Universidade Federal do Rio Grande do Sul
Faculdade de Medicina
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CARINA DE ARAUJO

EFEITO DA ESTIMULAÇÃO TRANSCRANIANA POR CORRENTE CONTÍNUA ASSOCIADA À DIETA HIPOCALÓRICA SOBRE A PERDA DE PESO E HOMEOSTASE GLICÊMICA EM INDIVÍDUOS COM EXCESSO DE PESO

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Tese apresentada como requisito parcial à obtenção do título de doutora em Medicina: Endocrinologia, da Faculdade de Medicina da Universidade Federal do Rio Grande do Sul.
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RESUMO

A obesidade é definida pelo acúmulo excessivo de gordura corporal e sua presença é um importante fator de risco para o desenvolvimento de resistência à insulina e disfunção da célula β-pancreática, aumentando as chances de desenvolver diabetes mellitus tipo 2 (DM2). Apesar dos diferentes tratamentos propostos para seu controle, sua prevalência, de uma maneira geral, tem aumentado de forma preocupante ao redor do mundo. Estudos demonstram que a fissura por alimentos está intimamente associada ao ganho de peso. Neste sentido, o aumento da atividade de circuitos cerebrais na região do córtex pré-frontal dorsolateral (CPFdl) poderia gerar benefícios terapêuticos no tratamento da obesidade, tendo em vista que esta região cerebral foi previamente associada à redução da fissura por alimentos, assim como no controle glicêmico, já que o aumento da excitabilidade neuronal está associado à abertura dos canais K_{ATP} nos neurônios hipotalâmicos, levando à supressão da gliconeogênese hepática. Portanto, a estimulação transcraniana por corrente contínua (ETCC) emerge como uma possível técnica coadjuvante ao tratamento do excesso de peso e da resistência à inslina. Trata-se de uma técnica de neuromodulação não-invasiva que permite um método seguro, indolor e de baixo custo para a indução de neuroplasticidade, ou seja, modular a função cortical e influenciar os processos cognitivos associados ao comportamento alimentar, mais especificamente, na regulação do apetite e do consumo alimentar. Diante do exposto, nesta tese avaliamos a segurança e o potencial terapêutico da ETCC combinada a uma dieta hipocalórica na redução de peso, redução do desejo de comer, do consumo alimentar e na melhora do perfil glicêmico de indivíduos com sobrepeso ou obesidade. Por meio de um ensaio clínico randomizado, duplo-cego e controlado por placebo, 28 indivíduos (79% com obesidade; 37,6 [5,8] anos) completaram quatro semanas (20 sessões, cinco dias consecutivos por semana) de ETCC anódica com dose fixa de 2 mA por 20 min, sobre o CPFdl direito, associada a uma dieta hipocalórica para perda de 3% do peso inicial. Os participantes foram randomizados na razão de 1:1 e estratificados por sexo em ETCC ativo ou ETCC placebo. A perda de peso foi avaliada por meio de medidas semanais do peso corporal. O desejo de comer foi avaliado por escala visual analógica no início e no final do estudo. O consumo alimentar foi medido por registro alimentar de três dias com pesagem de alimentos no início, durante e ao final do estudo. A glicose, a insulina e a albumina glicada foram avaliadas em jejum e as estimativas de

sensibilidade à insulina de Matsuda, secreção de insulina e função da célula β pancreática foram avaliadas por meio de um teste de tolerância à refeição de 4 horas, realizado antes e após a intervenção. Os aspectos de segurança e viabilidade foram avaliados por questionários de efeitos adversos, depressão, ansiedade, humor, qualidade de vida e do sono no início e no final do estudo. Os resultados demonstraram que, embora tenha havido maior perda de peso no grupo ativo do que no grupo placebo (ativo: –4,5 kg [95% IC: –9,4; 0,5] *vs*. placebo: –2,3 kg [–5,0; 0,3]), essa diferença não foi estatisticamente significativa (p = 0,786). No entanto, o grupo ativo apresentou menor desejo pelo consumo de doces (–23,7 pontos percentuais [–40,2; –7,1] *vs*. 1,0 ponto percentual [–13,3; 15,2]; p = 0,039), melhora significativa nos valores de glicose (–7,8 mg/dL [–13,9; –1,6] *vs.* –0,9 mg/dL [–4,0; 2,2]; p = 0,045) e insulina de jejum (–7,7 µIU/mL [–13,9; –1,6] *vs.* –1,3 µIU/mL [–3,3; 0,7]; p = 0,020) e no índice de sensibilidade à insulina de Matsuda (4,6 $pmol^{-1}$×$mmol^{-1}$ [1,7; 7,4] *vs.* 1,1 $pmol^{-1}$×$mmol^{-1}$ [–1,1; 3,2]; p = 0,024). O estudo se mostrou seguro e com mínimos efeitos adversos, tendo em vista que não houve alterações significativas nos escores de depressão, ansiedade e qualidade do sono, bem como entre as diferentes subescalas do questionário de qualidade de vida. Os resultados deste estudo piloto definem uma perspectiva de que esta modalidade terapêutica possa ser desenvolvida como uma estratégia potencial para o tratamento do excesso de peso e de anormalidades da homeostase glicêmica em indivíduos com excessod e peso. Além disso, confirmam a necessidade de protocolos futuros mais robustos, com um número maior de indivíduos investigados, a fim de identificar a eficácia da ETCC como uma ferramenta adjuvante no combate à obesidade e ao DM2 na prática clínica.

Palavras-chave: Obesidade. Sobrepeso. Estimulação transcraniana por corrente contínua. Neuromodulação não-invasiva. Homeostase glicêmica.

ABSTRACT

Obesity is defined as abnormal or excessive body fat accumulation, and it is a risk factor for insulin resistance and pancreatic β-cell dysfunction development, increasing the chances of developing type 2 diabetes (T2DM). Despite the different treatments proposed for its control, the prevalence of obesity, in general, has increased globally. Studies indicate that food craving is closely associated with weight gain. In this context, the increase in the brain circuit activity in the dorsolateral prefrontal cortex (dlPFC) region could generate therapeutic benefits in obesity treatment, considering that this brain region has previously been associated with reduced food cravings. In the same way, the increase in neuronal excitability is associated with the hypothalamic K_{ATP} channels opening, leading to hepatic gluconeogenesis suppression and glycemic control. Under these circumstances, transcranial direct current stimulation (tDCS) on the dlPFC emerges as a promising adjuvant technique in excessive weight and insulin resistance treatment. tDCS is a non-invasive neuromodulation technique that allows a safe, painless, and low-cost method for the induction of neuroplasticity, that is, to modulate the cortical function and influence the cognitive processes associated with eating behavior, more specifically, in the regulation of appetite and food consumption. Given the above, in this thesis, we evaluated the safety and therapeutic potential of tDCS combined with a hypocaloric diet in body weight, desire to eat and food intake reduction, and improvement of the glycemic profile in individuals with overweight or obesity. In a randomized, double-blind, placebo-controlled trial, 28 subjects (79% obese; 37.6 [5.8] years) completed four weeks (20 sessions, five consecutive days per week) of anodal tDCS with a fixed dose of 2 mA for 20 min, over the right CPFdl, associated with a hypocaloric diet to lose 3% of the initial weight. Participants were randomized in blocks in a 1:1 ratio and stratified by sex into active tDCS or placebo tDCS. Weight loss was assessed using weekly body weight measurements. A visual analog scale was used to evaluate the desire to eat at baseline and at the end of the study. Food consumption was measured by a three-day weighed dietary record at baseline, during, and at the end of the study. Glucose, insulin, and glycated albumin were evaluated in the fasting state, and Matsuda's estimates of insulin sensitivity, insulin secretion, and pancreatic β-cell function were evaluated using a 4-hour meal tolerance test, performed before and after the intervention. Safety and feasibility aspects were assessed by questionnaires on adverse effects, depression, anxiety,

mood, quality of life and sleep at baseline and the end of the study. The results showed that, although there was greater weight loss in the active group than in the placebo group (active: –4.5 kg [95% CI: –9.4 to 0.5] *vs*. sham: –2.3 kg [–5.0 to 0.3]), this difference was not statistically significant (p = 0.786). However, the active group had a lower desire for sweets (–23.7 percentage points [–40.2 to –7.1] *vs*. 1.0 percentage points [–13.3 to 15.2]; p = 0.039), significant improvement in fasting glucose (–7.8 mg/dL [–13.9 to –1.6] *vs.* –0.9 mg/dL [–4.0 to 2.2]; p = 0.045), fasting insulin (–7.7 µIU/mL [–13.9 to –1.6] *vs.* –1.3 µIU/mL [–3.3 to 0.7]; p = 0.020), and in Matsuda insulin sensitivity indices (4.6 $pmol^{-1}$×$mmol^{-1}$ [1.7 to 7.4] *vs.* 1.1 $pmol^{-1}$×$mmol^{-1}$ [–1.1 to 3.2]; p = 0.024). The study proved safe and with minimal adverse effects, given that there were no significant changes in depression, anxiety, and sleep quality scores, as well as between the different subscales of the quality of life questionnaire. The results of this pilot study define a perspective that this therapeutic modality could be developed as a potential strategy for overweight treatment and glycemic homeostasis abnormalities in individuals with excessive weight. In addition, they confirm the need for more powerful protocols with a greater number of individuals to identify the effectiveness of tDCS as an adjuvant tool in the fight against obesity and T2DM in clinical practice.

Keywords: Obesity. Overweight. Transcranial direct current stimulation. Non invasive neuromodulation. Glycemic homeostasis.

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LISTA DE ABREVIATURAS E SIGLAS

^{31}P-ERM – espectroscopia de fosforo por ressonância magnética

ADP – adenosina difosfato

AgRP – peptídeos relacionados à agouti

AKT – enzima da família das proteínas quinases

ARC – núcleo erqueado

ATP – adenosina trifosfato

BDNF – fator neurotrófico de crescimento cerebral

BHE – barreira hematoencefálica

BT – teste com refeição

CBM – teste de modificação de viés cognitivo

CMRgl – taxa metabólica cerebral de glicose

COMT – catecol O-metiltransferase

CPF – córtex pré-frontal

CPFdl – córtex pré-frontal dorsolateral

CPFvm - córtex pré-frontal ventromedial

D2/3R – receptor D2/3 de dopamina

DALYs – Anos de Vida Perdidos Ajustados por Incapacidade (do inglês, *Disability Adjusted Life Years*)

DM2 – diabetes mellitus tipo 2

ECNI – estimulação cerebral não invasiva

EMTr – estimulação magnética transcraniana repetitiva

ETCC – estimulação transcraniana por corrente contínua

EVA – escala visual analógica

FCI – inventário de food craving

FCQ-S – questionário de food craving – estado

FCT – testes de desafio alimentar

FDG-18F – 18F fluordesoxiglicose

FoxO – proteína Forkhead box O

FPT – testes com fotografias de alimentos

FSC – fluxo sanguíneo cerebral

GABA - ácido γ-aminobutírico

GBD – Estudo de Carga Global de Doença (do inglês: *Global Burden of Disease Study*)

GE – glicoexcitáveis

GI – glicoinibidores

GLUT-x – transportador de glicose tipo x

GSK3β – glicogênio sintase quinsae 3β

HOMA-IR - Modelo de Avaliação da Homeostase – Resistência à Insulina; do inglês, *Homeostatic Model Assessment of Insulin Resistence*)

IGF-1R – receptores do fator de crescimento semelhante a insulina

IMC (IMCs) – índice(s) de massa corporal

IRS – substrato do receptor de insulina

K_{ATP} – canais para potássio sensíveis a ATP

LH – hipotálamo lateral

LTD – depressão de longa duração

LTP – potenciação de longa duração

mA – miliampère

Met - metionina

mTOR1 – complexo 1 da proteína alvo mecanístico da rapamicina

MVA – Paradigma da Máquina de Venda Automática

NAc – núcleo accumbens

NMDA – N-metil D-aspartato

NMDAr – receptor de N-metil D-aspartato

PCr - fosfocreatina

PEG – produção endógena de glicose

PET – tomografia por emissão de pósitrons

Pi – fosfato inorgânico

PI3K – fosfatidilinositol 3-quinase

POMC – pró-opiomelanocortina

PVH – núcleo paraventrivular

RA – registro alimentar

RIs – receptores de insulina

RMf – ressonância magnética funcional

SAPK – proteínas quinases ativadas por estresse

SNC – sistema nervoso central

SNpc – substância nigra pars compacta

ST – teste com lanche

TH – tirosina hidroxilase

TrkB - receptor quinase B de tropomiosina (ou, receptor quinase B da tirosina)

TTOG – teste de tolerância oral à glicose

Val – Valina

VHM – núcleo ventromedial

VTA – área tegumentar ventral

SUMÁRIO

INTRODUÇÃO

1 INTRODUÇÃO

Indivíduos com excesso de peso e obesidade estão mais predispostos a desenvolver resistência à insulina e disfunção da célula β-pancreática, aumentando o risco de desenvolver diabetes mellitus tipo 2 (DM2) (1). O maior consumo de alimentos industrializados, processados, altamente palatáveis, ricos em gorduras e açúcares é um dos principais responsáveis pelo ganho de peso corporal (2). Apesar de sua etiologia ser multifatorial, deficiências no funcionamento executivo relacionadas ao córtex pré-frontal (CPF) – controle inibitório e valor de recompensa dos alimentos – estão associados ao desenvolvimento e à perpetuação do excesso de peso (3). Estes modelos neurocomportamentais associados à obesidade trazem a disfunção do sistema dopaminérgico de recompensa mesocorticolímbico e a alta suscetibilidade a pistas alimentares, como precursores cruciais do consumo alimentar excessivo, destacando a baixa atividade no CPF como um dos fatores responsáveis pela vulnerabilidade de um indivíduo para desenvolver obesidade (2, 4).

