Original Article

MOLECULAR METABOLISM

Interaction of circulating GLP-1 and the response of the dorsolateral prefrontal cortex to food-cues predicts body weight development

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ABSTRACT

Objectives: This study evaluated the impact of the interaction between the anorexigenic incretin hormone glucagon-like peptide-1 (GLP-1) and reward-related brain activity in the dorsolateral prefrontal cortex (DLPFC), a key area of behavioral control, on future weight loss in obese individuals.

Methods: We performed a weight loss-weight maintenance intervention study over 27 months. We applied an fMRI food-cue reactivity paradigm during which the participants were passively exposed to food pictures to evaluate neuronal activity in the DLPFC. Additionally, we measured concentrations of circulating GLP-1 levels during a standard oral glucose tolerance test. Phenotyping was performed consecutively before and after a 3-month low-calorie diet as well as after a randomized 12-month trial, investigating the effect of a combined behavioral intervention on body weight maintenance. Participants were then followed-up for another 12 months without further intervention.

Results: Using voxel-wise linear mixed-effects regression analyses, we evaluated 56 measurements and identified a strong interaction between circulating, endogenous GLP-1 levels and DLPFC activity predicting body weight change over the total observation period ($t = -6.17$, $p = 1.6 \cdot 10^{-7}$). While neither the GLP-1 nor the DLPFC response individually predicted the subsequent weight change, participants achieved body weight loss when the GLP-1 and the DLPFC responses occurred concurrently.

Conclusions: Our data demonstrate an interaction between a peripheral hormonal signal and central nervous activity as robust predictor of body weight change throughout the different periods of a long-term life-style intervention. The preeminent role of their interdependency compared to the partly ambivalent effects of the single components argues for integrative approaches to improve sensitivity and reliability of weight prediction conventionally based on individual biomarkers.

Keywords Obesity; Body weight regulation; GLP-1; DLPFC; Food-cue reactivity; Voxel-wise linear mixed-effects regression

1. INTRODUCTION

Obesity is currently one of the most substantial global health burdens. Especially the associated increase in cardiovascular disease puts people at risk [1]. Weight reduction by lifestyle interventions is possible and numerous trials have demonstrated that weight loss in obese individuals improves cardiovascular risk factors [2]. Unfortunately, weight regain was observed in the majority of obese individuals who underwent a lifestyle-based weight loss program [3]. In contrast, it seems that only a substantial and sustained weight loss is effective to prevent cardiovascular disease [4]. Current evidence indicates that the frequently observed body weight regain might be mediated by the persistence of regain promoting hormonal and neuronal adaptions [5–8]. Numerous neuroimaging studies aimed to identify factors determining successful body weight regulation. The use of different testing paradigms and the investigation of different dietary interventions provided a multitude of brain areas and functions potentially predicting body weight development [9–11]. The variety of the involved neuronal and hormonal processes, their interdependency and potentially divergent importance during different stages of long-term weight regulation renders the identification of factors determining successful body weight loss a challenging problem. We investigated two well establish processes relevant for body weight regulation: 1.) the response of GLP-1 after oral glucose intake and 2.) the neuronal food

