International Journal of Obesity
https://doi.org/10.1038/s41366-020-0545-3

ARTICLE

Clinical Research

Noninvasive neuromodulation of the prefrontal cortex in young women with obesity: a randomized clinical trial

Priscila Giacomo Fassini[1,2] · Sai Krupa Das[3] · Greta Magerowski[1] · Júlio Sérgio Marchini[2] · Wilson Araújo da Silva Junior[2] · Isabela Rozatte da Silva[2] · Rafaella de Souza Ribeiro Salgueiro[2] · Cássia Dias Machado[2] · Vivian Marques Miguel Suen[2] · Miguel Alonso-Alonso[1]

Received: 25 July 2019 / Revised: 9 January 2020 / Accepted: 3 February 2020

Abstract

Background/objectives Obesity is associated with reduced neurocognitive performance. Individuals with obesity show decreased activation in the left dorsolateral prefrontal cortex (DLPFC), a key brain region relevant to the regulation of eating behavior. Transcranial direct current stimulation (tDCS) has emerged as a potential technique to correct these abnormalities. However, there is limited information to date, particularly in clinical settings and regarding long-term effects of tDCS. This study aimed to investigate the effects of DLPFC-targeted tDCS in young women with obesity.

Subject/methods Randomized, double-blind, sham-controlled parallel-design clinical trial conducted in 38 women, aged 20–40 years, with BMI 30–35 kg/m^2. Study design: Phase I: *target engagement* (immediate effects of tDCS on working memory performance), Phase II: *tDCS only* (ten sessions, 2 weeks), Phase III: *t*DCS + hypocaloric *diet* (six sessions, 30% energy intake reduction, 2 weeks, inpatient), Phase IV: follow-up at 1, 3, and 6 months. Primary outcome: change in body weight. Secondary outcomes: change in eating behavior and appetite. Additional analyses: effect of Catechol-O-methyl transferase (*COMT)* gene variability. Data were analyzed as linear mixed models.

Results There was no group difference in change in body weight during the tDCS intervention. At follow-up, the active group lost less weight than the sham group. In addition, the active group regained weight at 6-month follow-up, compared with sham. Genetic analysis indicated that *COMT* Met noncarriers were the subgroup that accounted for this paradoxical response in the active group.

Conclusion Our results suggest that in young women with class I obesity, tDCS targeted to the DLPFC does not facilitate weight loss. Indeed, we found indications that tDCS could have a paradoxical effect in this population, possibly connected with individual differences in dopamine availability. Future studies are needed to confirm these findings.

Introduction

Neuroimaging and neuropsychology studies over the past two decades have reported associations between obesity and impairments in measures of brain and cognitive function [1–4]. These deficits may underlie common behavioral features of obesity, such as vulnerability to eating driven by external food cues or challenges to maintain lifestyle changes needed to lose weight successfully. The emerging conceptualization of obesity

These authors contributed equally: Vivian Marques Miguel Suen, Miguel Alonso-Alonso

Supplementary information The online version of this article (https://doi.org/10.1038/s41366-020-0545-3) contains supplementary material, which is available to authorized users.

✉ Miguel Alonso-Alonso
migalonsoalonso@alumni.harvard.edu

[1] Laboratory of Bariatric and Nutritional Neuroscience, Center for the Study of Nutrition Medicine, Beth Israel Deaconess Medical Center, Harvard Medical School, 330 Brookline Avenue, Boston, MA 02215, USA

[2] Department of Internal Medicine, Ribeirão Preto Medical School, University of São Paulo, Avenida Bandeirantes 3900, Bairro Monte Alegre, CEP, Ribeirão Preto, São Paulo 14049-900, Brazil

[3] Energy Metabolism Laboratory, Jean Mayer USDA Human Nutrition Center on Aging, Tufts University, 711 Washington Street, Boston, MA 02111-1524, USA

Published online: 19 February 2020

as a neurocognitive disorder [5–9] has therapeutic implications, as it offers a more expanded range of brain circuits, beyond the hypothalamus, that could be used as targets in the design of novel clinical interventions [10].

Individuals with obesity show reduced activation in prefrontal circuits, coupled with increased food cue reactivity in brain regions related to reward processing and salience [2, 10]. The dorsolateral prefrontal cortex (DLPFC) is one of the brain areas more consistently associated with obesity [7, 9, 11]. On the one hand, activation of the DLPFC during functional neuroimaging tasks has been linked to healthy food choice [12], inhibition of hunger and food cravings [13, 14], and successful weight loss [15–17]. Furthermore, variation in the recruitment of the DLPFC while viewing food images or during performance of a task that requires impulse control can predict successful response to a hypocaloric diet beyond behavioral measures [18, 19]. In addition, individuals with obesity typically show reduced DLPFC activation following a meal, compared with lean individuals, an effect that can be corrected by weight loss [20, 21]. Due to its prominent role in self-regulation, attentional control, decision making, and other complex executive functions, the DLPFC is well positioned to orchestrate cognitive regulation of eating behavior in humans, in conjunction with other high-order brain networks. Impaired activity in the DLPFC could contribute to the development and maintenance of maladaptive eating behaviors in obesity, hindering the translation of healthy dietary goals into daily behaviors and ultimately favoring overconsumption of calorically dense foods that can lead to weight gain. Finding novel brain-based strategies to rebalance DLPFC and cognitive control over food intake could help accelerate preventive and therapeutic efforts in obesity.

Noninvasive neuromodulation is a commonly used approach to alter human brain function in a safe, tolerable, and convenient manner [22]. By experimentally enhancing or disrupting the activity of specific brain areas, it may be possible to remediate neurocognitive deficits. Transcranial direct current stimulation (tDCS) is one of the available techniques for noninvasive neuromodulation. During tDCS, mild electric currents are applied over the scalp flow between two electrode pads. As currents enter the head, they can impact neuronal excitability, modulating neuronal resting membrane potential toward increased excitability (anodal tDCS) or decreased excitability (cathodal tDCS), in a direction-dependent manner [23, 24]. The use of tDCS has come of age in recent years with accumulated experience encompassing 10,000+ sessions and >1000 subjects receiving repeated sessions. tDCS has been used in a growing number of studies in the field of food craving, eating behavior, and obesity, with DLPFC as the target of choice. Anodal tDCS applied over the DLPFC can cause a reduction of food craving, appetite, and in some cases food intake and body weight [10, 25–28]. While some of these existing studies are promising, others have failed to identify an effect, possibly due to high interindividual variability. A better understanding of mechanisms of action and drivers of variability is needed to generate adequate knowledge to guide future directions [25]. The available data with tDCS in eating behavior, food craving, and obesity are limited to acute or short-term effects. It is unclear whether the observed effects can be sustained over time in the context of obesity, where demonstration of efficacy requires at least 6–12-month follow-up. In addition, the majority of studies have tested the effects of tDCS in isolation, without examining the impact of a diet or lifestyle change intervention, a necessary step to study the potential of this approach to facilitate weight loss. Overall, there is need for more evidence on the clinical potential of targeting the DLPFC with tDCS in individuals with obesity.

The present study was designed to address some of the limitations mentioned above. We conducted a clinical trial aimed at investigating the effects of DLPFC-targeted tDCS on change in body weight (primary outcome). As secondary outcomes, we examined behavioral effects regarding appetite, eating behavior, food craving, and neurocognitive performance in a DLPFC-related task. This article provides full results of the trial as prespecified; detailed analysis of appetite changes immediately before and after tDCS sessions has been reported in a recent publication [29]. The trial involved a 1-month intervention with tDCS alone and in combination with a hypocaloric diet, followed by 6-month follow-up. We selected young adult women as the study population based on prior evidence with tDCS at the time of study conception [10], and to reduce outcome variability. As an exploratory analysis, we also considered the effect of genetic polymorphism in a gene previously associated with different response to tDCS: Catechol-O-methyl transferase (*COMT*). We hypothesized that the active tDCS group would have more weight loss at the end of the study, in parallel with improvements in eating-behavior-related parameters and neurocognitive measures.