Paralelamente a isso, nos últimos anos, os estudos têm demonstrado que o cérebro também atua como um importante regulador da glicose sistêmica e do metabolismo energético (5). Embora o papel do hipotálamo na manutenção da homeostase glicêmica esteja bem estabelecido, outras áreas do cérebro também podem estar envolvidas neste mecanismo, como o núcleo accumbens (NAc), no estriado ventral. A glicose periférica é conhecida por modular a liberação de dopamina estriatal, ativando esta região de recompensa após o consumo de alimentos com alto índice glicêmico (6). No entanto, já foi demonstrado que a disponibilidade do receptor D2/3 de dopamina (D2/3R) estaria reduzida nesta região cerebral em humanos com obesidade e resistentes à insulina, em uma correlação inversa com o seu índice de massa corporal (IMC) (7).

Diante do exposto, é possível que a modulação/ativação de regiões cerebrais envolvias na homeostase glicêmica, no autocontrole do consumo alimentar e do desejo incontrolável (fissura ou *craving*) por alimentos altamente palatáveis, possa facilitar a aderência a intervenções dietéticas voltadas para a perda de peso e para o controle glicêmico. Neste sentido, o uso da estimulação transcraniana por corrente contínua (ETCC) vem sendo estudado como uma promissora ferramenta de neuromodulação não invasiva, com reconhecidos efeitos benéficos sobre o consumo

alimentar (8–10, 12, 15, 16), fissura por alimentos e desejo de comer (8, 9, 24, 10, 12, 16–20, 22) e metabolismo da glicose (25–27). A técnica é caracterizada por ser de baixo custo, com efeitos colaterais mínimos ou inexistentes, facilidade de operação, alta disponibilidade de dispositivos comerciais e com a possibilidade de uso de forma doméstica. Sendo assim, é possível que a sua utilização, associada a uma mudança de estilo de vida, exerça efeito adicional sobre a perda de peso e a sensibilidade à ação da insulina em indivíduos com sobrepeso ou obesidade.

Portanto, para tal investigação, essa tese se propõe a: 1) revisar os mecanismos de regulação cerebral, incluindo a regulação por sinalização cerebral de insulina, sobre o consumo alimentar, desejo de comer e sobre a homeostase glicêmica, assim como revisar a eficácia do uso da técnica de ETCC como ferramenta para o tratamento da obesidade, redução do consumo alimentar, do desejo de comer e da resistência à insulina; 2) descrever um protocolo de pesquisa para avaliação dos efeitos da ETCC associadas a uma dieta hipocalórica na aderência à dieta, emagrecimento e na alteração da homeostase glicêmica; 3) verificar aspectos de segurança e viabilidade da técnica proposta em indivíduos com sobrepeso e obesidade em um protocolo com sessões diárias de ETCC por quatro semanas; e, por fim, 4) analisar o efeito da ETCC associada a uma dieta hipocalórica sobre a perda de peso, consumo alimentar, desejo de comer e homeostase glicêmica nesta população.

CAPÍTULO I: REVISÃO DE LITERATURA

JUSTIFICATIVA

2 JUSTIFICATIVA

A prevalência global de obesidade vem aumentando a cada ano, juntamente com uma crescente demanda por tratamentos eficazes, seguros e acessíveis. Por ser uma doença crônica, está intimamente associada ao aumento da morbidade e da mortalidade (170). Os tratamentos de primeira linha para o tratamento da obesidade incluem intervenções abrangentes no estilo de vida, englobando dieta e atividade física (171, 172). No entanto, a eficácia deste tipo de tratamento permanece modesta, com a maioria dos indivíduos reduzindo menos de 5% de seu peso total (173). Para indivíduos com obesidade grave, a cirurgia bariátrica é o principal tratamento de escolha (174). Porém, embora muitos pacientes alcancem uma perda de peso expressiva com a cirurgia, trata-se de um procedimento altamente invasivo e relacionado a outras complicações, como deficiências nutricionais e de micronutrientes (175, 176). Mais recentemente, os medicamentos agonistas do receptor da GLP-1 (*glucagon-like peptide-1*), com ação sobre o apetite e a saciedade, vêm sendo recomendados de maneira promissora para o controle da obesidade. Porém são medicamentos injetáveis, caros e sujeitos a diversos efeitos colaterais, principalmente relacionados ao trato gastrointestinal (177). Neste sentido, os tratamentos com neuromodulação não invasiva têm sido propostos com a intensão de alterar o funcionamento dos elementos neurais envolvidos nos aspectos fisiológicos da ingestão de alimentos, fome e/ou saciedade, a fim de facilitar a aderência a intervenções dietéticas voltadas para a perda de peso e para o controle glicêmico (138). Sendo assim, é possível que a sua utilização, associada a uma mudança de estilo de vida, exerça efeito adicional sobre a perda de peso e sobre a sensibilidade à ação da insulina em indivíduos com sobrepeso ou obesidade.

OBJETIVOS

3 OBJETIVOS

3.1 OBJETIVO GERAL

O objetivo geral deste trabalho foi avaliar os efeitos de repetidas sessões de ETCC anódica sobre o CPFdl direito versus placebo associadas a uma dieta hipocalórica na aderência à dieta, emagrecimento e na alteração da homeostase glicêmica em indivíduos com sobrepeso e obesidade, durante quatro semanas de intervenção.

3.2 OBJETIVOS ESPECÍFICOS

a) Artigo I

Descrever um protocolo de pesquisa de um ensaio clínico randomizado, duplo cego e controlado por placebo para avaliação dos efeitos de repetidas sessões de ETCC anódica sobre o CPFdl direito versus placebo associadas a uma dieta hipocalórica na aderência à dieta, emagrecimento e na alteração da homeostase glicêmica em indivíduos com sobrepeso e obesidade, durante quatro semanas de intervenção.

b) Artigo II

Avaliar o estado de saúde psicossocial e a qualidade de vida de indivíduos com sobrepeso ou obesidade em tratamento com repetidas sessões de ETCC anódica sobre o CPFdl direito versus placebo associadas a uma dieta hipocalórica.

c) Artigo III

Avaliar o efeito de repetidas sessões de ETCC anódica sobre o CPFdl direito versus placebo associadas a uma dieta hipocalórica, sobre a perda de peso, consumo alimentar e desejo de comer de indivíduos com sobrepeso e obesidade.

d) Artigo IV

Avaliar os efeitos de repetidas sessões de ETCC anódica sobre o CPFdl direito associado a uma dieta hipocalórica, sobre a homeostase da glicose em indivíduos com sobrepeso e obesidade.

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CAPÍTULO II: ARTIGOS

The effect of transcranial direct current stimulation associated with hypocaloric diet on weight loss and metabolic profile in overweight or obesity: study protocol for a double-blind, randomized controlled clinical trial

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4 ARTIGO I

4.1 THE EFFECT OF TRANSCRANIAL DIRECT CURRENT STIMULATION ASSOCIATED WITH HYPOCALORIC DIET ON WEIGHT LOSS AND METABOLIC PROFILE IN OVERWEIGHT OR OBESITY: STUDY PROTOCOL FOR A DOUBLE-BLIND, RANDOMIZED CONTROLLED CLINICAL TRIAL[1]

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Abstract

Background: Diet interventions have limited success in promoting sustainable weight loss; new treatments allowing better compliance with hypocaloric diets should be developed. The aim of this trial is to describe the effects of a protocol combining repetitive active transcranial direct current stimulation (tDCS) with a hypocaloric diet on weight loss and food consumption in overweight or obese adults.

Methods: Overweight or obese adults between 20 and 50 years of age with stable weight over the last four months were selected for a four-week randomized clinical trial of fixed-dose tDCS (20 sessions; five consecutive weekdays/week, 2 mA, 20 min) over the right dorsolateral prefrontal cortex associated with a weight-loss diet. The subjects were randomly assigned (1:1) and stratified by sex to active tDCS + diet or sham tDCS + diet. The study was conducted at the Endocrine and Metabolism Unit of the Hospital de Clínicas de Porto Alegre, Brazil. The primary outcome was weight loss. Energy and macronutrient consumption, as well as adherence to the diet, were assessed using three-day weighted diet records (WDR). Changes in blood glucose and plasma insulin were assessed and participants completed self-report questionnaires to assess changes in mood and food behavior. All analyses were per protocol and intention to treat.

Discussion: This study explores the potential role of tDCS as an adjunct treatment with a hypocaloric diet for obesity management.

Trial Registration: ClinicalTrials.gov Identifier, NCT02683902. Registered on 11 January 2016.
https://clinicaltrials.gov/ct2/show/NCT02683902?term=NCT02683902&rank=1

Keywords: Clinical trial; Transcranial direct current stimulation; neuromodulation; weight loss; obesity; hypocaloric diet.

1 INTRODUCTION

Obesity is a complex and multifactorial condition characterized by adipose tissue accumulation that is strongly associated with multiple comorbidities, including type 2 diabetes, cancer, cardiovascular diseases, sleep apnea, and physical and social limitations [1–3]. Increased consumption of industrialized, processed, highly palatable foods rich in fat and sugar is being identified as a major contributor to the worldwide obesity epidemic [4]. As a consequence, limiting the consumption of foods with high caloric density could have an important impact in preventing and treating obesity [4, 5].

Calorie-restricted diets associated with lifestyle intervention are the first-choice treatment for weight loss [6]. However, weight loss through hypocaloric diets could induce neuroendocrine changes that trigger an intense, subconscious and powerful urge to eat, known as food craving [7, 8]. The behavioral drive to find and eat food is closely related to the rewarding feeling that this food provides, resulting in a strong motivation to engage in this behavior again, which frequently results in failure to maintain a hypocaloric diet [7, 9].

Transcranial direct current stimulation (tDCS) is a non-invasive neuromodulation technique that provides a safe, painless, inexpensive, and non-restrictive method to induce neuroplasticity [10, 11]. It has been previously shown that dorsolateral prefrontal cortex (DLPFC) neuromodulation controls the desire to eat and, thus, may be involved in food intake regulation. The use of tDCS has been studied as a promising treatment for food craving [12–15]. Moreover, two recent studies have demonstrated caloric intake reductions of approximately 32% after a single tDCS session [16] and 14.2% after eight consecutive tDCS sessions compared to controls [17]. This effect was related to reduced food intake, particularly carbohydrates [17].

Previous studies have demonstrated that repetitive tDCS sessions may have potential as a therapeutic intervention for decreasing the cravings associated with cocaine, alcohol, and smoking [18–20]. Because food and drug cravings share common biological mechanisms in the brain, repetitive tDCS sessions should yield similar results in overweight or obese individuals on a hypocaloric diet. However, despite the potential role of tDCS as a treatment for obesity, few studies have been conducted on overweight or obese individuals using this procedure [15, 21, 22]. To our

knowledge, no study has been published evaluating a daily tDCS protocol as an adjuvant tool for weight loss during a hypocaloric diet.