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cue-reactivity towards high caloric food items. Specifically we tested if the interaction between the two, would by a general predictor of subsequent body weight change in a weight loss-weight maintenance intervention study over 27 months. We choose to investigate GLP-1, since this gut-derived, postprandially released peptide has been identified as potentially satiety-promoting hormone reducing subjective appetite sensations and spontaneous energy intake [12]. Its anorexigenic effect is supposedly mediated through a modulation of multiple brain areas including hypothalamus, mesolimbic system, hippocampus and prefrontal cortex [13]. Several mechanisms seem to be involved conveying the effect on the brain. GLP-1 can cross the blood brain barrier and activate GLP-1 receptor expressing neurons directly or activate peripheral vagal nerve terminals projecting to the brain stem as well as preproglucagon neurons of the nucleus of the solitary tract projecting to the mesolimbic system and prefrontal cortex [13–16]. Several lines of clinical and experimental evidence underscore the potential of GLP-1 to reduce food intake and body weight [17–19]. Nevertheless, endogenous circulating GLP-1 levels itself have not been identified as independent predictor of successful body weight loss in lifestyle interventions. Besides hormonal changes, especially neural mechanisms of reward have been studied extensively in recent work on obesity [10,11,20]. We therefore choose a food-cue reactivity paradigm to investigate the reward system as well established approach in obesity research. This paradigm is inspired by findings showing that cue stimuli (e.g. the percept of a hamburger prior to consumption) predicting a reward, can convey the reinforcing properties and trigger the desire to actually consume [21]. During the test session, participants are passively exposed to food and neutral control stimuli. Application of this task showed that areas in the brain reward system including the prefrontal cortex and the striatum contribute to food wanting and that persons with obesity respond stronger to high-calorie food compared to neutral stimuli in these areas than lean persons [22,23]. Besides food wanting, impulse control is considered highly relevant for successful body weight maintenance. In particular, accumulating evidence indicates that an imbalance between enhanced food wanting and reduced impulse control impairs the regulation of appetitive behaviors in individuals with obesity [24]. Therefore, fMRI studies tested the role of neural processes underlying impulse control for short- and long-term body weight changes [9]. A key area underlying behavioral control in food choice and other domains such as social decision-making is the dorsolateral prefrontal cortex (DLPFC) [25]. Consistently, we found previously with an fMRI delay discounting paradigm that DLPFC activity predicts weight change for two specific constellations: 1.) weight loss under a low calorie diet and 2.) weight regain after an intervention [11,26]. With respect to interactions between hormone responses and the brain, few cross-modal studies have also already investigated neural mechanisms of food-cue perception together with the activity of the GLP-1 system. They found that endogenous as well as pharmacological activation of the GLP-1 system suppresses neuronal activity in reward-related brain areas as putamen, insula and orbitofrontal cortex [27–29]. This has given rise to the classical interpretation that the GLP-1 induced reduction in appetite and food intake might be mediated by modulating the activity within the brain's reward network. Interestingly, it was also demonstrated that GLP-1 concentrations correlate significantly with an increased regional cerebral blow in the DLPFC and that the GLP-1 receptor gene is expressed in this area [30,31]. Based in this background, we primarily focused our analysis on the DLPFC as region of interest to test the hypothesis that incorporating the interaction between GLP-1 and DLPFC activity enables to predict body weight change over 27 months throughout the different stages within a weight loss-weight maintenance intervention study. We performed a longitudinal analysis evaluating fMRI signals acquired with an fMRI food-cue reactivity paradigm and GLP-1 levels measured during a standard oral glucose tolerance test in persons with obesity [6]. We focused primarily on the postprandial GLP-1 response as parameter of interest since previous studies indicated that especially the glucose driven rise in GLP-1 is mediating the changes in neuronal activity detected in functional imaging studies [28,30]. In the fMRI task, participants passively viewed pictures from high-calorie food, low-calorie food, food utensils and natural scenes. fMRI sessions and blood sampling were performed for each participant at (up to) three time points: before ('T-3') and after a 12-week low-calorie diet ('T0') as well as after a 12-month randomized weight maintenance phase ('T12'). Moreover, an additional body weight measurement was conducted after another 12 months without intervention ('T24'). Finally, we used these data to model the effect of the interaction between DLPFC activity and GLP-1 (in contrast to their individual main effects) on longitudinal body mass changes obtained by all participants across the 27 months.

2. MATERIAL AND METHODS

2.1. Weight loss-weight maintenance study

In total 156 overweight or obese subjects (120 female and 36 male) with a BMI $\geq$ 27 kg/m^2 participated in a weight loss-weight maintenance intervention study. The details of the study design have been published previously [6,32]. Further details on the study design are summarized in the supplementary material. In short, the study consisted of a 12-week weight reduction program, followed by a randomized 12-month maintenance phase, comparing a multimodal lifestyle intervention to no active intervention. An additional body weight measurement was conducted 12 months later without further intervention in any of the two groups. The Institutional Review Board of the Charité Medical School approved the study protocol and all subjects gave written informed consent prior to inclusion in the study. The study was registered under ClinicalTrials.gov NCT00850629. A subset of the included individuals also participated in a complementary fMRI study to undergo repeated fMRI sessions immediately before the diet began ('T-3'), directly after the diet ('T0'), and 12 month after the end of the diet ('T12') [11,20,26]. 19 participants were measured at T-3, 23 at T0 and additionally 14 patients were measured at T12. Three consecutive measurements (fMRI & GLP-1) were available in eight participants, two consecutive measurements in 14 individuals and one measurement in four (Supplementary Table 2).

2.2. Food-cue reactivity task

We choose a standard food-cue reactivity design to measure neuronal activity while participants were watching food pictures. The specifications of the task design have been published previously [20]. In short, participants were measured between 8 and 11. a.m. after overnight fasting. Blocks of ten pictures from four different categories were presented: 1. high-calorie food, 2. low-calorie food, 3. food utensils, and 4. natural scenes. Individual pictures were shown for 3 s. Every picture block was separated by the presentation of a fixation cross for 0.49 s (Supplementary Figure 1). Blocks were presented in a pseudorandom fashion. Food blocks were shown twice followed by one control block. Our primary readout in this paradigm for analyzing reward process was the voxel-wise evaluation of the regression coefficient for high-caloric food items versus control pictures.