Material and methods

Participants

Women, aged 20–39 years with obesity BMI 30–35 kg/m^2 and stable weight for 3 months or longer prior to enrollment, were recruited from the state of São Paulo, Brazil. Exclusion criteria included pregnancy, diabetes, acute and chronic kidney disease, pancreatitis, or any other significant medical condition, any active psychiatric or neurological condition at the time of joining the study, intake of centrally acting medications that could interfere with tDCS effects, anemia (Hgb < 12 g/dl), and contraindications for tDCS, which include damaged skin at the site of stimulation, any electrically sensitive or metallic device and history of epilepsy [24].

Experimental design and randomization

This was a randomized, double-blind, sham-controlled clinical trial comparing active versus sham tDCS groups at three intervention phases (Fig. 1): Phase I, *target engagement*: immediate effects of a baseline tDCS session target (DLPFC)-related cognitive performance, Phase II, *tDCS only*: effects of ten daily tDCS sessions during a period of 2 weeks, and Phase III, tDCS + hypocaloric di*et*: combined effects of six tDCS sessions plus a hypocaloric diet administered at an inpatient setting over 2 weeks. After intervention, participants were followed for a period of 6 months, during which outcomes were measured at 1, 3, and 6 months.

Participants were randomized by the investigator (1:1: allocation ratio—active tDCS and sham tDCS) considering stratification by age. Participants and the investigators administering tDCS and collecting outcome data remained blinded to study group assignment (double-blind). All study procedures took place between January 2017 and September 2018 at the Metabolic Unit of Clinical Hospital from Ribeirão Preto Medical School, São Paulo University. The study was approved by the Hospital's Ethics Committee (number 8463/2016) and was registered at clinicaltrials.gov (NCT02953353), prior to enrollment. Informed consent was obtained from all subjects.

Interventions

Transcranial direct current stimulation protocol

We used a tDCS montage aimed at enhancing the excitability of the left DLPFC (anode placed over F3, based on the 10:20 EEG system, and cathode placed over the right supraorbital area). For each active tDCS session, stimulation was delivered at 2 mA intensity for 30 min, with 30 s fade in/fade out periods. For sham stimulation, the procedure was identical to active tDCS, except that the electric current flowed for only 30 s, to mimic the subjective sensations that occur with active tDCS [30]. To minimize variability, sessions were administered in the morning, after 2 h of fasting. All tDCS procedures strictly followed currently recommended standards regarding technical parameters and safety [23, 24, 31]. tDCS was delivered with a Soterix Medical 1 × 1 tDCS Stimulator Model 150x Clinical Trials that allows stimulation with participant codes for optimal blinding (Soterix Medical Inc., New York, NY), using 5 × 5 cm sponge electrodes (Soterix Medical EASY pads, Soterix Medical Inc.) soaked in a 0.9% sodium chloride solution.

Hypocaloric diet

The hypocaloric diet followed standard recommendations from the Institute of Medicine [32, 33] and consisted of 30% reduction in energy intake requirements based on measured resting energy expenditure (REE), 10% of thermic effect of food, and estimated level of physical activity by questionnaire [34].

REE was assessed by indirect calorimetry (IC) using the ParvoMedics TrueOne® 2400 Canopy System (Sandy, Utah, USA) [35]. Ethanol-burning tests were previously performed using the ethanol-burning kit from Cosmed® in order to guarantee the quality of the measurements. IC test was conducted in the morning with subjects having fasted for 12 h. Participants were asked to rest quietly in a temperature-controlled room for 30 min prior to beginning the test [36].

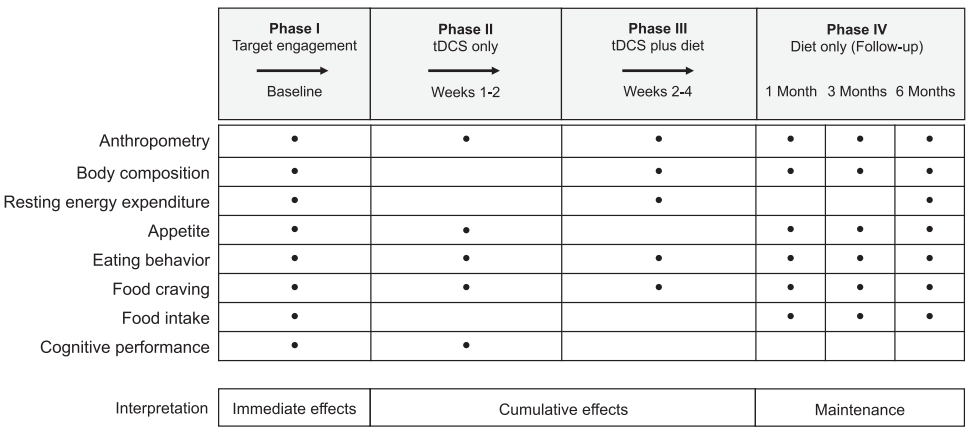

Fig. 1 Experimental design: randomized, double-blind, sham-controlled clinical trial comparing active versus sham tDCS, with four stages. Phase I, *Target engagement*: immediate effects of a baseline tDCS session on target (DLPFC)-related cognitive performance (working memory). Phase II, *tDCS only (2 weeks)*: effects of ten tDCS sessions. Phase III, *tDCS + hypocaloric diet (2 weeks)*: combined effects of six tDCS sessions plus a hypocaloric diet. Phase IV (follow-up)*:* Follow-up 1, Follow-up 3, and Follow-up 6**:** 1, 3, and 6 months after intervention, respectively. tDCS transcranial direct current stimulation, VAS visual analog scale, DLPFC dorsolateral prefrontal cortex.

Data were acquired for 40 min using a canopy hood as recommended [37]. Weir's formula [38] was used to calculate energy expenditure, and Frayn's formula [39] to calculate carbohydrate and lipid oxidation, excluding the first 10 min of data collection for volume of oxygen inspired (V_{O_2}) and volume of carbon dioxide expired (V_{CO_2}) analyses. Respiratory quotient was calculated using the ratio of V_{CO_2} to V_{O_2}.

Outcome measures

Anthropometrical and body composition measurements

The primary outcome was change in body weight throughout the study. Prior to body weight assessment, subjects were asked to empty their bladder, remove their shoes, and wear light clothing. Weight was obtained with a calibrated mechanical scale equipped with a stadiometer that was used to measure height (Filizola™, São Paulo, Brazil). BMI was calculated and classified using guidelines published by the World Health Organization [40].

Body composition was assessed using Biodynamics 450® (Biodynamics™ Corp., Shoreline, WA, USA), single frequency bioelectrical impedance analysis (50 kHz) method, according to recommendations [41].

Appetite

Changes in appetite were evaluated at Phase I and follow-up using anchored visual analog scales, with participants having fasted for 12 h. We used four standard questions: "How hungry are you?" "How full are you?" "How strong is your desire to eat?" "How much food do you think you could eat now?" to assess hunger, fullness, desire to eat, and prospective consumption [42].

Eating behavior

Changes in eating behavior were assessed during the different phases of the study using the Brazilian version of the three-factor eating questionnaire-R21 (TFEQ-21) [43]. The TFEQ-21 consisted of 21 items divided into three factors: cognitive restraint, uncontrolled eating, and emotional eating. Scores for each factor were transformed into a 0–100 range.