In this context, it was designed a randomized clinical trial in order to evaluate the effect of repetitive tDCS associated with a hypocaloric diet on food consumption and weight loss in overweight or obese adults. We hypothesized that subjects undergoing daily active tDCS associated with hypocaloric diet will have better adherence to the prescribed diet than those undergoing sham tDCS resulting in greater weight loss and improved metabolic profile.

2 METHODS

2.1 Design overview

Subjects in this four-week double-blind, randomized, single-center, placebo-controlled trial received one of two different types of intervention: (1) daily sessions of active tDCS + hypocaloric diet, or (2) daily sessions of sham tDCS + hypocaloric diet.

After screening and selection visits, included participants underwent a complete baseline assessment that included a clinical and nutritional interview, as well as screening questionnaires to assess eating behavior and psychiatric disorders such as depression and anxiety. The participants also underwent a standard protocol including a physical examination, anthropometric assessment, and metabolic and biochemical laboratory measurements. They also received individual counseling to improve dietary habits and were prescribed a low calorie diet by a dietician to reduce 3% of their initial body weight over a four-week treatment. The subjects also underwent one tDCS session each weekday (active or sham stimulation according to previous randomization) during the four weeks of treatment, i.e., a total of 20 sessions. During these sessions, the subjects were also exposed to a series of images of high calorie foods known to stimulate the DLPFC and the appetite [23]. A subgroup of participants used a flash glucose monitoring system (FreeStyle® Libre™) during the intervention to assess whether this form of brain stimulation had any effects on glycemic variability. At the end of the four-week intervention period, the participants underwent the same baseline assessments, and their weight, body composition and food behavior were then reassessed after three and six months.

The present protocol was written in accordance with Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) guideline, completing the SPIRIT checklist, and constructing a flow diagram in order to optimize the quality of reporting [24] (Figure 1 and Additional file 1).

Table 1. Inclusion and exclusion criteria.

Inclusion Criteria
Adults of either gender, aged between 20 and 50 years.
BMI 25 ≥ 35 Kg/m^2 at screening.
Stable weight for at least 12 weeks prior to screening.
Exclusion Criteria
Women who were pregnant, breastfeeding, trying to get pregnant or not using adequate contraception.
Women in perimenopause, menopause, postmenopause, or who have had early menopause (under 40 years) or a hysterectomy or oophorectomy.
A history of severe cranial trauma with changes in cranial anatomy or metallic intracranial implants.
Patients with preexisting irritations, cuts, or lesions where the tDCS electrodes would be placed.
Type 1 diabetes. Metabolic or acute complications of diabetes within the past six months.
A history of any acute or chronic intestinal disease (e.g., Crohn's disease, inflammatory bowel disease)
Having received nutritional counseling in the last six months by a nutritionist.
A history of severe depression or other serious psychiatric comorbidities.
A history of gastric bypass, antrectomy or small bowel resection.
A history of chronic pancreatitis or idiopathic acute pancreatitis.
Myocardial infarction, coronary artery bypass surgery, post-transplant cardiomyopathy, or stroke in the last six months.
Any abnormality in clinical laboratory tests that might prevent safe participation in the study.
Diagnosed and/or treated tumor (except basal cell skin cancer, carcinoma in situ of the cervix or prostate cancer in situ) in the last five years.
A history of known hemoglobinopathy or chronic anemia.
Blood donation of one unit or more (≥500 ml) or significant blood loss (≥ 500 ml) in the last two weeks, or blood transfusion in the past eight weeks.
Oral antidiabetic drug treatment and/or herbal preparations or non-prescribed medications that could affect glycemic control in the last 12 weeks.
Chronic oral or parenteral corticosteroid treatment (> 7 consecutive days) in the last four weeks.
Weight loss treatment agents (e.g., orlistat, sibutramine, topiramate, bupropion) in the last 12 weeks.
Ingestion of mineral oil or fiber supplements (e.g., Benefiber, Metamucil).
Unstable doses of lipid-lowering drugs in the past eight weeks.
Unstable doses of thyroid hormone replacement in the past 12 weeks.
Enrolled in another drug/device trial.
Any laboratory abnormalities identified in screening, such as alanine aminotransferase (ALT) and/or aspartate aminotransferase (AST) > 3 times the upper limit of normal, glomerular filtration rate estimated by the CKD-EPI equation ≤ 30 ml per min per 1.73 m^2, TSH outside the normal range, or triglycerides ≥ 400 mg/dL.
A history of active substance abuse (including alcohol) within the past year.
Any acute condition or exacerbation of a chronic condition that would, in the investigator's opinion, interfere with the procedures.

BMI, body mass index; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; tDCS, transcranial direct current stimulation; TSH, Thyroid-Stimulating Hormone.

2.2 Participants

Overweight or obese subjects were recruited through advertisements in the Hospital de Clínicas de Porto Alegre webpage, a local newspaper, and on television, or through referral by a physician or nutritionist from clinics in the metropolitan area of Porto Alegre, a large city in southern Brazil.

The study included men and women with a body-mass index (BMI: i.e., weight in kilograms divided by the square of the height in meters) between 25 and 35, aged 20-50 years, with stable body weight in the 12 weeks prior to screening, who had not received nutritional counseling six month prior to screening, and who were able to comply with the requirements of the study protocol. Table 1 shows further details of the inclusion and exclusion criteria.

All those who responded to the advertisements (n=322; 171 from the first campaign and 151 from the second) were contacted by telephone and the study's objectives were explained to them. Those interested in participating were assessed according to the inclusion and exclusion criteria. A total of 148 individuals were considered eligible and agreed to participate in a drawing. They were included in a Microsoft® Excel spreadsheet in alphabetical order according to gender. Each participant was randomly assigned a value between 0 and 1 using the "=RANDOM()" function, and they were then organized in ascending order according to this number. The first individuals from the women's and men's lists were invited for a face-to-face screening. If the randomly selected participant was ineligible for this study (i.e., failure to meet the inclusion criteria or withdrawal), he or she was replaced by the next person on the list.

2.3 Study protocol

After selection, the participants underwent a three-day assessment including a clinical and nutritional interview, anthropometric and laboratory measurements, and questionnaires to assess mood, appetite, and food intake behavior. This same assessment was performed at the end of the hypocaloric diet and tDCS intervention. The study timeline is shown in Figure 1.

STUDY PERIOD																											
	Enrolment/ Baseline			Post-allocation																						Close-out	
TIMEPOINT*	V1	V2	V3	t_1	t_2	t_3	t_4	t_5	t_6	t_7	t_8	t_9	t_{10}	t_{11}	t_{12}	t_{13}	t_{14}	t_{15}	t_{16}	t_{17}	t_{18}	t_{19}	t_{20}	t_{3mo}	t_{6mo}	V1	V2
Eligibility screen	X																										
Informed consent	X																										
Randomization			X																								
INTERVENTIONS																											
Hypocaloric Diet			X	♦	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	♦				
tDCS active/ tDCS sham 2mA, 20 min				♦	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	♦				
ASSESSMENTS																											
2h OGTT		X																									X
4h LMTT			X																							X	
Body weight			X					X					X					X					X	X	X	X	
Body composition			X																					X	X	X	
FreeStyle Libre				X										X													
Indirect calorimetry		X																									X
Nutritional visit								X					X					X					X				
Questionnaires and/ or VAS	X	X		X									X										X	X	X	X	X
Three- day WDR			X										X													X	

Figure 1. SPIRIT diagram.

*Timepoint: V1, V2, V3: visits one, two or three in the respective period (baseline or after four weeks of tDCS). t1 to t20 represent each tDCS/sham-tDCS session. t3mo and t6mo represent the evaluations performed three and six months after the end of the intervention period. LMTT: liquid meal tolerance test. OGTT: oral glucose tolerance test. VAS: visual analogue scale. WDR: weighted diet records.

2.3.1 Clinical interview

The participants underwent a standard evaluation that included their medical history and a physical examination. Ethnicity was classified as white or non-white according to official Brazilian methods of self-reported skin color [25]. Current smoking was defined as active consumption in the last three months. Habitual alcohol consumption was determined with a yes or no question. Sitting blood pressure was measured in triplicate with a one-minute interval between measurements after an initial cuff adjustment and five-minute rest period (Omron BP785, OMRON Healthcare Co. Bannockburn, IL, EUA). The mean of these measurements was used to estimate

systolic and diastolic arterial blood pressure. The clinical interview also included the following:

(a) CAGE (Cut down/Annoyed/Guilty/Eye-opener) Questionnaire screening to identify harmful alcoholic beverage consumption [26].

(b) Determination of socioeconomic status according to Brazilian economic classification criteria (ABEP 2015) [27].

(c) Application of the International Physical Activity Questionnaire (IPAQ) Short Form for physical activity [28]. Normal physical activity was estimated in steps/day and assessed for six consecutive days with a digital pedometer (HJ-152-E, OMRON Healthcare Co., Kyoto, Japan) [29].

(d) Application of the 36-item Short Form (SF-36) Health Survey to assess health-related quality of life (QOL). This instrument is a well-validated measure of health status and health-related quality of life, in which eight subscales are used to assess separate domains of health and related functioning. The survey provides a physical health summary score and a mental health summary score [30], with higher scores indicating better QOL.

(e) Application of the Epworth Sleepiness Scale (ESS-BR), which provides a simple standardized means of measuring general levels of sleepiness in ordinary life situations. In the validated Brazilian version, volunteers rate their chances of sleeping in six situations on a scale of 0 to 4, with a minimum score of 0 and maximum of 24. Normal scores range between 0 and 10 [31, 32].

2.3.2 Nutritional interview

The nutritional interview was conducted at baseline and consists of a detailed investigation of dietary habits. Habitual food intake was assessed using a three-day weighed diet records (WDR) technique (two nonconsecutive weekdays and one weekend day). The participants received a digital scale to weigh food (0 – 5 kg, Caumaq® EK3250) and a measuring cup (25 – 250 mL; Marinex®). A trained registered dietitian explained and demonstrated the proper use of the equipment to

each subject [33, 34]. The three-day WDR was performed the week before participants were prescribed a diet.

2.3.3 Mood assessment

The Beck's Depression Inventory (BDI), a 21-item self-report rating inventory, is probably the best known and most widely used depression scale. This tool was applied at baseline and at the end of the study, being used to measure characteristic attitudes and symptoms of depression [35, 36]. Scores range from zero to 63: zero, the minimal score, indicates no depression; 10 to 16 indicates mild to moderate depression; 17 to 29 indicates moderate to severe depression; and 30 to 36 indicates a state of severe depression.

The State-Trait Anxiety Inventory (STAI), specifically the State Anxiety Scale (A-State), requires a description of how the participant feels at "this very moment" in relation to 20 items presented on a four-point Likert scale (1 = "not at all," 2 = "somewhat," 3, = "moderately," 4 = "very much"). This tool assesses the intensity of feelings of anxiety using items that measure subjective feelings of apprehension, tension, nervousness, worry, and activation/arousal of the autonomic nervous system. Participants responded to this scale at baseline and after the intervention. The STAI, translated and validated for Brazil [37], has been revalidated in a number of studies [38, 39]. Scores range from 20 to 80 points, with higher values indicating higher anxiety levels [40].

The participants rated their attention and mood on a 100 mm visual-analogue scale (VAS) word-anchored at each end (terrible-very good) by answering two questions: "How is your [attention/mood] at this very moment?".

2.3.4 Eating behavior assessment

Hunger throughout the day was self-reported at baseline and at the end of the study (in the morning while the patient was still fasting) using a 100 mm VAS consisting of six word-anchored questions adapted from Haber et al. [41]. The participants recalled the intensity of their hunger upon waking, before lunch, three hours after lunch, before supper, three hours after supper and before bedtime.

Subjective satiety and hunger scores were assessed at baseline and at the end of the four-week intervention using a seven-point VAS described by Holt and Miller (1995) [42]. On this scale the participants rated their hunger at “this very moment”. The answers ranged from -3 “extremely hungry” to 0 “no particular feeling”, to +3 “extremely full”.