2.3. Brain imaging

We used a 1.5 T whole-body tomograph (Magnetom Sonata, Siemens, Erlangen, Germany) with a standard 12-channel head coil for the initial measurement at time point T-3. For the consecutive scans, we used a 3 T whole-body tomograph (Magnetom Trio, Siemens, Erlangen, Germany) also equipped with a standard 12-channel head coil. The switch to another scanner became necessary due to organizational reasons. We recorded a T2*-weighted gradient echo-planar imaging (EPI) blood-oxygenation level dependent (BOLD) sequence for the functional images. The sequence comprised 215 images (35 slices, slice thickness = 3 mm; 0.6 mm gap, interleaved; TR = 2000 ms; TA = 57.143 ms; TE = 40 ms; flip angle = 90°; field of view = 192 mm · 192 mm; matrix size = 64 · 64).

2.4. Within participant brain activity modeling

We followed a standard fMRI-preprocessing scheme with SPM12 (Wellcome Trust Centre for Neuroimaging, Institute of Neurology, UCL, London UK http://www.fil.ion.ucl.ac.uk/spm). The temporal preprocessing procedure contained high-pass filtering (cut-off 128 s) and an autoregressive model application. We performed a first-level general linear model (GLM) SPM analysis to compute voxel-vise markers of brain activity with multiple regression. We applied boxcar regressors for the different cue categories that modeled the sequence. Subsequently, we convoluted the regressors with the hemodynamic response function to use them as covariates of interest. We added six motion parameters and one constant to the model as covariates of no interest. We based subsequent analysis on the resulting regression coefficient voxel-maps for high-calorie food versus control pictures.

2.5. Cross modal between subject data analysis

In this study, we investigated the impact of interactions between hormonal and neural signals on longitudinal weight changes with voxel-wise LME regression models that reflected the different phases of the intervention study (weight loss, weight maintenance and follow-up) as well as the two different experimental groups (combined behavioral intervention versus control). One major advantage of the LME regression model, is the ability to handle participant-specific signal trends as well as unbalanced data due to study drop-outs [33]. LME regression models explain the criterion variance as linear combination of fixed and random effects. In the LME model, fixed effects represent factors that can be defined by the experimenter while random effects represent those that cannot be defined but vary in a participant-specific fashion. LME models are formulated in matrix notation as $\mathbf{y} = \mathbf{X} \cdot \mathbf{b} + \mathbf{Z} \cdot \mathbf{u} + \mathbf{e}$. y corresponds to the criterion vector, X to the design matrix of fixed effects, b to the fixed effects estimates, Z to the design matrix of random effect, u to the fandom effects estimates, and e to the unexplained criterion variance. To conduct the voxel-wise LME analyses we utilized in-house software. The approach was based on the FITLMEMATRIX algorithm included in Matlab 2014a (MathWorks, Natick, Massachusetts, USA). In this study, the interaction of brain activity and GLP-1 response was the fixed effect of interest used to model the variation in longitudinal BMI. The LME was constructed as follows:

Interaction: GLP-1 · Voxel

$$\begin{aligned}\Delta BMI = {} & b_1 + b_2 \cdot (\text{Voxel} \cdot \ln(\text{GLP\_30})) + b_3 \cdot \text{Voxel} + b_4 \cdot \ln(\text{GLP\_30}) \\ & + b_5 \cdot \text{BMI} + b_6 \cdot \text{Group} + b_7 \cdot \text{Sex} + b_8 \cdot \text{Age} + b_9 \cdot \text{Time} \\ & + b_{10} \cdot (\text{Time - 84}) + u_1 + e\end{aligned}$$

We computed the interaction as the element-wise product of the participants' log-transformed GLP-1 response (area under the curve from baseline GLP-1 to the level 30 min after oral 75 g glucose challenge) and a given voxel's food-cue responsivity parameter (i.e. the difference between regression coefficients computed for high-calorie food minus neutral stimuli) for a given time-point. In our model, the fixed effect regressors of no-interest reflected the given voxel's food-cue responsivity parameter, participants' log-transformed GLP-1 response, BMI, group membership, sex, age at T-3, time after diet onset and time after diet offset. We included those last two regressors to capture non-linear effects of the only temporary implementation of the initial weight loss program (Time on diet and after completion i.e. -84 days). This approach is called piecewise-linear regression and is typically used to model interrupted time series [34]. We also included a random intercept in our model to capture the participant-specific average ΔBMI across time points. Figure 1 shows a schematic representation of the model specifications. With respect to the individual analysis of the two main effects GLP-1 and food-cue induced neuronal activation, we used the described LME regression model in parallel including either GLP-1 or voxel activation with the same fixed effect regressors of no-interest and a random intercept (for specific matrix notation see Supplementary Material). We performed a hypothesis driven approach focused on the DLPFC as primary region of interest (ROI) and included a non-restricted whole-brain grey matter mask to reanalyze the initial findings. We used the MRIcro (http://www.mricro.com) BA template to construct the DLPFC and gray matter group masks as previously described [20,26]. We report brain voxels that show a significant positive t-statistic for the interaction effect according to a threshold corrected for family-wise error (FWE; $\alpha_{FWE} = 0.05$). We computed the threshold with the Bonferroni-method by dividing 0.05 by the number of voxels inside the used mask (DLPFC: 2752 voxels, Whole brain: 40320 voxels).