Food craving

Changes in food craving were assessed using the Brazilian validated version of the Food Craving Questionnaires State and Trait (FCQ-State and FCQ-Trait, respectively) [44]. The FCQ-State was assessed during the different phases of the study and consisted of 15 questions, grouped into five dimensions: an intense desire to eat (Desire); anticipation of positive reinforcement that may result from eating (Pos R); anticipation of relief from negative states and feelings as a result of eating (Neg R); thoughts of preoccupation with food and lack of control over eating (Lack Co); and craving as part of the sensation of hunger, a physiological state (Hunger). The full-scale score corresponds to the sum of the scores obtained in each dimension. For the FCQ-Trait, a total score was obtained by summing the items in this questionnaire during Phase I and at the end of the study.

Energy and macronutrients intake

Energy and macronutrient intakes were assessed via dietary recalls (two on weekdays, one on Saturday, and one on Sunday). To improve the quality of the portion size information obtained, participants were instructed to use a photographic record book by a trained dietitian [38]. Food consumption was converted into g or ml using a standardization of home measures and the picture booklet [45, 46]. Energy and macronutrient intakes were estimated using the software Virtual Nutri Plus®, updated with data from the Brazilian Food Composition Table [47] and the USDA American Table [48]. Macronutrient intake was assessed using the acceptable macronutrient distribution ranges recommendations [33].

Cognitive performance

Cognitive performance in a food-modified working memory test was evaluated during Phase II, immediately before the first tDCS session and immediately after the last tDCS session, to assess participants' working memory in the context of food. The task was comprised of three blocks of increasing difficulty level. The first block was a 1-back task, in which participants were instructed to press the spacebar of a computer if the image of food presented in a given trial matched the image presented one trial before. The second and third blocks consisted of a 2-back and 3-back task, respectively, in which participants were to press the spacebar when an image matched the image presented two and three images before, respectively. Each block had a total of 200 trials (trial duration: 2 s), of which 60 trials were match trials—defined as trials in which the current food figure matched the one presented before it (one, two, or three images, depending on block). For each block, we assessed accuracy rate—the number of correct match trials out of the total match trials, and speed (mean reaction time) for match trials that were performed correctly [49]. Food images were taken from a previously validated battery [50].

At target engagement (Phase I), subjects also performed a computerized working memory task immediately before and after the first tDCS session. The purpose of this task was to provide an indication of acute effects of tDCS on the target

(left DLPFC). This task was a letter N-back with 1-, 2-, and 3-back blocks. Full information and results in this task have been reported in another recent publication [29].

The rationale for using a working memory task was based on the tDCS target, DLPFC, an area associated with a broad range of cognitive operations, but with a central and necessary role in the support of working memory operations [51]. This direct relationship of DLPFC with working memory could facilitate detection of tDCS effects on the target more closely and precisely.

Genotyping

Genotyping was individually performed from genomic DNA extracted from whole blood using a DNA blood mini-kit DNA AS1010 (Promega, Madison, WI, USA) using the Maxwell® MDs automated nucleic acid extraction system (Promega, Madison, WI, USA) for screening of *COMT* Val158Met variation as previously described [29]. Detailed analysis is provided in Supplementary Information.

Statistical analysis

Kolmogorov–Smirnov test was used to assess the normality of the data. Baseline characteristics between the groups were compared using *t*-test for normal variables and Wilcoxon for nonnormal variables.

Primary outcome (change in body weight) and secondary outcomes were analyzed using linear mixed-effects models. Separate models were performed for each dependent variable. Models included treatment group (active tDCS versus sham tDCS). Additional analyses included genotypic status (Met carriers versus Met noncarriers) as well as their interaction as fixed effects and subjects as random effects.

Weight regain in the active group was compared with participants in the sham group considering only the follow-up period by using Fisher exact test.

Working memory task data were analyzed using mixed ANCOVA, with accuracy rate and mean reaction time as dependent variables, time (pre-tDCS and post-tDCS) and treatment (active and sham) as independent variables.

These analyses were conducted in IBM SPSS Statistics version 21.0. (IBM Corp., Armonk, New York). A two-sided significance level was set to 0.05 in all analyses. We did not adjust for multiple comparisons to avoid type II error (i.e., missing true effects) and due to the exploratory nature of this study. We determined that a total sample size of 30 participants ($n = 15$ per group) would be adequate to detect a significant difference between groups for the primary outcome of the trial (NCT02953353), given alpha = 0.05, two-tailed hypothesis, and 80% power. Effect size was estimated based on data from two previous studies with tDCS in obesity and appetite/food craving [52, 53]. We used G*Power 3, a validated statistical software [54], for these calculations. The final sample size was adjusted to account for potential dropouts.

Results

Participant characteristics

Participant's recruitment and screening is shown in the CONSORT Flowchart Supplementary Fig. 1. From 9667 inquiries, 85 subjects were assessed for eligibility and 47 declined to participate, giving a total of 38 women, aged 20–39 years, BMI 30–35 kg/m^2, enrolled in the study and randomized. Baseline characteristics did not differ between sham or active tDCS group (Table 1) nor did education and ethnicity.

Primary outcome

Anthropometric and body composition measurements by study stage are presented in Fig. 2 and Supplementary Table 1. We found no group difference in change in body weight during the intervention (sham tDCS: −0.37 ± 0.24%, active tDCS: −0.13 ± 0.27% for Phase II; sham tDCS: −2.27 ± 0.37%, active tDCS: −2.82 ± 0.32% for Phase III). However, linear mixed-effects models analysis (Supplementary Table 5) revealed a significant group effect (linear mixed-effects models, $p = 0.022$; *t*-test, $p = 0.030$ at 6-month follow-up): participants in the active group lost less weight than those in the sham group (sham tDCS: −7.41 ± 1.50%, active tDCS: −2.62 ± 1.41% for Follow-up 3). When considering only the follow-up period, we observed differences

Table 1 Baseline anthropometric and body composition characteristics between the groups.

Characteristic	Sham tDCS ($n = 18$)	Active tDCS ($n = 20$)	*p* value[b]
Age, years	30.61 ± 1.21[a]	32.16 ± 1.17	0.364
Weight, kg	86.31 ± 1.84	86.60 ± 1.85	0.914
Height, cm	1.62 ± 0.02	1.61 ± 0.01	0.821
BMI, kg/m^2	32.98 ± 0.21	33.27 ± 0.30	0.430
Waist circumference, cm	107.08 ± 1.22	107.10 ± 1.74	0.990
Fat-free mass, kg	53.44 ± 1.09	53.04 ± 1.06	0.794
Fat-free mass, %	61.98 ± 0.57	61.31 ± 0.50	0.381
Fat mass, kg	32.31 ± 0.91	33.50 ± 0.95	0.374
Fat mass, %	37.44 ± 0.69	38.62 ± 0.49	0.171

tDCS transcranial direct current stimulation, *BMI* body mass index.

[a]Values are expressed as mean ± SEM.

[b]Independent Student's *t* test for comparison between sham and active tDCS groups.