An additional 100 mm VAS, consisting of four word-anchored questions (“yes, very much”-“not at all”), was performed before and after the four-week intervention to assess the desire to eat something fatty, salty, sweet or savory (as previously described) [43, 44].

The Brazilian Food Craving Inventory (FCI-Br), consisting of 23 food items distributed in three dimensions (sweet foods, high-fat foods and traditional meals), was applied at baseline and at the end of the study. The validated FCI-Br [45] has been adapted to include important characteristics of Brazilian eating habits. Like the original version [46], this tool begins with a question “Over the past month, how often have you experienced a craving for the food listed below?’’. Each of the foods listed is evaluated using a five-point Likert scale ranging from “(1) never” to “(5) almost every day”. The total score for each FCI dimension is calculated by the sum of the scores for each related item.

2.3.5 Anthropometric assessment

Anthropometric assessment was performed with the participants barefoot and wearing light clothing. Height was measured using a wall-mounted stadiometer and recorded to the nearest 0.1 cm. An InBody 230 tetrapolar body composition analyzer (Biospace Co. Ltd, Seoul, South Korea) was used to measure body weight and composition via bioelectrical impedance. This procedure was performed in the morning, with the participant fasting and having avoided exercise in the last 24 hours. BMI was calculated by weight (kg)/height (m2). Midupper arm circumference was measured at the midpoint of the upper right arm, between the acromion process and the tip of the olecranon. Waist circumference was measured midway between the lowest rib margin and the iliac crest, near the umbilicus. Hip circumference was measured at the maximal gluteal protrusion. Flexible, non-stretch fiberglass tape was used for these measurements.

2.3.6 Metabolic assessment

Indirect calorimetry (Metacheck, Korr Medical Tchnologies, Salt Lake City, Utah, USA) was used to determine resting metabolic rate (RMR). This measurement was obtained at baseline and at the end of the study after a 12-hour fast and at least 24 hours without physical activity. The test required a single 20 min session, prior to which the participants rested for at least 30 min. Indirect calorimetry analyzes the respiratory gases of the patients to measure their oxygen consumption (VO2) and derives their resting metabolic rate (RMR) using Weir's equation with assumed respiratory quotient (RQ)=0.83 [47].

Adjusted resting metabolic rate (aRMR) was defined using a strategy for RMR normalization that involved multiple linear regression, a method that has been widely adopted in human obesity studies. The goal of normalizing RMR is to eliminate the influence of body size variation per se on RMR, such that the aRMR variable does not systematically vary with significant independent variables. Only then can RMR be compared across groups to determine the effect of an experimental intervention [48]. RMR is normalized by variables that, in multiple linear regression models, are shown to be independent determinants of RMR variation. To do this, the log10 of resting metabolic rate is estimated as a linear function of log10 using regression, as recommended by Kaiyala and Schwartz [48].

2.3.7 Glycemic homeostasis and control

2.3.7.1 Assessment of glycemic control

Glycemic control was assessed at baseline and at the end of the study, including a 75-g oral glucose tolerance test (OGTT) in which glucose was measured at 0 and 120 minutes, glycated hemoglobin (A1c) and glycated albumin (GA) measurements, and a liquid meal tolerance test (LMTT) in which both glucose and insulin were measured at -30, -15, 0, 30, 60, 120, 180 and 240 min.

For the LMTT, after a 12 hour fast, a flexible intravenous catheter was placed in the antecubital space of the arm and three blood samples (-30, -15 and 0 min) were drawn over 15 minutes to determine the mean fasting values. A standard liquid formula meal was then administered to all participants (400 mL Trophic 1.5, Prodiet, Curitiba,

PR, Brazil; 600 kcal, 53% carbohydrates, 16% protein and 31% fat), who were instructed to consume it within 10 min. Additional blood samples were collected at 30, 60, 120, 180 and 240 min after the meal [49]. Serum and plasma were divided into aliquots and immediately sent for analysis or frozen at -80 ºC for further analysis, as described below. Fasting glucose and insulin values were calculated as the mean of the three basal values (–30, –15 and 0) from the LMTT. The area under the curve was calculated for each LMTT using the trapezoidal rule [50]. Self-reported ratings of hunger and satiety and a self-reported rating of desire to eat were also answered immediately after each blood collection using the VAS described in item 2.3.5.

Glycated albumin is a laboratory test that reflects short-term glycemia due to the half-life of albumin, which is approximately three weeks. This test showed a better correlation with glucose excursions and post-prandial hyperglycemia than A1c, which makes it more suitable for assessing a short-term treatment efficacy [51].

2.3.7.2 Assessment of glycemic variability

Glycemic variability was assessed using flash-continuous glucose monitoring (FCGM) system technology. This factory-calibrated interstitial glucose monitoring system (FreeStyle® Libre™) uses a wired glucose oxidase enzyme co-immobilized on an electrochemical sensor that is worn on the back of the arm for up to 14 days [52]. Participants can obtain a real-time reading every minute by scanning the sensor with a reader. The data are transferred from the sensor to the reader memory and recorded automatically every 15 min. Glucose trends, rates, and direction for the past eight hours are shown on the screen and can be uploaded to a computer for summary reports. The participants' sensor data were uploaded using FreeStyle® Libre™ software (Abbott Diabetes Care Inc., version 1.0) and saved into five files: one general file and one for each of the four weeks of treatment.

The mean amplitude of glycemic excursion (MAGE), which is the mean of blood glucose values exceeding one standard deviation from the 24-hour mean blood glucose value, was used as an index of glycemic variability [53–55]. To adapt MAGE for interstitial glucose, glucose values were captured at 2 h intervals from the minimum and maximum points of the glucose excursion plot. WebPlotDigitizer (Austin, Texas, USA. Version 3.12) was used to calculate the minimum and maximum mean glucose

values. This software (http://arohatgi.info/WebPlotDigitizer) accurately transforms a variety of plot types and images to numerical data [56].

A subgroup of individuals enrolled in this study received a FreeStyle® Libre™ unit to be used during the course of the four-week intervention. Two sensors were required to monitor each participant: the first was placed on the first day of the intervention, one hour before the first tDCS session; the second was placed after 14 days. A trained researcher placed the sensors on the participants according to manufacturer instructions and provided a brief explanation of their operation. For this trial, the participants were instructed to check the sensor at least eight times a day: after waking up, just before and two hours after breakfast, lunch and supper, and before sleeping.

2.3.8 Laboratory measurements

Twelve-hour fasting blood samples were collected from all participants to analyze lipid profile (total cholesterol, HDL-c, and triglycerides), glycated hemoglobin (A1c), and to perform a 75g oral glucose tolerance test (OGTT), in which glucose was measured at 0 and 120 min. Total cholesterol and triglycerides were determined with an enzymatic colorimetric method (Cobas c702). HDL-cholesterol was determined with a homogeneous enzymatic colorimetric assay (Cobas c702). A1c levels were determined with an HPLC method (Variant II Turbo HbA1c, BioRad Laboratories, Hercules, CA, USA) certified by the National Glycohemoglobin Standardization Program (http://www.ngsp.org/prog/index.html), which is aligned with that of the International Federation of Clinical Chemistry. The Clinical Pathology department of the Hospital de Clínicas de Porto Alegre participates in the A1c External Quality Assurance Program, in which it has demonstrated adequate performance [57]. Plasma glucose from OGGT was determined with an enzymatic UV–hexoquinase method (Cobas c702).

On a different day from the OGTT, a LMTT was conducted to assess not only glucose and insulin response to a meal but also changes in the substances and hormones involved in weight regulation and glucose homeostasis. To assess this eight-point glucose-insulin curve, blood samples was collected in Vacuette® tubes with sodium fluoride for glucose and with separator gel serum tubes for insulin. Serum and plasma were obtained after centrifugation (3500 rpm, 15 min, room temperature) and

kept on ice until analysis. The measurements were taken at the Hospital de Clínicas de Porto Alegre Clinical Pathology Unit: glucose was determined using an enzymatic UV–hexoquinase method (Cobas c702), while insulin was determined using a chemiluminescent microparticle immunoassay (Architect Ci4100).

For hormonal analysis, two venous blood samples (4 mL) were collected in EDTA-treated tubes (Vacuette®). In one of the tubes, 40µL of DPP-IV inhibitor (K579, Tocris; Bio-techne, Minneapolis, USA) was immediately added to prevent degradation; plasma was obtained by centrifugation at 1500 rpm and 4ºC for 10 min, with the aliquots stored at -80ºC. The other plasma sample was centrifuged at 3500 rpm and room temperature for 15 minutes, aliquoted and stored at -80 ºC for further analysis.

For glycated albumin (GA), venous blood samples (5 mL) were collected in separator gel serum tubes (Vacuette®). Serum was obtained after centrifugation (3500 rpm, 15 min, room temperature) and stored at -80ºC for subsequent determination. GA measurement involved the “GlycoGap” enzymatic method (Diazyme®, California, EUA), which measures the amount of glycated serum protein (mmol/L) but not GA specifically. However, due to similar reaction principles, GA levels can be calculated from the total albumin concentration. This method presented more analytical precision, with intra- and inter-assay coefficient of variations of 3.5 % and 8.7%, respectively, in previous analyses performed in our laboratory [58].

2.3.9 Assessment of insulin sensitivity and β-cell function

Insulin sensitivity and β-cell function were estimated using static and dynamic measures based on glucose, C-peptide and insulin measurements taken during the LMTT. The homeostasis model assessment (HOMA) index described by Matthews et al. [59] can be calculated in two ways: estimating IR, in which HOMA-IR = fasting plasma insulin (FPI; µUI/mL) x fasting plasma glucose (FPG; mmol/L) / 22.5; and estimating β-cell function, in which HOMA-β = (20 x FPI) / (FPG - 3.5). The calculator used for this purpose is available at http://www.dtu.ox.ac.uk, (accessed March 2017) [60].

The LMTT provides a means of assessing insulin secretory patterns in more physiological conditions, since these tests include the incretin effect, which follows oral nutrient ingestion [61]. Insulin sensitivity and β-cell responsivity to glucose were determined as previously described [62]. Plasma glucose and insulin concentrations

were used to determine LMTT indices of insulin sensitivity (MISI) and secretion (ISI). Pancreatic β-cell function was determined by calculating the disposition index (LMTT-DI).

2.3.10 Intervention procedures

At the end of the baseline evaluation, each participant was prescribed a hypocaloric diet aimed at reducing his or her initial weight by at least 3% in four weeks. While on the diet, the participants received five weekly visits for tDCS intervention, either active or sham according to prior randomization. A registered dietitian blinded to the tDCS intervention visited each participant weekly to confirm diet adherence and to make any necessary adjustments to the diet. Participants were instructed to not change their current physical activity during the trial. Body weight was also assessed weekly with the same bioelectrical impedance scale used in the baseline evaluation (3-hour fast, light clothes, barefoot).

If a participant withdrew or was removed from the study for any reason, all research-related activities for that individual were immediately discontinued. Final clinical, anthropometric and laboratory exams were collected from willing participants. Intention to treat and per protocol analysis were performed.

2.3.10.1 Hypocaloric diet

The energy deficit was calculated for each participant individually using the online Body Weight Planner program, which is a validated dynamic simulation model of human metabolism [63, 64] recommended by NIDDK for prescribing weight loss diets. Using information on age, sex, height, weight, and physical activity level, the number of kcal per day needed to alter a specific amount of weight in a certain number of days can be determined.

The prescribed hypocaloric diets conformed to American Diabetes Association specifications for overweight or obese patients with type 2 diabetes or prediabetes. The planned macronutrient profile was: 45 to 50% of total energy intake as carbohydrates, with emphasis on a low glycemic index; 15 to 25% as protein; ≤30% from fat, with unsaturated fats being the main source (saturated fat was limited to <7%); and ≥20 g/day or 14 g/1000 kcal/day as fiber [65].

Compliance with this diet and changes in food consumption from baseline were assessed using the three-day WDR technique at three different times: at baseline, as previously described (2.3.4), at the end of the two first weeks, and again during the last two weeks of the study, totaling nine WDRs. The diet composition, obtained from the prescribed diet and the three-day WDRs, was analyzed (USDA table [66]) in NutriBase 17 Pro Edition software (version 17.2, CyberSoft, Inc., Phoenix, Arizona). The mean values for energy and nutrients consumed during the three-day WDR were calculated. Patients were classified as adherent or non-adherent according to whether or not they reached their calculated energy goals and diet recommendations.