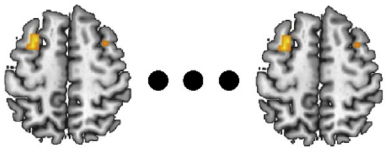
2.6. Laboratory analysis

Total Serum GLP-1 levels (including active GLP-1 (7-36) and degraded GLP-1 (9-36)) were measured in plasma samples containing aprotinin (Bayer, Leverkusen, Germany) using fluoroimmunometric assay (Merck Millipore; Cat.No. # EZGLP1T-36K; inter-assay CV 6.7—7.5%, intra-assay CV 2.9—4.4%). Total and 30-minute post meal area under the curve (AUC) GLP-1 levels were calculated from blood sampling during a standard oral glucose tolerance test after overnight fasting (0, 30, 60, 90, 120 and 180 min).

3. RESULTS

3.1. Sample characteristics

In total, 56 fMRI measurements with additional blood sampling could be included in the analysis. Baseline characteristics are summarized in Table 1. The number of participants ranged from 14 to 23 individuals per time point. In reflection of the complete study group (156 subjects) [6], the subset of participants in the fMRI study showed a marked reduction in body weight during the initial 3 month weight loss period with a mean difference in body-mass index (ΔBMI) of −4.3 kg/m^2. During the maintenance phase the intervention group was able to stabilize the intended weight loss (ΔBMI − 0.4 kg/m^2) while the control group started to regain (ΔBMI + 1.4 kg/m^2). During the subsequent observation period without further intervention, both groups regained weight, with a more pronounced body weight change in the preceding intervention group (ΔBMI + 0.9 kg/m^2 and +1.3 kg/m^2).

voxel-wise LME analyses		
Participant / time	ID: 4 / T-3	ID: 42 / T24
ΔBMI = b_1	- 6.95 [kg/m²]	0.91 [kg/m²]
+ b_2 · (**Voxel** · **ln(GLP_30)**)	- 0.92 [a.u.]	- 1.14 [a.u.]
+ b_3 · **Voxel**	- 0.376 [a.u.]	0.381 [a.u.]
+ b_4 · **ln(GLP_30)**	2.45 [a.u.]	2.98 [a.u.]
+ b_5 · **BMI**	30.2 [kg/m²]	39.2 [kg/m²]
+ b_6 · **Group**	0: control group	0: control group
+ b_7 · **Sex**	0: male	1: female
+ b_8 · **Age**	16501 [days]	15647 [days]
+ b_9 · **Time**	- 1 [days]	487 [days]
+ b_{10} · (**Time - 84**)	0 [days]	403 [days]
+ u_1		
+ **e**		

Voxel: difference between regression coefficients for high caloric food minus neutral stimulus

ln(GLP_30): GLP-1 response (log-transformend AUC 0 to 30 minutes after 75 mg oral glucose)

Figure 1: Schematic representation of the voxel-wise LME regression model for longitudinal changes in body-mass predicted from the interaction between DLPFC activation and GLP-1 response. ΔBMI: BMI difference computed for each participant and each pair of consecutive time-points, Voxel: the difference between regression coefficients computed for high-calorie food minus neutral stimuli (see Methods section Within participant brain activity modeling for details), ln (GLP_30): log-transformed GLP-1 area under the curve from baseline GLP-1 to the level 30 min after oral 75 g glucose challenge, BMI: recent body weight in kg/m^2, Group: 1 = intervention group, 0 = control group, sex: 1 = male, 0 = female, age: age at T-3 in days, Time - after diet onset and Time - after diet offset in days.