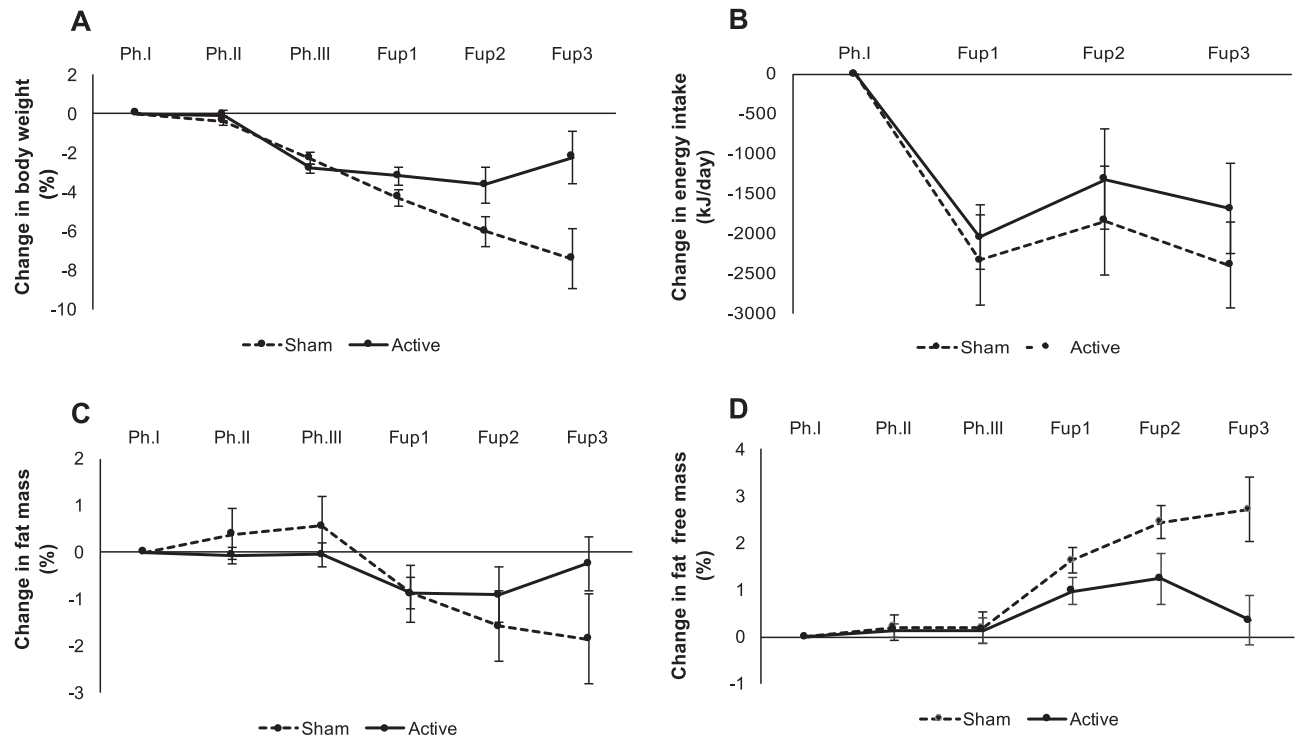

Fig. 2 Primary outcome. a Change in body weight (%). **b** Change in energy intake (kJ/day). **c** Change in percent of fat mass. **d** Change in percent of fat-free mass, compared with Phase I for sham versus active tDCS groups. Ph.I Phase I, Ph.II last day of Phase II, Ph.III last day of Phase III, Fup1 1-month follow-up, Fup2 3-month follow-up, Fup3 6-month follow-up, tDCS transcranial direct current stimulation.

in weight regain. Specifically, 77% of participants in the active group regained weight, compared with 17% of participants in the sham group (Fisher's exact, $p = 0.005$).

Change in body composition differed between groups throughout the study (linear mixed-effects models $p = 0.039$ and $p = 0.033$, respectively, for percentage of fat mass and fat-free mass). At 6-month follow-up, participants in the sham group had higher % of fat-free mass (*t*-test, $p = 0.011$ at Follow-up 3) and lower % of fat mass compared with participants in the active group.

Supplementary Table 2 shows metabolic profile data. There was a significant group effect (linear mixed-effects models $p = 0.038$ and $p < 0.01$, respectively, for REE and REE/kg). However, we only observed a significant difference for change in REE and REE/kg between groups after the intervention with tDCS plus hypocaloric diet (*t*-test, $p = 0.028$ and $p = 0.023$, respectively, at final of Phase III). Participants in the sham group had a larger reduction in REE, compared with participants in the active group. There was no significant difference in the change in respiratory quotient for carbohydrate and lipid oxidation rates.

Secondary outcomes

Figure 3 illustrates appetite and eating behavior outcomes, and Supplementary Table 3 reports the results for food craving.

We found no difference in appetite change between sham and active tDCS groups at baseline as well as throughout the follow-up period (Supplementary Table 5).

Eating behavior did not differ significantly between groups at baseline. We also did not find significant differences for change in uncontrolled eating, emotional eating, and cognitive restraint between the groups (Supplementary Table 5). However, change in cognitive restraint showed a trend for a higher reduction in the active tDCS group during the follow-up period (sham tDCS: 7.52 ± 3.63, active tDCS: 5.93 ± 4.25 for Phase II; sham tDCS: 26.98 ± 5.11, active tDCS: 36.30 ± 6.79 for Phase III; sham tDCS: 30.56 ± 4.46, active tDCS: 28.15 ± 5.85 for Follow-up 1; sham tDCS: 24.54 ± 4.46, active tDCS: 13.25 ± 6.88 for Follow-up 2; sham tDCS: 20.83 ± 5.60, active tDCS: 9.83 ± 5.87 for Follow-up 3).

Food craving did not differ between groups for any of the dimensions at baseline. Change in food craving (state) did not differ between groups throughout the study (Supplementary Table 5).

Energy and macronutrients intake

Figure 1 illustrates change in energy intake, and Supplementary Table 4 reports the results for energy and macronutrient intake by study stage. We found no group difference in change in energy and macronutrient intake