2.3.10.2 Transcranial Direct Current Stimulation

The tDCS technique consists of applying a weak constant electrical current (typically 1.0 to 2.0 mA) through two surface electrodes placed on the scalp to modulate brain activity in a polarity-dependent fashion, i.e., anodal stimulation typically increases spontaneous neuronal excitation (depolarization), while cathodal stimulation has the opposite effect (hyperpolarization)

A battery driven, direct current stimulator called the Chattanooga IontoTM Dual Channel Electrophoresis System (Chattanooga Group, Hixson, TN, USA) was used in this protocol. Two mA of direct current was delivered over the right dorsolateral prefrontal cortex (DLPFC) through a pair of 35 mm2, 0.9% saline-soaked surface sponge electrodes placed according to the 10-20 International System of Electrode Placement (EEG 10/20). The right DLPFC position was found according to Beam et al.'s methods [67]. This system accounts for variability in subject skull size by using certain percentages of the circumference and distances between four basic anatomic landmarks (right and left tragus, nasion and inion). We used Beam F3 Locator software (www.clinicalresearcher.org), which was developed to more accurately determine where the electrodes should be placed on the skull [68].

The participants were randomized to receive either active or sham tDCS treatment. While following the hypocaloric diet, they underwent 20-min daily tDCS sessions each weekday (including holidays) for four weeks (20 total sessions). If a scheduled tDCS session was missed for any reason, it was made up the following weekend. The participants were instructed not to consume food three hours prior each

stimulation session. Each session was conducted at the same time of day for each participant, and only trained technicians performed tDCS.

For active tDCS, the electrodes were placed with the anode electrode over the right DLPFC (F4) and the cathode electrode over the left DLPFC (F3), using the technique described above. The current was ramped up for 15-s until it reached 2 mA, and the participants were then stimulated for 20 min. The current was then ramped down for another 15-s and turned off. For sham tDCS, the electrodes were placed identically to the active group, but the current was ramped up to 2 mA and then back down in the first and last 30-s of the 20-min session. As a result, the participants could feel the initial itching sensation associated with turning on the device, but received no stimulation for 19-min of the session.

During the sessions, the subjects were also exposed to a series of high caloric food images previously demonstrated to stimulate the appetite. Three different sets of 200 pictures of high-calorie and highly palatable foods such as sweets, high-fat foods and traditional meals were shown to participants as a video presentation during the 20-min sessions, with a 6-s interval between pictures. The participants were asked to pay attention to these images, and turn off phones, pagers or other devices that might distract their attention.

In addition, the participants' subjective feelings of desire to eat, mood and attention are assessed immediately before and after the first, tenth, and twentieth sessions with a VAS, as described in 2.3.5. The aim was to verify the presence of acute effects on their desire to eat after visual stimuli associated with tDCS.

Since effects such as headache, scalp burning, tingling, dizziness has been previously associated with tDCS, the participants were asked about adverse effects twice each week at the end of a stimulation session, as well as whether they had experienced any such effects after the previous session. No patient reported any adverse effects during the intervention period or in subsequent follow-up.

2.4 Outcomes

The primary outcomes were reductions in body weight and BMI from baseline and reduction or maintenance of the prescribed energy and macronutrient intake during the four-week intervention. The secondary outcomes were reduced hunger, desire to eat and food craving scores compared to baseline; reductions in fasting and

postprandial responses of plasma glucose, insulin, glycated albumin, hunger hormones, such as ghrelin, and an increase in the blood levels of hormones that participate in the regulation of satiety, such as PYY3-36, GLP-1, PP [49]. These hormones were measured if a significant tDCS impact was detected regarding weight, desire to eat, food cravings, or glycemic control and variability.

The exploratory outcomes included: the number of participants achieving a 3% reduction or more in body weight compared to baseline; improvements in insulin sensitivity and β-cell function compared to baseline; changes in body composition, such as reduced waist and hip circumferences and reduced body fat mass compared to baseline; changes in depression and anxiety scores compared to baseline; and acute changes in subjective feelings of desire to eat, mood and attention between pre- and post-tDCS sessions.

2.5 Statistical Procedures

2.5.1 Sample size

The sample size was calculated based on a significant reduction in the amount of calories ingested after a single 20-min session of active tDCS ($F_{1,8} = 8.4$, $p = 0.02$, $\eta p2 = 0.5$) [16]. Three types of analysis were performed in G*Power 3.1.9.2® software, considering a power of 80% and an α of 0.05, as follows: a) effect size (f) = 1.0: 10 subjects; b) effect size (f) = 0.8: 12 subjects; and c) effect size (f) = 0.6: 20 subjects. In another study, a 14.2% reduction in the amount of calories ingested was observed after 8 consecutive days of active tDCS sessions. The authors considered a medium effect size (d) > 1.09 as sufficient for their findings [17]. Following these assumptions, a total of ≥10 subjects would be required in each group to detect a medium-sized effect in this study.

2.5 2 Randomization

The block randomization method is designed to randomize subjects into groups that result in equal sample sizes [69]. In the present study, two blocks of six participants and four blocks of four participants were used, stratified by gender (three female and three male blocks) in a 1:1 ratio to receive one of two interventions: daily sessions of

active tDCS associated with hypocaloric diet (active group) or daily sessions of sham tDCS associated with hypocaloric diet (sham group). Each block consisted of opaque, sealed envelopes, identified as female or male, which contained sealed tickets labeled “Diet + active tDCS” or “Diet + sham tDCS” in a 1:1 ratio. Only one ticket was drawn for each volunteer, which occurred near the beginning of the study, after the hypocaloric diet had been prescribed. The participants, as well as all other investigators except those who applied tDCS (i.e., the investigators who performed the screening and clinical assessment visits and those who prescribed the hypocaloric diet) were blinded to the different interventions. Randomization (DAN), patient selection, dietary intervention (CA, RCF) and tDCS sessions (DAN) were performed by different researchers to ensure study blindness. The raters (CA, RCF, FG) neither administered nor were present during tDCS sessions.

2.5.3 Data analysis

To compare the participants’ baseline characteristics, Student’s t-test and the chi-square test were used as appropriate. The results were expressed as mean (± standard deviation), means (95% CIs), or the number of patients with the characteristic (percentage). The Shapiro-Wilk test was used to verify data normality; variables with non-Gaussian distribution, such as insulin and the HOMA index, were log transformed before analysis when necessary. The aRMR was performed using log-transformed data, as recommended by Kaiyala and Schwartz (2011) [48].

The effects of tDCS between groups over time were analyzed using the generalized estimated equation (GEE), including tDCS group, time, and the group and subgroup-by-time interaction as predictors. All GEE models were followed by a Bonferroni post hoc and the least significant difference test to identify significant differences detected by the GEE. Gamma distribution with log link function was used for variables with non-Gaussian distribution. After treatment of the first 10 participants, an interim security analysis was planned, and the trial would have been stopped prematurely based on safety concerns, such as severe psychiatric and neurologic reactions (FG). The outcomes were presented as p-values for the included factors and estimated marginal means, with corresponding 95% confidence intervals for each tDCS group at each time point. A p-value <0.05 was considered statistically significant. SPSS 19.0 statistical software (SPSS, Chicago, IL) was used for the analyses.

3 DISCUSSION

This is the first study to investigate whether sessions of repetitive tDCS over the right DLPFC may add additional benefits to weight loss in subjects undergoing a calorie-restricted diet. The evidence-based treatment options for overweight and obesity control currently available are lifestyle intervention, pharmacotherapy, and bariatric surgery [6]. However, the first two modalities have limited effects on weight loss. As a result, new treatment modalities must be developed. Lifestyle interventions are the first option for weight loss, given their low cost and minimal risk of adverse effects and complications. Bariatric surgery is frequently recommended in accordance with strict treatment criteria for patients who do not respond sufficiently to lifestyle therapy and/or pharmacotherapy [6]. Thus, the use of tDCS, a well-tolerated, safe, and non-invasive technique, would act as a complementary treatment to lifestyle therapy for managing overweight and obesity.

The positioning of the electrodes is considered an important determinant of stimulation effectiveness. For this protocol, the choice of anodal stimulation on the right DLPFC and cathodal stimulation on the left DLPFC (AR/CL) was based on previous studies that found a significant effect in reducing food intake and desire to eat associated with this position, both in healthy [14, 16] and in overweight or obese individuals [15]. The right DLPFC is linked with weight regulation and cognitive processes related to food intake [70], such that increasing its activity results in reduced appetite by reestablishing inhibitory control [12, 22]. To date, most studies reporting a reduction in food craving have used the AR/CL electrode positioning over the DLPFC, which presumably increases activity in the right DLPFC (F4 position), and inhibits activity in the left DLPFC (F3 position) [14–16, 21, 71–73].

Reduced caloric intake is the essential component in the majority of lifestyle interventions for treating obesity. However, reducing food intake leads to a negative energy balance and to compensatory and adaptive biological mechanisms designed to prevent weight loss, such as reductions in energy expenditure and increases in hypothalamic reward responsivity within hours of caloric restriction [74–76]. These mechanisms could hinder the weight loss efforts of obese individuals attempting even modest weight reduction, especially in an environment that induces food overconsumption [75]. Thus, increasing the activity of the right DLPFC might improve adherence to hypocaloric diets, a major determinant of success in treating obesity [70].

This protocol was designed to include a total of 20 tDCS sessions on 20 consecutive weekdays, each lasting 20 min. The effects of a single tDCS session have been assessed in several studies, showing significant reductions in the desire to eat and food consumption [14–16, 21, 71–73, 77], with the exception of two studies [78, 79]. The use of 20 repetitive tDCS sessions has not yet been observed in the literature for this purpose. However, a European group of experts recently reported that daily tDCS sessions (between 5 and 30 sessions) have been commonly used for other clinical conditions, such as chronic pain, stroke, schizophrenia, substance abuse, and other addictions [80]. Only one study so far has found a reduction in the desire to eat after 5 days of repetitive tDCS over the right DLPFC in overweight and obese individuals [22], although the cathode electrode was not positioned over left DLPFC, but over forehead (supraorbital). Long term-effects studies in which the cumulative effects of tDCS can be analyzed are still sparse. This study was also motivated by evidence that the magnitude of the behavioral change might be associated with the number of sessions needed to observe a change in eating habits [81].

Although daily contact between the research team and participants could positively influence weight loss and adherence to the hypocaloric diet, as well as improve depressive and anxiety aspects, randomization to either real or sham tDCS treatment should mitigate this potential bias.

This study has may provide important data on tDCS as a potential complementary treatment for hypocaloric diet in overweight and obesity management and as an alternative to medications or bariatric surgery. A recent meta-analysis concluded that non-invasive brain stimulation has significant effects on food craving [13]. Since eating behavior is an important component that can enhance adherence to prescribed diets, we believe that the potential of tDCS to modulate eating behavior could contribute to better adherence to dietary treatment and, thus, to weight loss and better quality of life.

Trial Status

This trial is currently ongoing. The RCT recruitment started in March 2016.

Additional file

Additional file 1: SPIRIT 2013 checklist: recommended items to address in a clinical trial protocol and related documents. (DOC 49.6 kb)

Abbreviations

aRMR, adjusted resting metabolic rate; BDI, back depression inventory; DLPFC, dorsolateral prefrontal cortex; FCGM, flash-continuous glucose monitoring; FCI-Br, food craving inventory; GA, glycated albumin; HOMA, homeostasis model assessment; LMTT, liquid meal tolerance test; MAGE, mean amplitude of glycemic excursion; OGTT, oral glucose tolerance test; RMR, resting metabolic rate; STAI, state trait anxiety inventory; tDCS, transcranial direct current stimulation; VAS, visual analogue scale; WDR, weighted diet records.

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Availability of data and materials

Not applicable.

