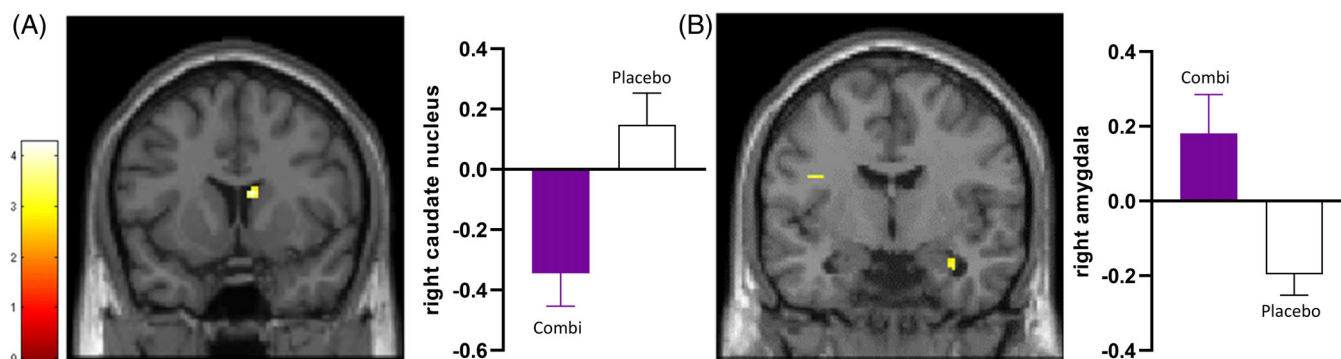
(A)

(B)

FIGURE 2 Differences in central nervous system activation after 16 weeks of treatment. A, Axial slices showing average differences between dapagliflozin plus exenatide and placebo in right caudate nucleus ($p_{FWE} < 0.01$, t = 4.3) in response to the anticipation of chocolate milk versus tasteless solution, including the bar charts for the effect of anticipation of chocolate milk versus tasteless solution (purple bar, the effect of combination treatment; white bar, the effect of placebo treatment). B, Axial slices showing average differences between dapagliflozin plus exenatide and placebo ($p_{FWE} < 0.05$, t = 3.4) in response to the receipt of chocolate milk versus tasteless solution, including the bar charts for the effect of chocolate milk versus tasteless solution (purple bar, the effect of combination treatment; white bar, the effect of placebo treatment). Left side of the axial slices is the left side of the brain. Colour scale reflects the t value of the functional activity. Results are presented at the threshold of p < .05, familywise error corrected on cluster extent. In the graphs, blood oxygen level-dependent signal intensity (effect size) is plotted (arbitrary unites), mean and SEM. Combi, combination treatment with dapagliflozin plus exenatide

3.3.5 | Ad libitum lunch buffet

As previously published,[25] there was no significant difference in mean energy intake at the ad libitum lunch buffet between the groups after 10 days or 16 weeks of treatment. However, there was a significant increase in carbohydrate intake after 16 weeks in the dapagliflozin group (21.3 ± 9.6 g, p = .029).

3.4 | Correlations between central nervous system activation and clinical parameters

There were no significant correlations between the changes in brain responses and changes in body weight, fat percentage, HbA1c, glucose or carbohydrate intake.

4 | DISCUSSION

Previous studies found that people with obesity and T2D relative to healthy control subjects have increased brain activation in response to anticipation of palatable food, but decreased brain activation to the receipt of palatable food in areas regulating satiety and reward.[10-12] To our knowledge, in this double blind randomized placebo controlled trial, we are the first to show that dapagliflozin further increased brain activation to anticipation of palatable food, and further decreased activation to the receipt palatable food. Importantly, when dapagliflozin was combined with exenatide, there was no increase in activation to anticipation of chocolate milk after short- and long-term treatment, and a reduction in brain activation in the caudate nucleus and the amygdala after long-term treatment was observed. Furthermore, with the combination of dapagliflozin and exenatide there was

no reduced activation to the receipt of chocolate milk reward anymore. The combination even increased brain activation to the receipt of chocolate milk in the right amygdala after long-term treatment.