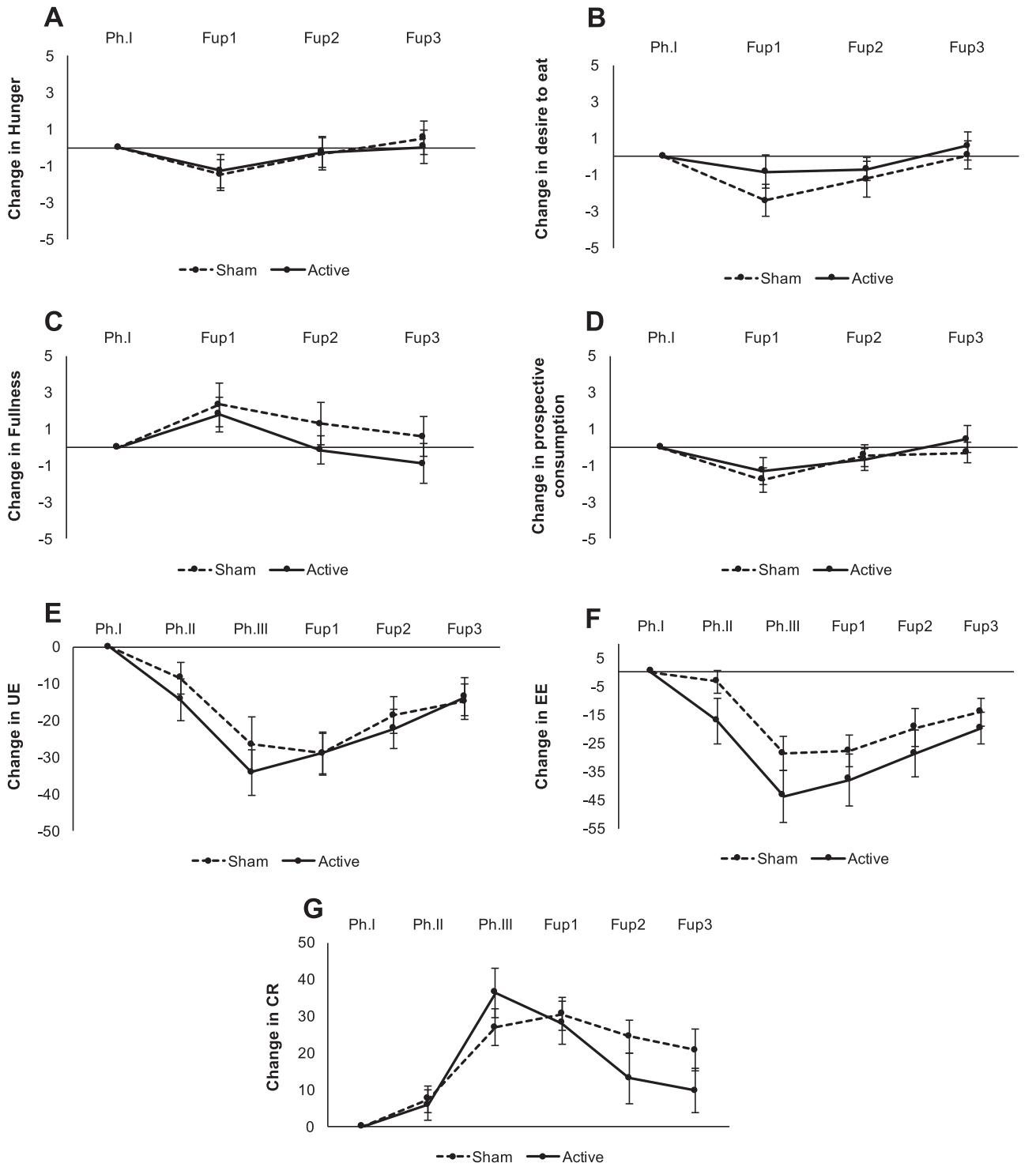

Fig. 3 Secondary outcome. Change in appetite and eating behavior compared with Phase I for sham versus active tDCS groups. **a** Hunger; **b** Desire to eat; **c** Fullness; **d** Prospective consumption; **e** Uncontrolled eating; **f** Emotional eating; **g** Cognitive restraint. Ph.I Phase I, Ph.II last day of Phase II, Ph.III last day of Phase III, Fup1 1-month follow-up, Fup2 3-month follow-up, Fup3 6-month follow-up, tDCS transcranial direct current stimulation.

(Supplementary Table 5). Both groups showed a macronutrient distribution within recommended ranges (IOM, 2005): carbohydrate 45–65% of daily value (DV), protein 10–35% of DV, lipids 20–35% of DV.

Cognitive performance

Cognitive performance in the food-modified working memory test did not change significantly between groups for

speed measures (mean reaction time) and accuracy rate in the 1-back, 2-back, and 3-back blocks of task during the tDCS intervention period (Supplementary Table 5). However, as shown in Fig. 4, average values seemed to be in the direction of better cognitive performance in the active group.

Effect of genotype

Genotype distribution of Met carriers and Met noncarriers of *COMT* Val158Met polymorphism was: sham tDCS → 6 Met noncarriers and 12 Met carriers ($n = 18$), active tDCS → 8 Met noncarriers and 12 Met carriers ($n = 20$).

Figure 5 shows data for change in body weight and appetite for Met carriers and Met noncarriers (sham and active groups). Linear mixed-effects models analysis revealed an overall interaction between group × time × *COMT* genotype ($p = 0.030$). Active Met noncarriers differed significantly, compared with all participants in the sham group at Follow-up 3 (*t*-test, $p = 0.026$), representing a subgroup that regained weight at 6-month follow-up. We observed a trend for higher levels of appetite for Met noncarriers of COMT Val158Met polymorphism during the follow-up period (Wilcoxon test, $p = 0.052$ for prospective consumption at Follow-up 1).

Discussion

In this clinical trial, we examined the effects of DLPFC-targeted tDCS on body weight, appetite, and eating behavior in young adult women with obesity. We studied immediate and cumulative effects of an intervention consisting of tDCS only, followed by tDCS combined with a hypocaloric diet. We also studied maintenance effects over a follow-up period of 6 months. Contrary to our hypothesis, we did not find that active tDCS was beneficial for weight loss in this study population. In fact, we observed that, over time, the active group showed a tendency to lose less weight than the sham group, with a clear difference emerging only at the end of the study (6-month follow-up). Genetic

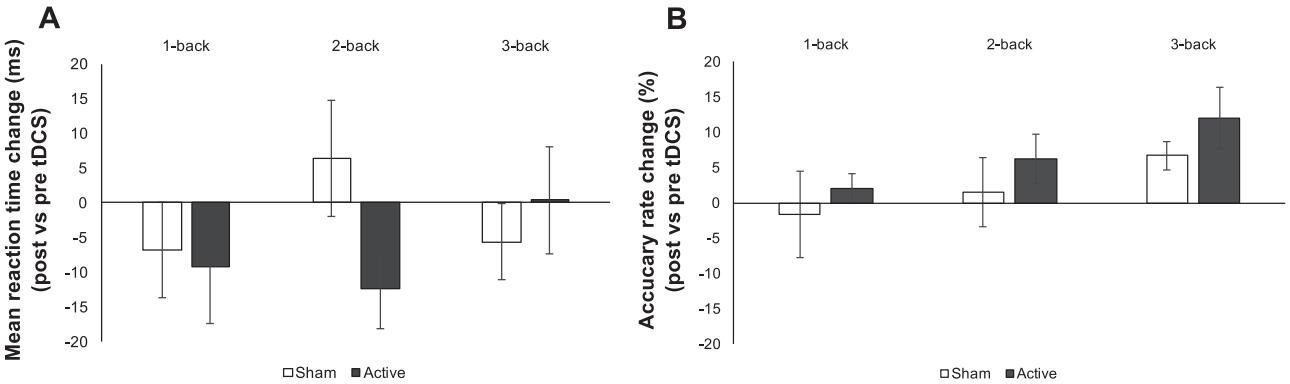

Fig. 4 Cognitive performance. a Mean reaction time change (ms). **b** Accuracy rate change (ms) 1-back, 2-back, 3-back, between sham and active tDCS groups (day 1 versus day 10 of Phase III). Error bars indicate standard error of the mean. tDCS transcranial direct current stimulation.

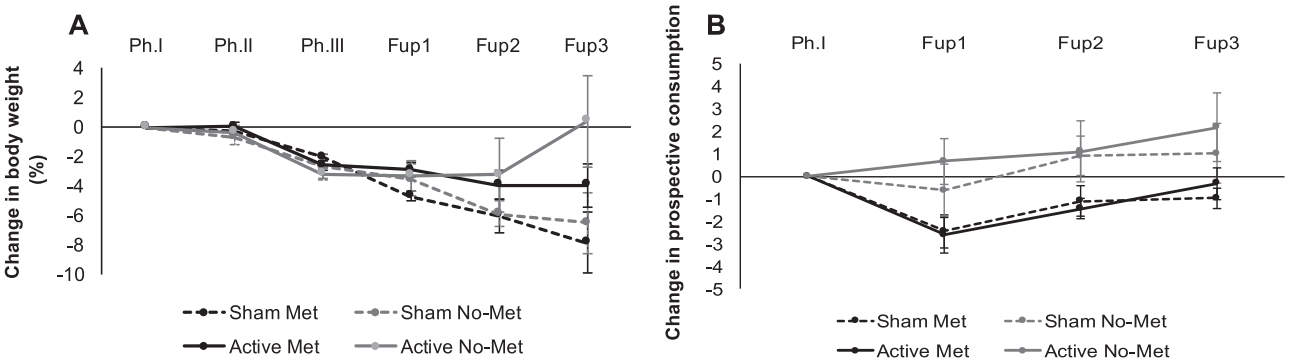

Fig. 5 Effect of COMT polymorphism. a Change in body weight (%). **b** Change in prospective consumption between sham and active tDCS for Met carriers and Met noncarriers for *COMT*. Ph.I Phase I, Ph.II last day of Phase II, Ph.III last day of Phase III, Fup1 1-month follow-up, Fup2 3-month follow-up, Fup3 6-month follow-up, tDCS transcranial direct current stimulation.

analysis indicated that *COMT* Met noncarriers were the subgroup that accounted for this paradoxical response in the active group, showing a pattern of weight regain.