Authors' contributions

CA, PS, and FG conceived the study protocol. CA and FG wrote the first draft of the manuscript. RCF and FG wrote the first draft of the glycemic control and variability studies. DAN is responsible for the randomization. CA, DAN, and FG wrote the final manuscript. CA, RCF, DAN, and FG, PS critically read the protocol and revised and wrote the methods/analysis. CA, RCF, and FG will participate in the data analysis. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The protocol was approved by the Internal Review Board of the Hospital de Clínicas de Porto Alegre, the Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil and conforms to the principles of Helsinki Declaration. All participants provided written informed consent, obtained by the main researchers (CA, RCF), and approval was obtained from the research ethics committee of Hospital de Clínicas de Porto Alegre (GPPG-HCPA protocol Nos. 150119 and 160417, and CAAE Nos. 42996915.0.0000.5327 and 54832016.1.0000.5327). The present trial has been registered at clinicaltrials.gov as NCT02683902. Personal information about potential and enrolled patients will be maintained in a database in order to protect patients confidentially. Investigators will communicate trial results to participants, healthcare professionals, and other relevant groups via publication.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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APÊNDICE A - ADDITIONAL FILE

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	01
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	02
	2b	All items from the World Health Organization Trial Registration Data Set	___
Protocol version	3	Date and version identifier	02
Funding	4	Sources and types of financial, material, and other support	28
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	01, 29
	5b	Name and contact information for the trial sponsor	01
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	28
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	29
Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	04-05
	6b	Explanation for choice of comparators	04-05
Objectives	7	Specific objectives or hypotheses	05
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	05
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	06-07
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	07 (Table 1)

Section/item	Item No	Description	Addressed on page number
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	18-22
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	___
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	19-20
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	18, 21
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	22
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	08 (Figure 1)
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	23
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	06-07
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	23-24
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	23-24
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	24
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	24
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	___
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	08-18

Section/item	Item No	Description	Addressed on page number
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	19
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	___
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	24-25
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	25
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	19
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	29
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	24-25
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	22
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	___
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	29
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	29
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	29
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	___
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	29
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	29
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	29
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	___

Section/item	Item No	Description	Addressed on page number
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	29
	31b	Authorship eligibility guidelines and any intended use of professional writers	_____ __
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	29
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	_____ __
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	_____ __

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

Psychiatric profile and quality of life of subjects with excess weight treated with transcranial direct current stimulation combined with a hypocaloric diet

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5 ARTIGO II

5.1 PSYCHIATRIC PROFILE AND QUALITY OF LIFE OF SUBJECTS WITH EXCESS WEIGHT TREATED WITH TRANSCRANIAL DIRECT CURRENT STIMULATION COMBINED WITH A HYPOCALORIC DIET

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The effect of transcranial direct current stimulation along with a hypocaloric diet on weight loss in excessive weight people: A pilot randomized clinical trial

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6 ARTIGO III

6.1 THE EFFECT OF TRANSCRANIAL DIRECT CURRENT STIMULATION ALONG WITH A HYPOCALORIC DIET ON WEIGHT LOSS AND FOOD INTAKE IN EXCESSIVE WEIGHT PEOPLE: A PILOT RANDOMIZED CLINICAL TRIAL[2]

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[2] Trata-se da versão submetida à revista, sem as alterações solicitadas pelos revisores.

[3] In Memorium.

ABSTRACT

Background/Objectives: The dorsolateral prefrontal cortex (DLPFC) plays an important role in the desire to eat and food intake regulation and may be a target for transcranial direct current stimulation (tDCS) to promote weight loss. Our aim was to test the effect of repeated, active tDCS along with a hypocaloric diet (HD) on weight loss in overweight adults.

Methods: This was a randomized, placebo-controlled, double-blind pilot study conducted in Porto Alegre, Brazil. Twenty-eight overweight adults were selected to receive 4-week (20 sessions, t0 to t20; 5 weekdays) fixed-dose tDCS along with an HD. Subjects were randomly assigned to active (AG) or sham (SG) tDCS groups. The primary outcome was weight loss as determined via body weight measurements at baseline (t0), weekly (t5, t10, t15, and t20), and after the intervention (tF). A visual analogue scale was used to assess desire to eat at t0 and at tF. Registered under ClinicalTrials.gov Identifier no. NCT02683902.

Results: Although there was a greater weight loss in the AG (mean −4.5kg [95%CI: −9.4, 0.5]) than in the SG (−2.3kg [−5.0, 0.3]), this difference was not statistically significant. However, the AG showed a significant reduction in the desire for sweet foods (*P*=0.005).

Conclusions: Although this pilot study did not show that repeated tDCS is able to optimize weight loss, it was able to reduce the desire to eat sweet foods. These findings suggest that a protocol with a larger sample size could determine whether tDCS may be an adjunctive treatment of obesity.

Keywords

obesity; transcranial direct current stimulation; tDCS; weight loss; desire to eat.

Abbreviations used: aRMR, adjusted resting metabolic rate; DLPFC, dorsolateral prefrontal cortex; GEE, generalized estimated equation; HD, hypocaloric diet; ITT, intention-to-treat; PFC, prefrontal cortex; RMR, resting metabolic rate; tDCS, transcranial direct current stimulation; VAS, visual analogue scale; WDR, weighted dietary records.

1. Introduction

The rates of obesity have increased globally, and no country to date has been able to reverse this epidemy [1]. Food cravings and desire to eat are important phenomena involved in weight gain, and it has been proposed that highly palatable foods may be addictive and that obesity could be conceptualized as a form of addiction [2,3]. In particular, high-caloric foods seem to affect the brain's reward system like substance addictions, and similar to persons addicted to drugs, obese individuals present increased cravings [4]. Additionally, the reduction in dopamine D2 receptors in the striatum observed in obesity is associated with prefrontal cortex (PFC) dysfunction, which is linked to compulsive behaviors and a decrease in impulse control [5]. Considering this context, it was postulated that enhancing the activity in the dorsolateral prefrontal cortex (DLPFC), a critical area involved in cognitive control of food intake, may strengthen inhibitory control, thus suppressing the reward-related activity in the reward-cognition neural circuits that drive food cravings and overeating [6,7].

Transcranial direct current stimulation (tDCS) is a noninvasive neuromodulation technique that can induce neuroplasticity, modulate cortical function, and influence cognitive processes [8]. This technique consists of applying a low intensity, constant electrical current to modulate brain activity [9]. The DLPFC is a target because of its role in suppressing food cravings and consumption [6,10–13].

However, despite the potential role of tDCS in reducing overeating and thus treating obesity, only two of many tDCS studies have aimed to reduce the desire to eat and food intake assessed weight loss in a repeated protocol [6,14]. Although recent publications have used protocols with repeated tDCS sessions in obese people and have assessed its effect on body weight, the electrode montage used was unilateral, with the anode on the left side [14–16]. No study published to date has evaluated a more intensive and frequently repeated tDCS protocol adjunctive to a hypocaloric diet (HD) in individuals with obesity with the aim of reducing body weight. Therefore, in view of the above, the aim of this study was to compare the effect of multiple sessions (4 weeks, 20 sessions) along with an HD on the weight loss in overweight or obese individuals. It was hypothesized that subjects undergoing active tDCS in conjunction with HD would achieve greater weight loss than those undergoing sham tDCS.

2. Subjects and methods

2.1. Study design

This was a 4-week double-blind, randomized, single-center, sham-controlled pilot study. This protocol was performed at the Hospital de Clínicas de Porto Alegre, Brazil, in conformity with the principles of the Helsinki Declaration and previously published in accordance with Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) guidelines (https://doi.org/10.1186/s13063-018-2776-3) [17]. All the participants provided written informed consent prior to their enrolment, with approval from the IRB of Hospital de Clínicas de Porto Alegre. The present trial is registered with ClinicalTrials.gov (NCT02683902).

2.2. Participants

Eligible participants were men and women with a measured BMI between 25 and 35 kg/m², aged 20−50 years, with stable body weight, who were not given nutritional counselling prior to screening and who were able to comply with the requirements of the study protocol. Table S1 shows details of the exclusion criteria.

Participants were randomly assigned to one of two types of intervention: active tDCS plus HD or sham tDCS plus HD (placebo). Randomization was stratified by sex and was performed in blocks consisting of opaque, sealed envelopes that contained sealed tickets labelled “Diet + active tDCS” or “Diet + sham tDCS”, in a 1:1 ratio. The participants, as well as all investigators, were blinded to the assigned intervention. All the tDCS sessions were performed by trained technicians who knew the randomization allocation. The investigators neither administered nor were present during the tDCS sessions to maintain blindness. Further details regarding the protocol are provided in a previous publication [17].

2.3. Procedures

After selection, the participants underwent a standard baseline evaluation that included demographic status, medical history, and physical examination. Physical activity was estimated before and after the intervention with a digital pedometer (HJ-

152-E; OMRON Healthcare Co., Kyoto, Japan) in steps per day. The resting metabolic rate (RMR) and the severity of depressive and anxiety symptoms were assessed at baseline and at the end of the study. Additional information is provided in previous publications [17,18].

An anthropometric assessment was performed at baseline and at the end of the study; assessment was done in the morning, with the participant barefoot and wearing light clothing, after the participant had fasted and avoided exercise for the last 24 hours. An InBody 230 tetrapolar body composition analyzer (Biospace Co. Ltd, Seoul, South Korea) was used to measure body weight and composition via bioelectrical impedance, and BMI was calculated. Waist and hip circumferences were measured using a flexible, nonstretch fiberglass tape. Additional measurements of body weight were assessed weekly with the same bioelectrical impedance scale used in the baseline evaluation (3-hour fast, light clothes, barefoot).

Food intake was assessed three times as follow: at baseline, during the first fortnight, and during the second fortnight of the intervention. Habitual food intake (baseline) was assessed using a three-day weighed dietary record (3-day WDR) technique (two nonconsecutive weekdays and one weekend day), totaling three WDRs; this was done prior to participants being prescribed an HD. Adherence with the HD was assessed by a registered dietitian blinded to the tDCS intervention using both the expected weekly weight reduction and the 3-day WDRs (first and second fortnights) as references. Participants were classified as adherent or non-adherent according to whether they reached 80% of their calculated energy goals.

Eating behaviors were assessed while participants were still fasting, in the morning, at baseline and at the end of the study. Current hunger scores were assessed using a 7-point visual analogue scale (VAS) in which participants rated how intense their hunger was at that moment. A paper-based 100-mm VAS was used to assess the desire to eat specific categories of foods (sweets, salty, savory, or fatty).

The HD was prescribed by a dietitian a day before the beginning of the tDCS sessions, with the aim of reducing initial body weight by at least 3% in four weeks. The participants received individual counselling to improve their dietary habits over the 4-week intervention and to maintain their physical activity. The energy deficit was calculated individually using the online Body Weight Planner program recommended by the NIDDK (National Institute of Diabetes and Digestive and Kidney Disease) [19]. The diet compositions from the prescribed diet (Table 1) and from the 3-day WDRs

were analyzed with NutriBase 17 Pro Edition software (version 17.2, CyberSoft, Phoenix, Arizona).

Table 1. Nutritional composition of the prescribed hypocaloric diet according to the intervention.

	Active	**Sham**	***P* value**
Energy (kcal/day)	1882.6 (420.3)	1929.7 (322.1)	0.742
Energy (kcal/kg)	21.2 (3.2)	21.0 (2.2)	0.842
Carbohydrate (g/day)	236.3 (52.5)	246.7 (44.4)	0.577
Protein (g/day)	113.2 (24.0)	116.6 (18.7)	0.680
Total fat (g/day)	59.7 (5.5)	59.8 (11.7)	0.981
Fiber (g/day)	38.7 (10.4)	42.0 (8.5)	0.352

Data are the mean (SD). The *P* value for comparisons between two groups was tested by Student's t test.

While following the HD, participants underwent 20-minutes tDCS sessions each weekday (including holidays) for four weeks (20 sessions). A battery-driven direct current stimulator (Chattanooga Ionto™ Dual Channel Electrophoresis System, DJO Global, Guildford, UK) was used in this protocol and a direct current of 2 mA was delivered through a pair of 35 cm2, 0.9% saline-soaked surface sponge electrodes placed according to the 10–20 International System of Electrode Placement . For active tDCS, the electrodes were placed with the anode electrode over the right DLPFC (F4) and the cathode electrode over the left DLPFC (F3). For sham tDCS, the electrodes were placed identically to those in the active group, but the current was ramped up to 2 mA and then back down in the first and last 30-s of the 20-min session. As a result, the participants in the sham group were not receiving stimulation for 19-min of the session. During the 20-min tDCS sessions, participants were exposed to a video presentation with high-calorie food images such as sweets, high-fat foods, and traditional meals, presented in sets of 200 pictures each session. They were asked to pay attention to these images and turn off phones, pagers, or other devices that might distract their attention. The study timeline is shown in Figure 1.