It has been suggested that the discrepancy between the observed and expected weight loss with SGLT2 inhibitors may be because of an increase in caloric intake.[4,5,22,23] Our hypothesis was that treatment with dapagliflozin would further increase brain activation to the anticipation of food intake, and would decrease brain activation in response to actual food receipt, leading to an increased food intake and less weight loss than expected. We indeed found increased brain activation in the amygdala and caudate nucleus to the anticipation of chocolate milk after short-term treatment, which may further increase cravings for food. In addition, dapagliflozin tended to decrease activation to the receipt of chocolate milk in the amygdala after short-term treatment, which may result in overeating. As previously published, we found that dapagliflozin increased carbohydrate intake and appetite scores.[25] Together, this could partially explain the discrepancy between the observed and expected weight loss with SGLT2 inhibition. These results expand our previous findings in which dapagliflozin increased brain responses to visual food cues after short-term treatment.[25]

In the current study, it was not possible to distinguish between the direct and the indirect effects of dapagliflozin on the brain. Central (but not intraperitoneal) administration of tofogliflozin, empagliflozin and dapagliflozin increased food intake in rats, suggesting that SGLT2i induce hyperphagia via direct effects on the brain.[23] However, chronic glycosuria could also induce metabolic adaptions or changes in afferent neuronal signalling, which may also contribute.

In line with previous findings, we found that exenatide reduced brain activation in response to the anticipation of a food reward, and increased brain activation to actual food receipt.[12,13] We expected that, because of these effects of GLP-1RAs, combining a GLP-1RA

with an SGLT2 inhibitor would lead to beneficial effects on the brain, resulting in less craving for foods and increased reward feelings to the consumption of food. As ascribed above, central (but not intraperitoneal) administration of SGLT2 inhibitors increased food intake in rats, suggesting that SGLT2i induce hyperphagia via direct effects on the brain. Interestingly, peripheral administration of liraglutide blunted this SGLT2i-induced hyperphagia.[23] In our study in humans, after short-term treatment with dapagliflozin plus exenatide, there was no dapagliflozin-induced increase in brain activation anymore in response to the anticipation of food. This may be best explained as a combined effect of dapagliflozin (increase) and exenatide (decrease), resulting in a net zero effect. After 16 weeks, combination therapy even reduced activation in the right caudate nucleus. These findings expand our previous findings, in which we also found a reduction in the right amygdala after 16 weeks of treatment with dapagliflozin plus exenatide in response to the viewing of food pictures.[25] For the actual receipt of food, after 10 days of treatment with dapagliflozin plus exenatide there was no decreased brain activation in response to food receipt anymore, and a non-significant increase in the left caudate nucleus. After 16 weeks of treatment, there was an increase in the right amygdala. Our findings suggest that exenatide blunts the increased brain activation observed with dapagliflozin monotherapy after 10 days. In addition, exenatide may play an important role in the CNS changes with combination treatment after 16 weeks. These findings suggest that adding exenatide to dapagliflozin leads to less craving for food and less compensatory overconsumption, and may partially explain the almost additive weight loss with the combination.

The effects of treatments were observed in the amygdala, caudate nucleus and insula. These areas are part of a complex reward circuitry and are similar to those in which we observed effects to visual food cues.[17,30-32] The important role of the hypothalamus and brainstem (homeostatic regulation of feeding) should not be disregarded.[33] As visualization of the hypothalamus is, however, hampered by its location in the brain we did not include the hypothalamus as ROI in our analyses.