Several reasons may explain the unexpected findings in body weight in our study. First, recent research suggests that tDCS targeted to the DLPFC can induce dopamine release in the striatum [55]. Thus, it is possible that the paradoxical response of Met noncarriers may involve dopaminergic effects. Whether tDCS-triggered dopamine impacts DLPFC and becomes inactivated more rapidly in Met noncarriers is currently unknown. If that were the case, our results would fit well with the notion that excessive fluctuation of dopamine levels could facilitate overeating and hinder weight loss efforts. Alternatively, through other mechanisms, tDCS could enhance the expression of obesity risk status posed by low dopamine levels genetically determined [56]. Another factor that may have partially contributed to our findings is participants' age (20–40 years old). Young adults may be more vulnerable to the influence of dopamine tone and potential fluctuations triggered by tDCS, due incomplete brain maturity and developmental mismatches between brain areas supporting high-level cognitive processing and those related to reward and emotional processing [57, 58]. Last, it is also important to note that the paradoxical effects of tDCS only emerged during Phase IV, when subjects were following the diet at home, with no supervision. This suggests that the tDCS intervention may have induced a decrease in self-regulatory capacity. In support of this possibility, our behavioral data showed a reduction in cognitive restraint over time in the active group.

Previous research with tDCS in the field of obesity has reported mixed results. A small trial conducted in a well-controlled inpatient setting found that three sessions of tDCS caused a significant reduction of body weight over the course of a week [52]. However, this finding could not be replicated in a larger follow-up trial, despite observing significant reductions in appetite and a snack intake test [11]. The study population in these investigations was broad in terms of age, gender, and ethnic groups, and large interindividual variability was noted. Besides these two trials, another recent similar, albeit shorter, trial examining the effects of tDCS in combination with a hypocaloric diet in 40 midlife women found a beneficial effect on weight loss and food-related neurocognitive performance [59]. Given the main difference in the age profile of participants, and similarities in many other aspects of the trial (tDCS target, procedure, hypocaloric diet, etc.), this discrepancy may support the critical role of age in the response to tDCS in women with obesity.

Regarding secondary outcomes, we did not find any improvement in food craving or energy intake in the active group, contrary to some previous studies [28, 60]. However, a recent meta-analysis reported that, based on the available data, there is insufficient evidence supporting an effect of tDCS in food craving, noting the importance of individual variability [26]. Similarly, we did not see any group difference in appetite during the study from measures collected under 12 h of fasting. However, using appetite measures collected under 4 h of fasting, pre- and post-tDCS, we showed, in a recent publication related to this trial, both a reduction of appetite in Met carriers and a delayed paradoxical increase in appetite in Met noncarriers during the intervention period. This publication investigated the influence of *COMT* Val158Met genotype variability on appetite effects of DLPFC-targeted tDCS [29]. The observed discrepancy between our appetite measures suggests that the effects of tDCS on appetite may be more prominent under short fasting hours, suggesting the involvement of nonhomeostatic pathways regulating feeding. Last, we did not see a significant effect of group on performance in the food-related cognitive task, even though the averages were all in the direction of an improvement in task speed and accuracy in the active group. This would be compatible with target engagement and immediate facilitatory effects on the brain target, as expected with the tDCS montage and parameters used [61].

The findings of this study are also compatible with identified differences in the response to tDCS based on dopamine availability, determined by *COMT* genotype and characterized by an inverted U-shape (reviewed in [62]). Thus, Met carriers and Met noncarriers typically behave differently when they receive anodal tDCS targeted to DLPFC [62].

Our study represents the longest and most comprehensive investigation to date evaluating the effects of tDCS in obesity. Strengths of our study include a clinical trial design with 6-month outcome data, systematic delivery of tDCS sessions and data collection, simultaneous assessment of clinical, behavioral and cognitive parameters, and the use of multiple phases in the design as a window into potential mechanisms. Importantly, this study allowed direct comparison between the effects of a diet delivered under direct supervision (inpatient unit), versus a diet that was self-managed by participants (Phase IV). Our data confirmed that all subjects were able to lose weight under direct supervision, with no difference between groups at the end of the intervention. However, under free living conditions, participants varied in their ability to continue the diet. We identified, for the first time, *COMT* Met noncarrier status as a potential genetic profile in young women with obesity in whom tDCS may be used with caution, e.g., in combination with pharmacological manipulations of COMT, or not used at all. Overall, our study is a first step toward understanding the clinical relevance and potential mechanisms of tDCS targeted to the DLPFC in obesity.

This study has some limitations including the restriction of the study sample to young adult women with BMI ranging from 30 to 35 kg/m^2, which could potentially impact the generalizability of our findings. Furthermore, we have only evaluated *COMT* as source of tDCS response variability, while there are other contributors that not only influence tDCS response, but also overeating and changes in body weight. Overall, our findings should be considered preliminary, particularly for the case of exploratory analysis comparing *COMT* genotype subgroups. A larger trial will be needed to confirm the null active tDCS effect or possibly support the paradoxical effect on weight observed in this subpopulation of young women with obesity. Future studies should also evaluate the effects of tDCS in obesity for different subgroups of age, gender, and BMI, while also addressing sources of variability. Understanding drivers of response variability is a priority in the field of tDCS, in general [63]. In addition, we assessed working memory processing with a food-modified N-back task. We did not measure cognitive control directly; however, there are known complex relationships between working memory capacity and cognitive control, e.g., through flexible control of attention. We also collected self-reported behavioral measures that can be influenced by cognitive control, such as food craving and uncontrolled eating behavior (TFEQ-21). Due to this limited assessment, it is difficult to make conclusions on cognitive mediators and cognitive-behavioral relationships underlying our results. Another limitation of our study is a 6-month follow-up period, which did not allow for a more extensive period to address aspects of weight maintenance variability. An unmet need in obesity treatment is to reverse the compensatory drive to regain lost weight [46, 47]. More studies are needed to advance the knowledge of techniques to promote the maintenance of weight loss.

In conclusion, we recommend caution in the administration of DLPFC-targeted tDCS in young women with obesity. This approach should perhaps not be used in this subpopulation, as it can induce paradoxical responses, possibly related to *COMT* Val158Met genotype status. Our results suggest that tDCS effects on appetite and body weight may be mediated by dopamine neurotransmission. Future clinical trials with tDCS in obesity should examine in detail individual sources of response variability.

Acknowledgements This study was funded by a research grant from FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo); São Paulo Research Foundation (Funding reference 2016/04766-6, 2016/10785-3, 2016/14592-5); and FAEPA (Fundação de Apoio ao Ensino, Pesquisa e Assistência do Hospital das Clínicas da Faculdade de Medicina de Ribeirão Preto—USP, Funding reference 27/2018). Additional support came from Harvard Catalyst, The Harvard Clinical and Translational Science Center (National Center for Research Resources and the National Center for Advancing Translational Sciences, National Institutes of Health Award UL1 TR002541), and financial contributions from Harvard University and its affiliated academic health care centers. The content is solely the responsibility of the authors and does not necessarily represent the official views of Harvard Catalyst, Harvard University and its affiliated academic health care centers, or the National Institutes of Health.