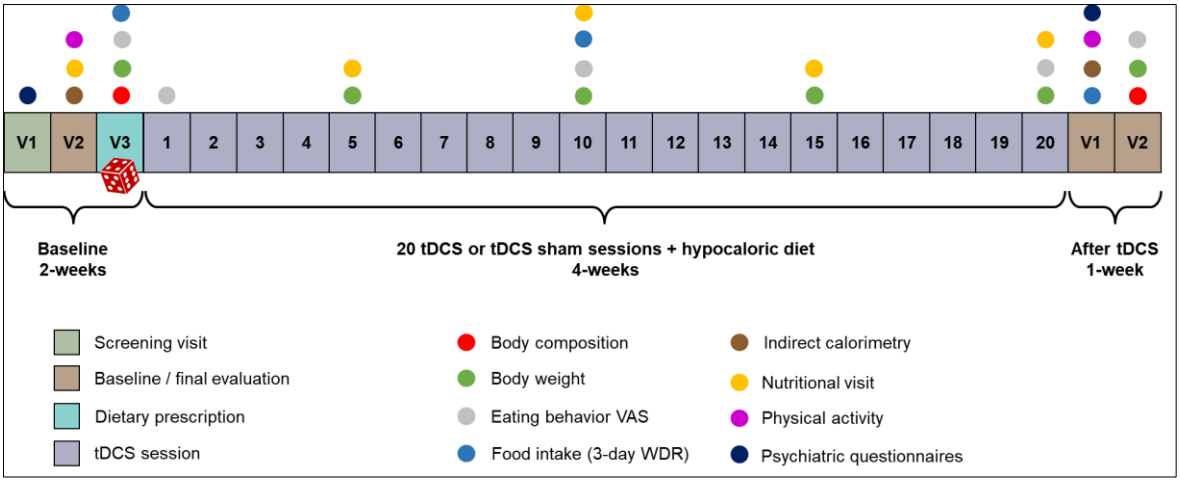

Figure 1. Study timeline. V1, V2, and V3: visits one, two, and three in the respective period (baseline or after tDCS). Numbers 1 to 20 represent each tDCS or sham-tDCS session. tDCS, transcranial direct current stimulation; VAS: visual analogue scale; 3-day WDR: three-day weighted diet records.

The participants were asked not to consume food 3 hours before each stimulation session. Each session was conducted by trained technicians at the same time of the day for each participant. If a scheduled tDCS session was missed for any reason, the session was made up the following weekend. At the end of the 4-week intervention, the participants were assessed about study blindness and were asked if they could deduce the treatment they received. The results have been previously published [18].

2.4. Outcomes

The primary outcome of the study was the change in body weight from baseline to week four. The secondary outcomes include the following: reduction in actual energy intake, hunger, and desire to eat. The exploratory outcome was the change in body composition.

Weight loss and changes in body weight (kg), relative weight loss (%) and BMI (kg/m^2) were examined weekly (sessions 0 [baseline], 5, 10, 15, 20, and F [final]). For the analysis of the effect of tDCS on energy and macronutrient consumption, the average of the 3-day WDRs was used for each time point: baseline, first (intermediate) and second fortnight (final). Participants whose energy intake, derived from the baseline 3-day WDR, was outside the specified range (500 kcal/d to 3500 kcal/d for women and 800 kcal/d to 4000 kcal/d for men) were excluded from the baseline

analysis from the calculations of food energy and nutrient intake. Hunger was assessed using a 7-point VAS, with participant answers ranking from -3 (extremely hungry) through +3 (extremely full). Desire to eat was assessed using a 100-mm VAS, in which 0 mm described a very high desire for and 100 mm described no desire for sweet, salty, savory, or fatty foods. The results are expressed as percentage points of changes from baseline.

Treatment-emergent adverse events were determined through a standard questionnaire regarding possible adverse events commonly related to tDCS; the questionnaire was administered twice each week to assess the acute effects of tDCS after a session and the post-effect between sessions. Details and results are published elsewhere [18].

2.5. Statistical procedures

Baseline characteristics were summarized by using means and SDs for normally distributed continuous variables, medians and IQR for nonnormally distributed continuous variables, and frequencies and percentages for categorical variables. The Shapiro-Wilk test was used to verify the data normality.

The effectiveness of the intervention on all outcomes was evaluated between the two groups over time using the generalized estimating equation (GEE). All the GEE analyses were conducted in two ways: a crude analysis determined the effect of the intervention over time without any adjustment, and an adjusted analysis determined the intervention effect over time while adjusting for the baseline value of the outcome and for the number of tDCS sessions completed. Variables with a skewed distribution were performed on a gamma distribution with a log link function. At all stages of data analysis, an intention-to-treat (ITT) analysis was performed, thus including all participants who received at least one tDCS session. For the primary outcome, a per-protocol (PP) analysis was performed, including only participants who completed the study in the group that they were allocated. Outcomes were presented as P values for the group-by-time interaction and estimated marginal means, with corresponding 95% confidence intervals for each tDCS group at each time point. A $P < 0.050$ was considered statistically significant. All the statistical tests were performed using the software IBM SPSS Statistics, version 19.0 (IBM, Armonk, NY, USA).

3. Results

Between March 21, 2016 and July 19, 2018, 28 subjects were randomized and included in the ITT analysis. Twenty-three participants received all 20 planned sessions; one withdrew after 4 sessions, one after 5 sessions, and three participants after 8 sessions (Figure 2). The participants who did not complete the study had a higher BMI, a higher percentage of body fat, and a lower percentage of body skeletal muscle at baseline compared to the participants who completed the study (data not shown).

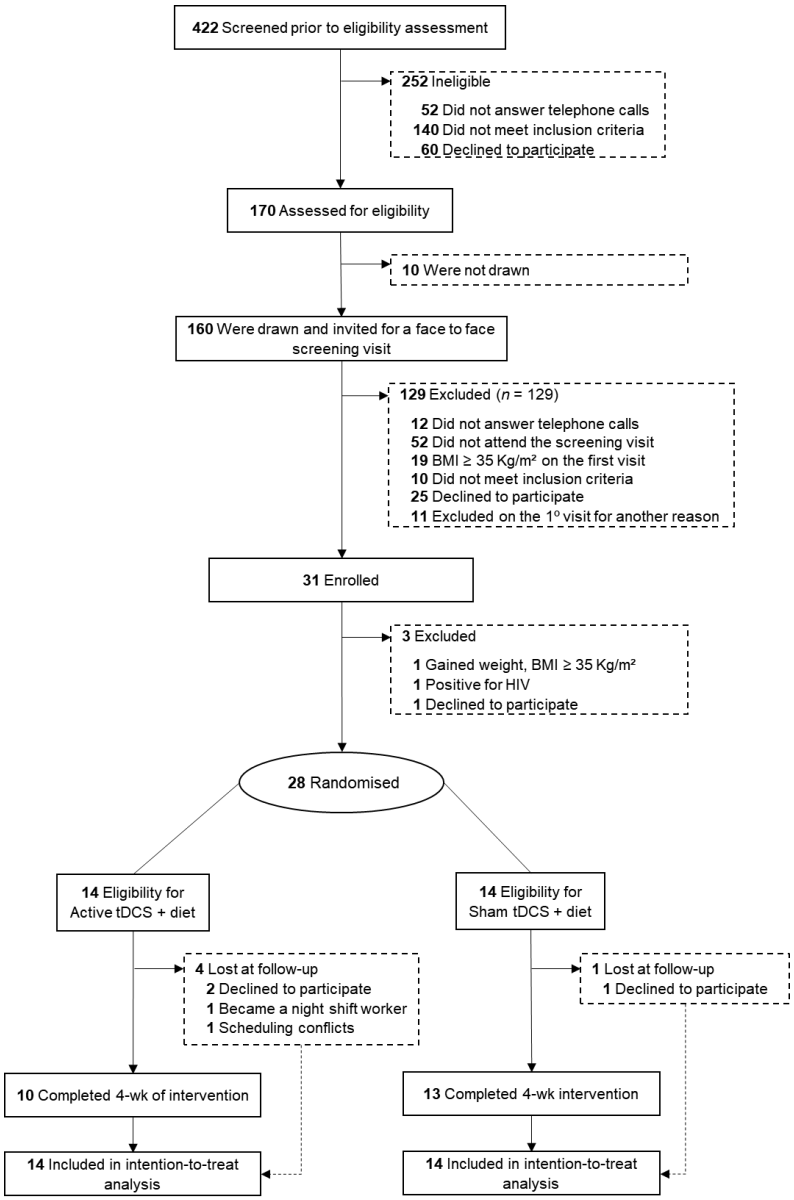

Figure 2. CONSORT flow chart. tDCS, transcranial direct current stimulation.

Participants receiving active vs. sham tDCS did not differ regarding baseline demographic, clinical, and anthropometric characteristics. Six participants were using antidepressants, five of whom were in the sham group. Neither the drugs nor their doses were changed during the study. The energy and macronutrient composition from habitual food intake was similar between groups (Table 2). Two participants from the active group were excluded from the energy and macronutrient baseline calculations because they reported unrealistic energy intakes. Their exclusion from the analysis did not affect the results (data not shown).

Table 2. Baseline characteristics

	Active tDCS (n = 14)	Sham tDCS (n = 14)
Age (years)	37.5 (7.0)	37.7 (4.7)
Sex		
Male	7 (50%)	7 (50%)
Ethnicity		
White	10 (71%)	11 (79%)
Black	1 (7%)	0
Other	3 (21%)	3 (21%)
Physical activity		
Pedometer (steps/day) *	3718.6 (2448.4 – 6607.2)	4767.5 (4153.6 – 5553.5)
Systolic blood pressure (mmHg)	114.6 (15.3)	118.6 (11.2)
Diastolic blood pressure (mmHg)	79.0 (12.3)	81.5 (8.3)
aRMR (kcal/day)	1676.9 (288.1)	1745.1 (301.5)
Anthropometric characteristics		
Weight (kg)	88.8 (12.8)	92.0 (11.2)
BMI (kg/m^2)	31.8 (2.6)	31.3 (2.4)
Waist circumference (cm)	100.3 (11.7)	101.4 (7.9)
Waist-to-hip ratio	0.91 (0.10)	0.91 (0.07)
Skeletal muscle mass (%)	35.4 (4.8)	35.8 (4.8)
Body fat (%)	37.3 (7.6)	36.6 (7.5)
Habitual food intake †		
Energy (kcal/day)	2537.4 (535.6)	2422.5 (492.1)
Energy (kcal/kg/day)	29.0 (6.1)	26.5 (5.5)
Carbohydrate (g/day)	290.6 (68.4)	260.6 (65.0)
Protein (g/day)	106.1 (28.8)	116.9 (33.2)
Total fat (g/day)	98.8 (29.8)	95.7 (30.1)
Fiber (g/day)	20.4 (7.8)	19.4 (7.6)

Data are frequency (%), median (IQR), or mean (SD). aRMR = adjusted resting metabolic rate. tDCS = transcranial direct current stimulation. *Median of six consecutive days. †Habitual food intake was assessed by 3-day weighed dietary records; n = 12 in the active group; two participants were excluded because of unrealistic energy intake reports.