3.2. Interaction of GLP-1 and DLPFC activity in food-cue reactivity is associated with subsequent body weight loss

We modeled ΔBMI obtained by all participants across the 27-months period using linear mixed-effects (LME) regression based on interactions between neural response differences to high-calorie food minus neutral pictures in DLPFC voxel coordinates measured at (up to) three time points on one hand and the corresponding GLP-1 response after oral glucose consumption on the other. Methods section Within participant brain activity modeling provides details with respect to computation of neural response differences and Figure 1 describes the LME approach including covariates of no interest. Using this strategy, we found a strong association between the interaction of the GLP-1 system and food-cue induced brain activity in the DLPFC with longitudinal variations in ΔBMI (MNI -30, 5, 59: $t = -6.17$, $p = 1.63 \cdot 10^{-7}$, voxel size 171 mm^3). When performing the LME analyses in parallel but restricted to the individual main effects (same fixed effect regressors of no-interest: bmi, age, group, sex, time on diet, time after + random intercept) neither the GLP-1 response nor DLPFC activation showed a significant association to subsequent body weight change (GLP-1: $t = -1.2$, $p = 0.23$, DLPFC no significant voxel detected). To evaluate the identified association between the GLP-1 · DLPFC interaction and the subsequent body weight change, we re-analyzed the dataset with respect to baseline GLP-1 levels and GLP-1 levels after 30 min separately to delineate whether the spontaneous or the stimulated GLP-1 level dominates the association. This revealed a weaker but still significant effect, mainly driven by the postprandial GLP-1 level rather than the baseline constellation (MNI: −30, 5, 59; $t = -5.75$, $p = 7.23\ 10^{-7}$). To review our hypothesis driven approach focused on the DLPFC (as alleged key component for the behavioral control system), we repeated the analyses with a whole-brain grey matter mask as region of interest. For this non-restricted approach, we found again the DLPFC as sole larger cluster of significantly associated voxel activity with respect to the interaction effect and subsequent body weight development (MNI −30, 5, 59: $t = -6.17$, $p = 1.63\ 10^{-7}$, voxel size 63 mm^3). Additionally a discreet activation in the whole brain approach was detected in the medial temporal cortex (MTL) bilaterally (right MTL: MNI −24, −43, −7: $t = -5.60$, $p = 1.15\ 10^{-6}$, voxel size 9 mm^3, left MTL: MNI 51, −52, 2: $t = -5.38$, $p = 2.44\ 10^{-6}$, voxel size 9 mm^3). With respect to the unrestricted main effect analysis of food-cue induced neuronal activation, only a small area in the ventrolateral prefrontal cortex (VLPFC) showed a significant individual association to subsequent body weight development (MNI −57, 26, 8: $t = 6.18$, $p = 1.33 \cdot 10^{-7}$, voxel size 9 mm^3) - for a summarized overview of the individual results, see also Supplementary Table 1. Predominantly the interaction between the postprandial GLP-1 response and the left

Table 1 — Demographic characteristics.

	Weight loss	Maintenance			Follow-up		
	T-3 → T0	T0 → T12			T12 → T24		
	total	total	cont.	int.	total	cont.	int.
BMI [kg/m^2]	34.5 (2.7)	31.4 (3.3)	31.1 (3.6)	31.7 (2.9)	31.9 (3.8)	32.6 (4.7)	31.2 (2.6)
ΔBMI [kg/m^2]	−4.3 (1.6)	0.5 (2.7)	1.4 (1.2)	−0.4 (3.5)	1.1 (1.3)	0.9 (1.0)	1.3 (1.5)
Age [years]	48.5 (13.0)	46.8 (12.9)	45.7 (11.5)	48.0 (14.2)	47.2 (14.0)	46.5 (14.3)	47.9 (13.7)
GLP-1: 0 min [pmol/l]	11.2 (8.0)	12.2 (8.9)	12.7 (8.3)	11.6 (9.5)	11.8 (10.9)	16.3 (9.6)	7.4 (10.3)
GLP-1: 30 min [pmol/l]	20.1 (10.2)	22.1 (13.5)	21.8 (9.9)	22.4 (16.6)	25.2 (10.1)	26.5 (6.7)	23.9 (12.5)
AUC GLP-1 30 min [a.u.]	470 (257)	514 (318)	517 (257)	511 (374)	556 (290)	642 (240)	470 (310)
Female [N]	14	15	6	9	8	3	5
Male [N]	5	8	6	2	6	4	2

Baseline characteristics as well as GLP-1 baseline measures and responses after oral glucose challenge (mean ± standard deviation) for the three measurements time points T-3. T0 and T12. The three columns "total". "cont." and "int." depict the corresponding values for all participants (total) in the corresponding phase respectively sub-divided in the control (cont.) and intervention (int.) group according to the randomization at T0. Note that ΔBMI values do not correspond precisely to BMI values at different time points since not all participants are included in the analysis at all instances.