Author contributions PGF and MAA had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: MAA, PGF, SKD, VMMS, and GM. Acquisition, analysis, or interpretation of data: PGF and MAA. Drafting of the paper: PGF and MAA. Critical revision of the paper for important intellectual content: PGF, MAA, SKD, VMMS and GM. Statistical analysis: PGF and MAA. Obtained funding: VMMS and PGF. Administrative, technical, or material support: PGF, MAA, SKD, VMMS, GM, JSM, WAS, IRS, RSRS, and CDM. Study supervision: MAA and VMMS.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

References

1. García-García I, Michaud A, Dadar M, Zeighami Y, Neseliler S, Collins DL, et al. Neuroanatomical differences in obesity: meta-analytic findings and their validation in an independent dataset. Int J Obes. 2019;43:943–51.
2. Vainik U, Dagher A, Dubé L, Fellows LK. Neurobehavioural correlates of body mass index and eating behaviours in adults: a systematic review. Neurosci Biobehav Rev. 2013;37:279–99.
3. Veronese N, Facchini S, Stubbs B, Luchini C, Solmi M, Manzato E, et al. Weight loss is associated with improvements in cognitive function among overweight and obese people: a systematic review and meta-analysis. Neurosci Biobehav Rev. 2017;72:87–94.
4. Prickett C, Brennan L, Stolwyk R. Examining the relationship between obesity and cognitive function: a systematic literature review. Obes Res Clin Pract. 2015;9:93–113.
5. Vainik U, Baker TE, Dadar M, Zeighami Y, Michaud A, Zhang Y, et al. Neurobehavioral correlates of obesity are largely heritable. Proc Natl Acad Sci USA. 2018;115:9312–7.
6. O'Rahilly S, Farooqi IS. Human obesity: a heritable neurobehavioral disorder that is highly sensitive to environmental conditions. Diabetes. 2008;57:2905–10.
7. Lowe CJ, Reichelt AC, Hall PA. The prefrontal cortex and obesity: a health neuroscience perspective. Trends Cogn Sci. 2019;23:349–61.
8. Stoeckel LE, Birch LL, Heatherton T, Mann T, Hunter C, Czajkowski S, et al. Psychological and neural contributions to appetite self-regulation. Obes Silver Spring. 2017;25:S17–25.
9. Alonso-Alonso M, Pascual-Leone A. The right brain hypothesis for obesity. J Am Med Assoc. 2007;297:1819–22.
10. Val-Laillet D, Aarts E, Weber B, Ferrari M, Quaresima V, Stoeckel LE, et al. Neuroimaging and neuromodulation approaches to study eating behavior and prevent and treat eating disorders and obesity. Neuroimage Clin. 2015;8:1–31.
11. Heinitz S, Reinhardt M, Piaggi P, Weise CM, Diaz E, Stinson EJ, et al. Neuromodulation directed at the prefrontal cortex of subjects with obesity reduces snack food intake and hunger in a randomized trial. Am J Clin Nutr. 2017;106:1347–57.

12. Hare TA, Camerer CF, Rangel A. Self-control in decision-making involves modulation of the vmPFC valuation system. Science. 2009;324:646–8.
13. Kober H, Mende-Siedlecki P, Kross EF, Weber J, Mischel W, Hart CL, et al. Prefrontal-striatal pathway underlies cognitive regulation of craving. Proc Natl Acad Sci USA. 2010;107:14811–6.
14. Wang G-J, Volkow ND, Telang F, Jayne M, Ma Y, Pradhan K, et al. Evidence of gender differences in the ability to inhibit brain activation elicited by food stimulation. Proc Natl Acad Sci USA. 2009;106:1249–54.
15. DelParigi A, Chen K, Salbe AD, Hill JO, Wing RR, Reiman EM, et al. Successful dieters have increased neural activity in cortical areas involved in the control of behavior. Int J Obes. 2007;31:440–8.
16. McCaffery JM, Haley AP, Sweet LH, Phelan S, Raynor HA, Del Parigi A, et al. Differential functional magnetic resonance imaging response to food pictures in successful weight-loss maintainers relative to normal-weight and obese controls. Am J Clin Nutr. 2009;90:928–34.
17. Hassenstab JJ, Sweet LH, Del Parigi A, McCaffery JM, Haley AP, Demos KE, et al. Cortical thickness of the cognitive control network in obesity and successful weight loss maintenance: a preliminary MRI study. Psychiatry Res. 2012;202:77–9.
18. Weygandt M, Mai K, Dommes E, Leupelt V, Hackmack K, Kahnt T, et al. The role of neural impulse control mechanisms for dietary success in obesity. NeuroImage. 2013;83:669–78.
19. Neseliler S, Hu W, Larcher K, Zacchia M, Dadar M, Scala SG, et al. Neurocognitive and hormonal correlates of voluntary weight loss in humans. Cell Metab. 2019;29:39–49.e4.
20. Le DS, Pannacciulli N, Chen K, Del Parigi A, Salbe AD, Reiman EM, et al. Less activation of the left dorsolateral prefrontal cortex in response to a meal: a feature of obesity. Am J Clin Nutr. 2006;84:725–31.
21. Le DSN, Pannacciulli N, Chen K, Salbe AD, Del Parigi A, Hill JO, et al. Less activation in the left dorsolateral prefrontal cortex in the reanalysis of the response to a meal in obese than in lean women and its association with successful weight loss. Am J Clin Nutr. 2007;86:573–9.
22. Bikson M, Grossman P, Thomas C, Zannou AL, Jiang J, Adnan T, et al. Safety of transcranial direct current stimulation: evidence based update 2016. Brain Stimul. 2016;9:641–61.
23. Nitsche MA, Paulus W. Transcranial direct current stimulation-update 2011. Restor Neurol Neurosci. 2011;29:463–92.
24. Nitsche MA, Cohen LG, Wassermann EM, Priori A, Lang N, Antal A, et al. Transcranial direct current stimulation: state of the art 2008. Brain Stimul. 2008;1:206–23.
25. Alonso-Alonso M. Translating tDCS into the field of obesity: mechanism-driven approaches. Front Hum Neurosci. 2013;7:512.
26. Hall PA, Vincent CM, Burhan AM. Non-invasive brain stimulation for food cravings, consumption, and disorders of eating: a review of methods, findings and controversies. Appetite. 2018;124:78–88.
27. Lowe CJ, Vincent C, Hall PA. Effects of noninvasive brain stimulation on food cravings and consumption: a meta-analytic review. Psychosom Med. 2017;79:2–13.
28. Mostafavi SA, Khaleghi A, Mohammadi MR, Akhondzadeh S. Is transcranial direct current stimulation an effective modality in reducing food craving? A systematic review and meta-analysis. Nutr Neurosci. 2018;23:55–67.
29. Fassini PG, Das SK, Miguel Suen VM, Magerowski G, Marchini JS, Araújo da Silva Junior W, et al. Appetite effects of prefrontal stimulation depend on COMT Val158Met polymorphism: a randomized clinical trial. Appetite. 2019. https://doi.org/10.1016/j.appet.2019.05.015.
30. Gandiga PC, Hummel FC, Cohen LG. Transcranial DC stimulation (tDCS): a tool for double-blind sham-controlled clinical studies in brain stimulation. Clin Neurophysiol. 2006;117:845–50.
31. Woods AJ, Antal A, Bikson M, Boggio PS, Brunoni AR, Celnik P, et al. A technical guide to tDCS, and related non-invasive brain stimulation tools. Clin Neurophysiol. 2016;127:1031–48.
32. Das SK, Gilhooly CH, Golden JK, Pittas AG, Fuss PJ, Dallal GE, et al. Long term effects of energy-restricted diets differing in glycemic load on metabolic adaptation and body composition. Open Nutr J. 2007;85:1023–30.
33. Institute of Medicine. DRI dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein and amino acids (Macronutrients). 2005. http://www.nap.edu/read/10490/chapter/1. Accessed 3 Oct 2016.
34. Matsudo S, Araujo T, Matsudo V, Andrade D, Andrade E, Oliveira LC, et al. Questionário internacional de atividade física (IPAQ): estudo de validade e reprodutibilidade no Brasil. 2001;6:5–18.
35. Cooper JA, Watras AC, O'Brien MJ, Luke A, Dobratz JR, Earthman CP, et al. Assessing validity and reliability of resting metabolic rate in six gas analysis systems. J Am Diet Assoc. 2009;109:128–32.
36. Fassini PG, Silvah JH, Lima CMM, Brandao CFCCM, Wichert-Ana L, Marchini JS, et al. Indirect calorimetry: from expired CO_2 production, inspired O_2 consumption to energy equivalent. J Obes Weight Loss Ther. 2015;S5:1–3.
37. Compher C, Frankenfield D, Keim N, Roth-Yousey L, Evidence Analysis Working Group. Best practice methods to apply to measurement of resting metabolic rate in adults: a systematic review. J Am Diet Assoc. 2006;106:881–903.
38. Weir JBDB. New methods for calculating metabolic rate with special reference to protein metabolism. J Physiol. 1949;109:1–9.
39. Frayn KN. Calculation of substrate oxidation rates in vivo from gaseous exchange. J Appl Physiol. 1983;55:628–34.
40. World Health Organization. Physical status: the use and interpretation of anthropometry. Report of a WHO expert committee. World Health Organ Tech Rep Ser. 1995;854:1–452.
41. Kyle UG, Bosaeus I, De Lorenzo AD, Deurenberg P, Elia M, Manuel Gómez J, et al. Bioelectrical impedance analysis-part II: utilization in clinical practice. Clin Nutr Edinb Scotl. 2004;23:1430–53.
42. Blundell JE, De Graaf K, Finlayson G, Halford JCG, Hetherington M, King NA, et al. Measuring food intake, hunger, satiety and satiation in the laboratory. In: Allison DB, Baskin ML, editors. Handbook of assessment methods for eating behaviours and weight-related problems: measures, theory and research. 2nd ed. Newbury Park, California: Sage; 2009. p. 283–325.
43. de Medeiros ACQ, Yamamoto ME, Pedrosa LFC, Hutz CS. The Brazilian version of the three-factor eating questionnaire-R21: psychometric evaluation and scoring pattern. Eat Weight Disord. 2017;22:169–75.
44. Queiroz de Medeiros AC, Campos Pedrosa LF, Hutz CS, Yamamoto ME. Brazilian version of food cravings questionnaires: psychometric properties and sex differences. Appetite. 2016;105:328–33.
45. Monteiro JP, Pfrimer K, Tremeschin MH, de Camargo Molina M, Chiarello P. Consumo alimentar—visualizando porções. São Paulo: Guanabara Koogan, 2007.
46. Pinheiro ABV, Lacerda EMA, Benzecry EH, Gomes MCS, Costa VM. Tabela para avaliação de consumo alimentar em medidas caseiras (Table for evaluation of food consumption in home measures). 5th ed. São Paulo: Atheneu; 2008.
47. Tabela brasileira de composição de alimentos—TACO. 4th ed. Universidade Estadual de Campinas—UNICAMP, Núcleo de Estudos e Pesquisas em Alimentação—NEPA: Campinas, 2011. http://www.nepa.unicamp.br/taco/contar/taco_4_edicao_ampliada_e_revisada.pdf?arquivo=taco_4_versao_ampliada_e_revisada.pdf.
48. USDA nutrient database for standard reference. Release 12. 1998. https://ndb.nal.usda.gov/ndb/search.