All active treatments resulted in significant weight loss. Weight loss was largest, but not fully additive in the dapagliflozin plus exenatide group, and comparable with weight loss in previous studies assessing the effects of combination treatment with SGLT2i and GLP-1RA.[34-36] Weight reduction per se may be associated with changes in brain activation in reward-related areas in response to food stimuli.[37,38] After 16 weeks of treatment with dapagliflozin, we could not find changes in brain activation anymore. Therefore, an effect of weight loss may have blunted the changes with dapagliflozin on brain activation after 16 weeks of treatment.

We did not measure changes in insulin, glucagon or other hormones, and therefore cannot rule out if changes in these hormones influenced our results. SGLT2i show a small reduction in circulating plasma insulin levels.[3] Insulin might be a negative feedback signal in the regulation of reward-related food intake, resulting in reduced food cue reactivity, appetite and caloric intake in healthy lean individuals.[39] However, in people with obesity, this response to insulin is impaired, possibly reflecting brain insulin resistance, and associated with a

higher, not lower, preference for palatable food.[39,40] In people with prediabetes, 8 weeks of treatment with empagliflozin improved insulin sensitivity in the hypothalamus.[40] Therefore, it could be suggested that improved insulin sensitivity explained the absence of changes in CNS activation after longer-term treatment. This improved insulin sensitivity is unlikely to explain the difference between the observed and expected weight loss with SGLT2i, but adaptive mechanisms may be important after longer-term treatment. Consistent energy loss via the urine may trigger an anabolic response, thereby inducing enhanced appetite and (carbohydrate) craving, which may partially offset body weight loss.[4,41] SGLT2i also exhibit a glucagonotropic effect.[3] However, higher levels of glucagon are associated with higher satiating effects, as intravenous infusion of glucagon in humans, and intracerebroventricular glucagon administration in animals is associated with reduced food intake.[42] Therefore, higher levels of glucagon during dapagliflozin treatment cannot explain the observed changes in brain responses to anticipation of food and food receipt. To substantiate further if the observed changes in brain activation to food cues with dapagliflozin are independent of changes in hormones, a trial using somatostatin pancreatic-pituitary clamp technique should be performed.

The double-blinded, double-dummy, randomized four-armed design is a major strength of this study, but there are some limitations. To assess the rewarding effect of chocolate milk, the receipt of chocolate milk was compared with the receipt of a tasteless solution. Although not designed to do so, the receipt of the tasteless solution may also have a certain rewarding effect. This will decrease the quantitative difference in activation within the contrast chocolate milk versus tasteless solution. Secondly, the sample size may have been too small for several secondary outcomes, and to detect correlations between changes in brain activation and clinical parameters.

In conclusion, dapagliflozin increased brain responses to the anticipation of food and decreased brain responses to actual food receipt after short-term treatment. These findings may contribute to the discrepancy between observed and expected weight loss with SGLT2 inhibitors. Importantly, exenatide blunts the dapagliflozin-induced changes in brain activation, which contributes to more weight loss with the combination. These findings provide further insights in the weight-lowering mechanisms of SGLT2i and GLP-1RAs and in combination, and may lead to the optimization of treatment strategies for obesity and T2D.

AUTHOR CONTRIBUTIONS

CCvR designed the study, conducted the experiments, performed the data analysis and wrote the article. DJV designed the fMRI paradigm, performed the data analysis,. and contributed towards writing the article. JStK designed the fMRI paradigm and contributed towards writing the article. MW performed the processing of (functional) MRI scans and contributed to the writing of the article. MHHK contributed to writing the article. MN contributed towards writing the article. RGIJ designed the study and the fMRI paradigm, performed the data analysis and wrote the manuscript All authors have seen and approved the final version of the manuscript. CCvR and RGIJ are the guarantors of

PEER REVIEW

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DATA AVAILABILITY STATEMENT

This clinical trial data can be requested by any qualified researchers who engage in rigorous, independent scientific research, and will be provided following review and approval of a research proposal and statistical analysis plan and execution of a data sharing agreement by the corresponding author. Data requests can be submitted by email from 3 months after publication of this report and data will be made accessible for 24 months, with possible extensions considered.

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