DLPFC activity was predictive for subsequent body weight change (Figure 2A). The association between the neuro-endocrine interaction and the ΔBMI showed no signs for a clustering of measurements from the same study period as indicated in Figure 2B. As delineated from the main effects LME analyses there is no individual association of neither DLPFC activity nor GLP-1 response to subsequent body weight change. Furthermore, there is no correlation between GLP-1 and DLPFC activity when exclusively considering the two variables without taking body weight change into account (supplementary figure S2). In order to disassemble the interaction effect identified in the LME analysis, we separated the dataset into tertiles ranked along increasing GLP-1 response levels (Figure 3). These data suggested a contrasting relationship of DLPFC voxel activity and subsequent body weight development in constellations with a high compared to a low GLP-1 response. In cases with low GLP-1 levels (bottom 33%), high DLPFC activity predicted an unfavorable body weight development, while in constellations with high GLP-1 levels (upper 33%), DLPFC activity inversely predicted a successful body regulation. In order to provide an alternative illustration for the interaction effect of GLP-1 and DLPFC with respect to ΔBMI, we show in Figure 4 a 3-dimensional representation with a fitted polynomial surface with 2 degrees of freedom indicating the opposing direction of the associations between DLPFC activity and ΔBMI along different GLP-1 levels. To further elaborate this aspect, we analyzed the association between the GLP-1 and DLPFC response separately for weight loss compared to weight gain. We ranked the dataset along ΔBMI values and did a near median split: negative ΔBMI (N = 29) and positive ΔBMI (N = 27). The results generated from the LME analyses revealed a positive association for the weight loss constellation (t = 3.017, p = 0.007) but not in the context of a subsequent weight gain (t = −0.198, p = 0.845)

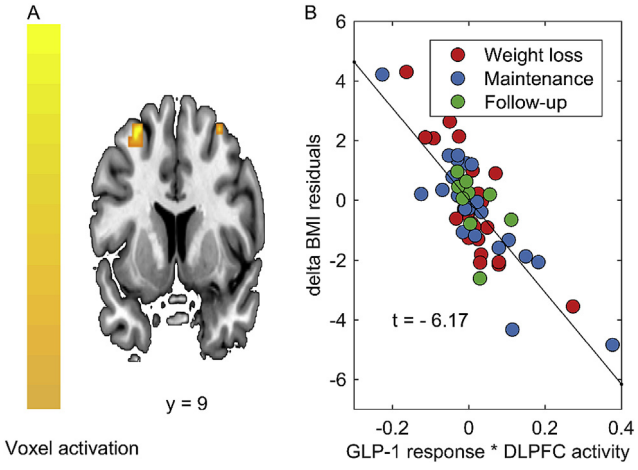


Figure 2: A. shows a representation of the differential voxel activity for high calorie food vs. control items in the dorsolateral prefrontal cortex. B. The scatterplot depicts the association between the interaction of DLPFC voxel activity and postprandial GLP-1 increase and ΔBMI (corrected for fixed and random covariates of no interest) Data points are color code for the specific phase of the study program.

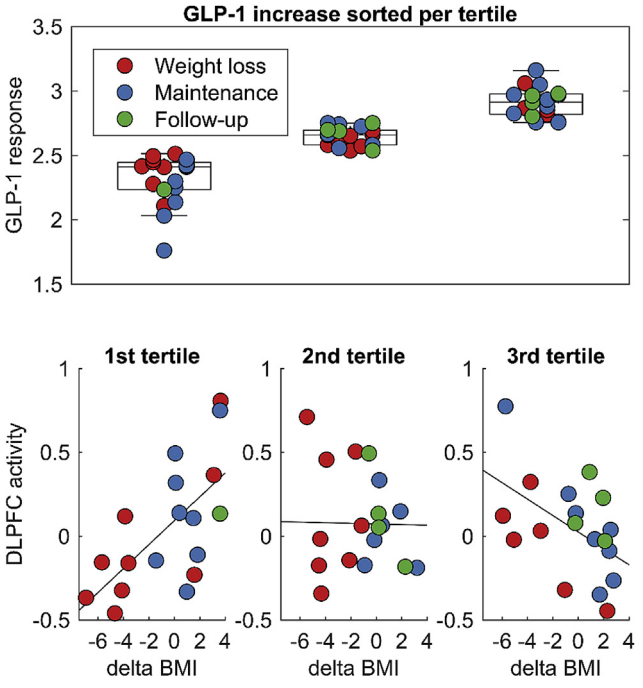


Figure 3: The upper column represents the postprandial GLP-1 increases measured as 30 min AUC values of all available samples throughout the study period ranked into tertiles from lowest to highest subset. The bottom column represents the three corresponding scatterplots depicting the associations between DLPFC activity and subsequent body weight development separately for the low, medium and high GLP-1 response constellation. A positive correlation between DLPFC activity and ΔBMI (successful weight loss) consists for the 1st tertile while an opposing significant negative association is found in the 3rd tertile of postprandial GLP-1 levels.