49. Marron EM, Viejo-Sobera R, Cuatrecasas G, Redolar-Ripoll D, Lorda PG, Datta A, et al. Prefronto-cerebellar neuromodulation affects appetite in obesity. Int J Obes. 2018. https://doi.org/10.1038/s41366-018-0278-8.
50. Blechert J, Meule A, Busch NA, Ohla K. Food-pics: an image database for experimental research on eating and appetite. Front Psychol. 2014;5:617.
51. Barbey AK, Koenigs M, Grafman J. Dorsolateral prefrontal contributions to human working memory. Cortex J Devoted Study Nerv Syst Behav. 2013;49:1195–205.
52. Gluck ME, Alonso-Alonso M, Piaggi P, Weise CM, Jumpertz-von Schwartzenberg R, Reinhardt M, et al. Neuromodulation targeted to the prefrontal cortex induces changes in energy intake and weight loss in obesity. Obes Silver Spring. 2015;23:2149–56.
53. Fregni F, Orsati F, Pedrosa W, Fecteau S, Tome FAM, Nitsche MA, et al. Transcranial direct current stimulation of the prefrontal cortex modulates the desire for specific foods. Appetite. 2008;51:34–41.
54. Faul F, Erdfelder E, Buchner A, Lang A-G. Statistical power analyses using G*Power 3.1: tests for correlation and regression analyses. Behav Res Methods. 2009;41:1149–60.
55. Fonteneau C, Redoute J, Haesebaert F, Le Bars D, Costes N, Suaud-Chagny MF, et al. Frontal transcranial direct current stimulation induces dopamine release in the ventral striatum in human. Cereb Cortex. 2018;28:2636–46.
56. Stice E, Yokum S, Zald D, Dagher A. Dopamine-based reward circuitry responsivity, genetics, and overeating. Curr Top Behav Neurosci. 2011;6:81–93.
57. Somerville LH, Jones RM, Casey BJ. A time of change: behavioral and neural correlates of adolescent sensitivity to appetitive and aversive environmental cues. Brain Cogn. 2010;72:124–33.
58. Welter MW, Welter CJ. Evaluation of killed and modified live porcine rotavirus vaccines in cesarean derived colostrum deprived pigs. Vet Microbiol. 1990;22:179–86.
59. Amo Usanos C, Valenzuela PL, de la Villa P, Navarro SM, Melo Aroeira AE de, Amo Usanos I, et al. Neuromodulation of the prefrontal cortex facilitates diet-induced weight loss in midlife women: a randomized, proof-of-concept clinical trial. Int J Obes. 2019. https://doi.org/10.1038/s41366-019-0486-x.
60. Kekic M, McClelland J, Campbell I, Nestler S, Rubia K, David AS, et al. The effects of prefrontal cortex transcranial direct current stimulation (tDCS) on food craving and temporal discounting in women with frequent food cravings. Appetite. 2014;78:55–62.
61. Truong DQ, Magerowski G, Blackburn GL, Bikson M, Alonso-Alonso M. Computational modeling of transcranial direct current stimulation (tDCS) in obesity: Impact of head fat and dose guidelines. NeuroImage Clin. 2013;2:759–66.
62. Wiegand A, Nieratschker V, Plewnia C. Genetic modulation of transcranial direct current stimulation effects on cognition. Front Hum Neurosci. 2016;10:651.
63. Bikson M, Brunoni AR, Charvet LE, Clark VP, Cohen LG, Deng ZD, et al. Rigor and reproducibility in research with transcranial electrical stimulation: an NIMH-sponsored workshop. Brain Stimul. 2018;11:465–80.