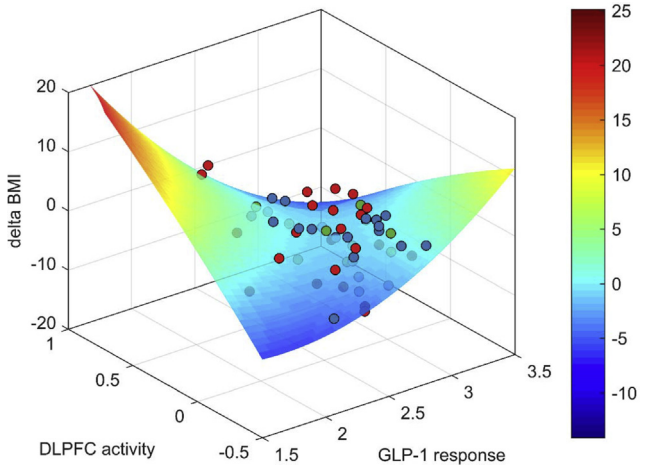


Figure 4: 3-dimensional representation of the GLP-1 response and DLPFC activity with respect to ΔBMI. We fitted a polynomial surface with 2 degrees of freedom in the x- and y-axis (GLP1 and DLPFC). Z-axis and corresponding color indicate ΔBMI value. Positive (red) ΔBMI values represent weight gain while negative (blue) ΔBMI values represent weight loss.

(supplementary material figure S3 depicts the corresponding GLP-1/DLPFC residual plots).

4. DISCUSSION

Our analysis identified the interaction between the postprandial GLP-1 response and food-cue induced brain activation in the DLPFC to predict body weight change consistently throughout a 27-month weight loss-weight maintenance intervention study. We based our approach on a LME model to incorporate these two parameters as interaction term to analyze the interplay between a humoral and neuronal regulatory system with respect to successful body weight regulation. The analysis revealed that a strong activation in the brain's behavioral control system in response to a food-cue can predict a positive as well as a negative body weight change depending on the hormonal constellation in terms of the accompanying GLP-1 response. Our approach is not suitable to establish a causal relationship or to evaluate the direction of this interaction. Nevertheless, whenever hormonal and neuronal reactions occurred concurrently, they predicted a successful body weight regulation in terms of the intended weight loss. Notably, this effect appeared to be independent of their common directionality, meaning that a combination of a low DLPFC with a low GLP-1 response as well as a high DLPFC with a high GLP-1 response predicted body weight loss. In conclusion, the concurrence of the neuro-humoral response rather than the individual reactivity enabled successful body weight control. In line with this interpretation, in recent studies an opposing peripheral and central GLP-1 reactivity has been identified following sugar consumption [35] and in obesity [36]. In a caloric restriction setup similar to the first period of our intervention trial, DLPFC cue reactivity was identified to predict weight loss, while hormonal adaptation of leptin and ghrelin were not predictive. These findings further support the conclusion that neurocognitive aspects are decisive for successful body weight regulation rather than hormonal adaptions occurring alongside an intended body weight change [37]. Yet especially with respect to GLP-1 and cue reactivity, a relevant hormone-dependent modulation of the neuronal response was detected in numerous trials [28,29,38]. Pharmacological modulation of the GLP-1 receptor as well as the endogenous GLP-1 rise after glucose challenge have been investigated in these trials. We choose to determine the AUC of baseline and 30 min post-glucose GLP-1 levels as measure for the GLP-1 response. Since most participants showed peaked GLP-1 concentrations at that time point, this approach enabled us to use a standardized response measure, incorporating baseline and post-prandial GLP-1 concentrations. Our data point out, that the interdependency between hormonal and neuronal regulations might be pivotal for body weight regulation. In our dataset, a linear association between the DLPFC and GLP-1 response was detected only in constellations with subsequent body weight loss. One major obstacle for a reliable long-term prediction of body weight development might be the variability between the involved psychological, metabolic and hormonal changes along any long-term weight intervention. Therefore, we propose to choose an LME approach enabling the inclusion of the different constellations manifesting throughout weight loss as well as weight maintenance and eventually regain periods in order to search for robust biomarkers of body weight regulation. Far more than in obesity, DLPFC activation in cue reactivity paradigms has been studied in the context of drug craving [39]. Active drug users showed an enhanced cue elicited DLPFC response but in participants, seeking to quit consumption, this effect was absent. This striking discrepancy has been interpreted as reflection of a differential cue induced processing within the DLPFC depending on the cognitive appraisal framework (i.e. whether or not the presented cue is linked to the actual intend to consume it). Integration of mutable, relevant information seems to be an important function of the DLPFC in goal-directed behavior and motor performance [40,41]. An increased activity in the DLPFC is observed when context changes require a reevaluation of a given stimulus [42]. This underscores the importance of DLPFC activation for context dependent stimulus valuation. Interestingly, numerous studies examining the metabolic effects of circulating GLP-1, indicate a highly context dependent action as well. The modulation of insulin and glucagon secretion via GLP-1 for example is highly dependent on the accompanying glucose levels [43,44]. Similarly, pharmacological treatment in obesity with GLP-1 receptor agonists shows a striking heterogeneity ranging from substantial body weight loss to persistent increase under therapy. Weather this heterogeneity can be explained by a different brain response to GLP-1 remains an open question [45]. It seems intriguing to interpret our data as a similar context dependent neuronal processing in the DLPFC depending on a "hormonal framework" or vice versa. A potential mechanism might be that, the capacity of the DLPFC to adequately integrate the GLP-1 response is an important physiological process to achieve body weight loss. While these processes individually have been demonstrated to play an important role in body weight regulation, we suggest, that their usability as biomarkers in a long-term setting featuring a high contextual variability can be limited. Our data suggest that adequate coupling of the neuro-humoral systems is relevant for body weight loss. We suggest that investigating interaction effects is crucial to advance our understanding of body weight regulation. In our secondary, unconstrained analyses using a whole brain grey matter mask as region of interest, we detected a discreet, additional activation in the MTL to predict body weight loss in interaction with GLP-1. The MTL is involved in visual perception and memory processing [46] and an increased MTL activation in response to food-cues has been demonstrated [47]. In this context, the observed interaction effect might affect body weight development in part by modulation the visual perception and processing of food cues. When performing the whole brain analysis with respect to the individual main effect of cue induced neuronal activation to predict body weight beyond the DLPFC, we detected only a small brain volume in the VLPFC to feature a statistically significant

association to a subsequent change in body weight. Former cross-sectional studies pointed out, that a higher BMI is associated with a higher VLPFC response potentially characterizing an attention bias towards high-calorie food in obesity [48]. Both of these latter findings are restricted to very small brain volumes and therefore should be interpreted with caution. Our study comes with some limitations. One of them is the small number of participants per group. When subdividing the different periods, the number of individuals per time point ranges from 14 to 23. Additionally the number of participants that completed all three consecutive fMRI and endocrine test sessions is only eight (Table S2). While the LME allows the incorporation of these segmented time series to generate a dataset based on 56 measurements, the experimental raw data is limited at this scale. Nonetheless it has been demonstrated, that the accuracy of LME models for longitudinal analysis that include participants with only one available data point is higher than the accuracy of models excluding these measurements [34]. Thereby this approach offers the potential to increase sensitivity especially in long-term studies by allowing inclusion of participants with limited longitudinal data availability. A second limitation consists in the data collection delay between the two interacting variables. We based the evaluation of the interaction between the GLP-1 response and the DLPFC activity on measurements made in two different recording sessions due to organizational reasons. The mean difference between an fMRI sessions and the GLP-1 sampling during the OGTT was 14.0 (SD $\pm$ 29.7) days, see also Supplementary Table 2. The variability of the time passed between the two measurements adds a potential confounder and might reduce the accuracy of our target measure. It seems that the modulation of the GLP-1 response during dietary intervention studies changes at a rather slow pace over several weeks to months [5,49]. Therefore, the mean 14 days delay between measuring the DLPFC and GLP-1 response should still provide a suitable frame for investigating the interplay between the two systems. In any case, we would expect that the delay should increase the variability of interaction rather than inducing an interaction. Accordingly, the here described interaction estimate may, despite being highly significant, even underestimate the signal. Finally, although GLP-1 is likely to play an important role in this context, it is only one endocrine parameter. Apparently, a complex pattern of various circulating endocrine factors will influence body weight regulation rather than one specific hormone and the relation between other endocrine parameters and brain activity patterns with respect to body weight control should be addressed in future studies.

5. CONCLUSIONS

In summary, we describe the interaction of GLP-1 and DLPFC activity as predictor for successful body weight regulation. We would like to emphasize that the ongoing pursuit to establish robust biomarkers for body weight regulation, may be improved by parallel considering neuronal and hormonal processes since their interaction might be superordinate to each phenotype alone.

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AUTHOR CONTRIBUTIONS

L.M., M.W., J.S., K.M., J.D.H., H.K. researched data and wrote the manuscript; L.M. and M.W. were responsible for data analysis. K.M., J.S., J.D.H., M.W., H.K. designed the research. All authors contributed to interpretation of the results. All authors critically read and edited several drafts before submission. All authors read and approved the submitted version.

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CONFLICT OF INTEREST

None declared.

APPENDIX A. SUPPLEMENTARY DATA

Supplementary data to this article can be found online at https://doi.org/10.1016/j.molmet.2019.08.014.

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