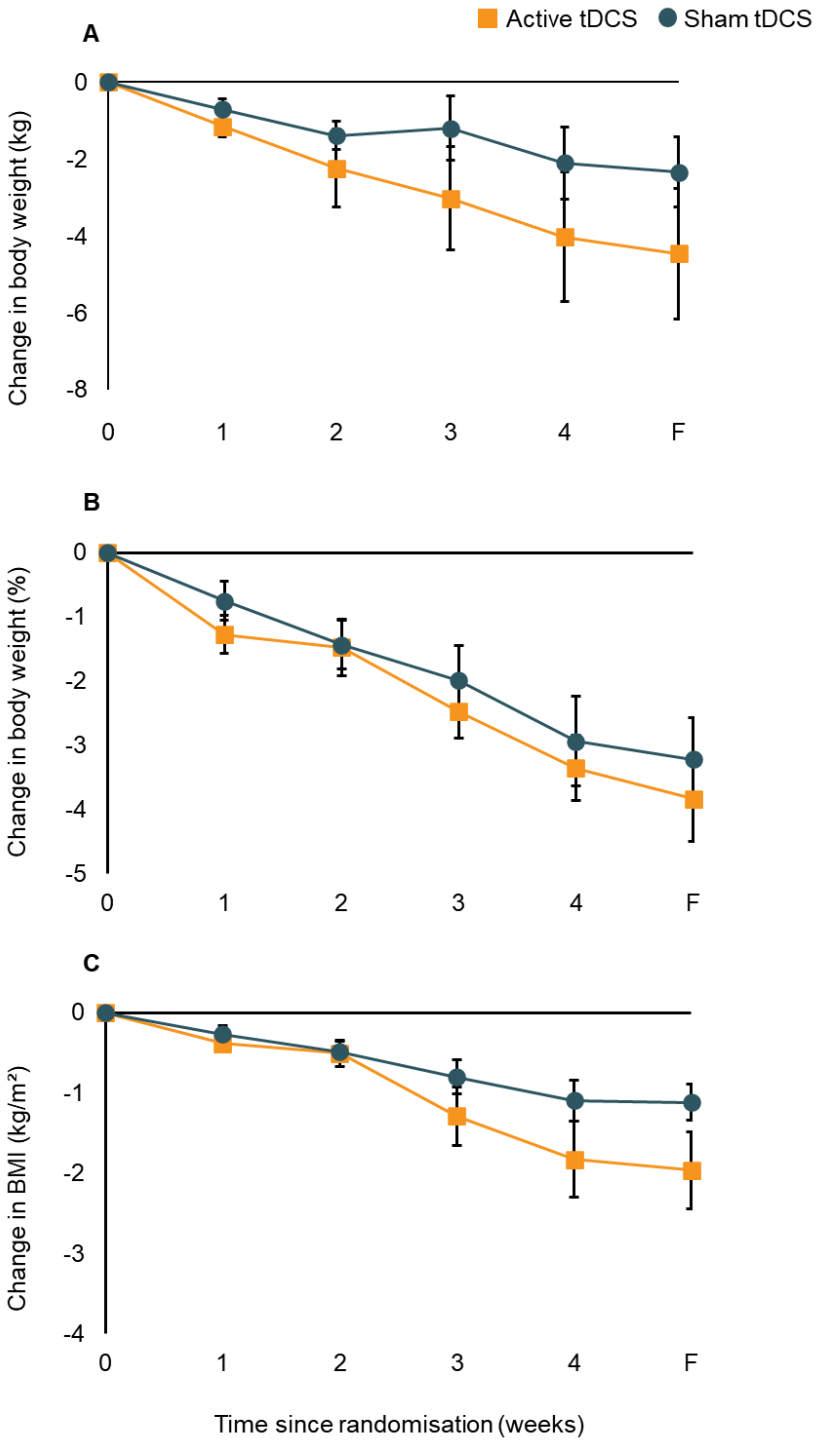

Figure 3. Weight loss by tDCS group relative to baseline.
Data are estimated marginal means [SEE]. The p value for interaction (tDCS by time) was tested by the generalized estimating equation (GEE) at six time points, 0, 5, 10, 15, 20, and F. (A) P = 0.786. (B) P = 0.605. (C) P = 0.412. BMI = body mass index. F = final evaluation assessed three days after the last tDCS session. tDCS = transcranial direct current stimulation.

The mean body weight reduction for the pooled sample was −3.4 kg (95% CI: −6.2, −0.6) (P = 0.000). However, although the active group reached a greater reduction of −4.5 kg [95% CI: −9.4, 0.5] in body weight compared with the reduction in

the sham group (−2.3 kg [95% CI: −5.0, 0.3]), this difference was not statistically significant in either ITT (P = 0.786; Figure 3A) or PP analysis (P = 0.455; Figure S1). Neither changes in relative weight loss (P = 0.605; Figure 3B and Figure S1) nor changes in BMI (P = 0.412; Figure 3C and Figure S1)) were significantly different between the active and sham groups over time. Adjustments for the baseline values of the outcome (weight or BMI) and number of tDCS sessions did not affect these results (Figure S2).

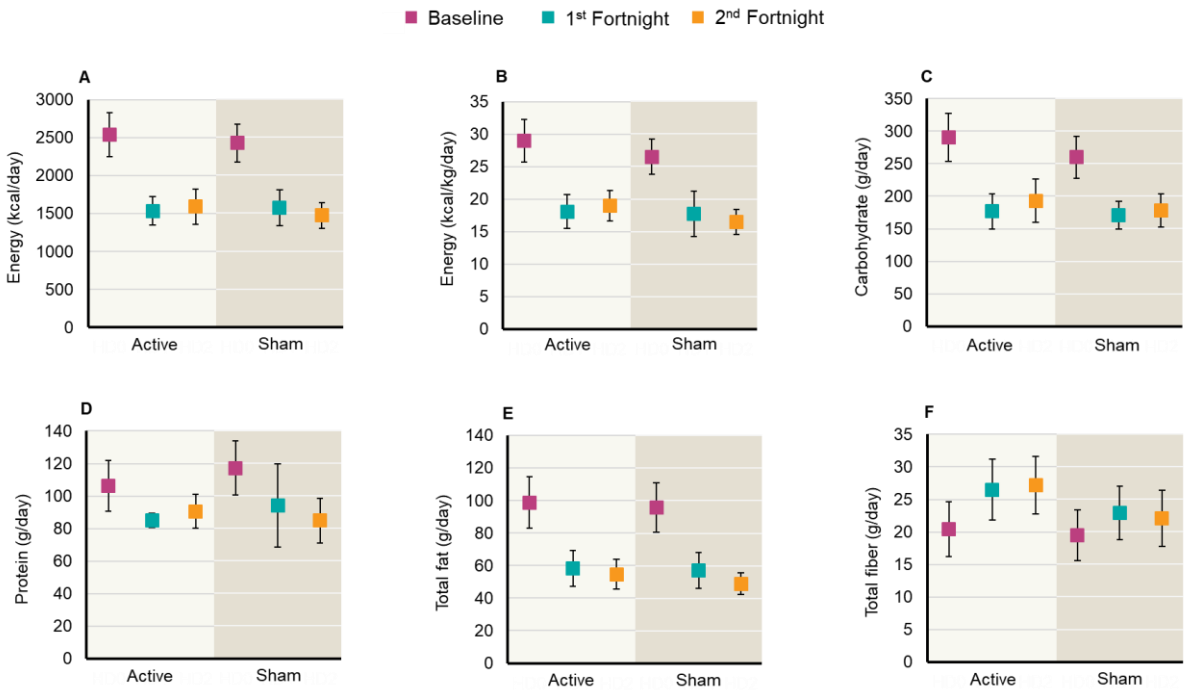

Figure 4. Energy and macronutrient intake by tDCS group relative to baseline.
Data are means [95% CI]. Energy and macronutrient intake were assessed by 3-day weighed dietary records (WDR). Responses were analyzed using the generalized estimating equation (GEE). In panels B, D, and F, GEE analysis was performed on a gamma distribution with a log link function. 1st Fortnight: period between the first and second weeks of intervention. 2nd Fortnight: period between the third and fourth weeks of intervention.

Regarding energy intake, participants had a similar adherence over the study, with 83.0% (20.8) for the active group and 80.1% (25.5) for the sham group (P = 0.774). In comparison with the baseline, all the participants reduced their energy intake by −926.9 kcal/day (95% CI: −1187.7, −666.1) after the 1st fortnight and by −949.0 kcal/day (95% CI: −1216.8, −681.3) in the 2nd fortnight, with a significant difference over time (P < 0.0001). These reductions appeared to be clinically relevant to promoting weight loss in both groups. The energy intake (kcal/day) and relative to body

weight (kcal/kg/day) was not significantly different between the active and sham groups throughout the study (Figure 4A−B; P = 0.598 and P = 0.549).

Regarding macronutrients, changes in the consumption of carbohydrates (P = 0.686), protein (P = 0.264), total fat (P = 0.872), and total fiber (P = 0.593) were not different between groups over time (Figure 4C−F). The inclusion of the baseline values of the outcomes (energy, carbohydrates, protein, total fat, or fiber) and number of tDCS sessions as adjustment covariates did not affect the results (Table S2). In comparison to baseline intake, the participants in both groups ate smaller amounts of carbohydrates (P < 0.001), protein (P < 0.001), and total fat (P < 0.001), while eating more fibers (P = 0.011) during the intervention.

Hunger ratings were compared between groups during the 4-week intervention. The hunger ratings in the active and the sham groups did not differ throughout the study, or at the end of the study (Table 3). Regarding desire to eat, there was a significant difference in the reduction in the desire for sweet foods between the active and sham groups over time. The active group showed a significant −23.7 percentage points (95% CI: −40.2, −7.1; P = 0.005) reduction in the desire for sweet foods, whereas the sham group had a non-significant 1.0 percentage point (95% CI: −13.3, 15.2; P = 0.895) increase at the end of the study. There were no differences between groups over time for salty, savory, or fatty foods (Figure 5 and Table 3). The adjusted analysis did not affect any of these results (Table S3).

There was a significant decrease of −0.04 (95% CI: −0.06, −0.01) in the waist-to-hip ratio over time in the active group (P = 0.009) but not in the sham group (0.00 [95% CI: −0.02 to 0.02]; P = 0.744) (Table S4). However, we found no differences between the active and sham groups regarding changes in waist circumference, body fat percentage, or skeletal muscle percentage throughout the study period (Table S4).Compared to the sham group, neither the energy expenditure assessed by indirect calorimetry nor the physical activity measured with the pedometer differed significantly over time or between groups during the study.

The most frequent adverse effects during the tDCS sessions were burning sensations (67.9%), local itching (60.7%), and tingling (57.1%). The rates of adverse effects did not differ between the groups, as described in a previous publication (18). The blindness assessment showed that neither the participants in the active group (25% correct) nor the participants in the sham group (33.3% correct) distinguished real from sham tDCS (P = 0.087).

Table 3. Effects of tDCS associated with a hypocaloric diet on eating behavior according to the intervention.

	Active		Sham		
	Baseline (n= 14)*	After intervention (n=10)†	Baseline (n= 14)	After intervention (n= 14)	p value
Subjective satiety and hunger VAS					
How is your hungry/satiety at this very moment? (-3= “extremely hungry” / +3= “extremely full”)	−0.8 (−1.3 to −0.2)	−0.8 (−1.2 to −0.4)	−0.9 (−1.3 to −0.6)	−1.4 (−1.8 to −1.0)	0.195
Desire to eat VAS					
Would you like to eat something sweet? (0 = “yes, very much” / 100 = “not at all”)	60.4 (43.2 to 77.7)^{Aa}	84.1 (72.9 to 95.4)^{Ab}	75.0 (59.4 to 90.6)^{Aa}	74.1 (58.9 to 89.3)^{Aa}	0.039
Would you like to eat something salty? (0 = “yes, very much” / 100 = “not at all”)	34.1 (18.1 to 50.1)	37.1 (19.7 to 54.5)	37.6 (21.1 to 54.2)	44.4 (27.7 to 61.2)	0.791
Would you like to eat something savory? (0 = “yes, very much” / 100 = “not at all”)	26.0 (10.0 to 42.1)	34.1 (5.6 to 52.6)	22.3 (6.7 to 37.9)	35.6 (20.6 to 50.7)	0.644
Would you like to eat something fatty? (0 = “yes, very much” / 100 = “not at all”)	73.7 (57.1 to 90.4)	85.9 (77.2 to 94.6)	74.3 (58.6 to 90.0)	71.0 (54.8 to 87.1)	0.241

Data are estimated marginal means (95% CI). Responses were analyzed using generalized estimating equation (GEE). For subjective satiety and hunger, desire for sweet, savory, and fatty foods, the GEE analysis was performed on a gamma distribution with a log link function. Means without a common lowercase letter differ in time, $P < 0.050$. Means without a common capital letter differ by group, $P < 0.050$. *For subjective satiety and hunger VAS, n= 13. †For desire to eat 100-mm VAS, n=11. VAS, visual analogue scale.

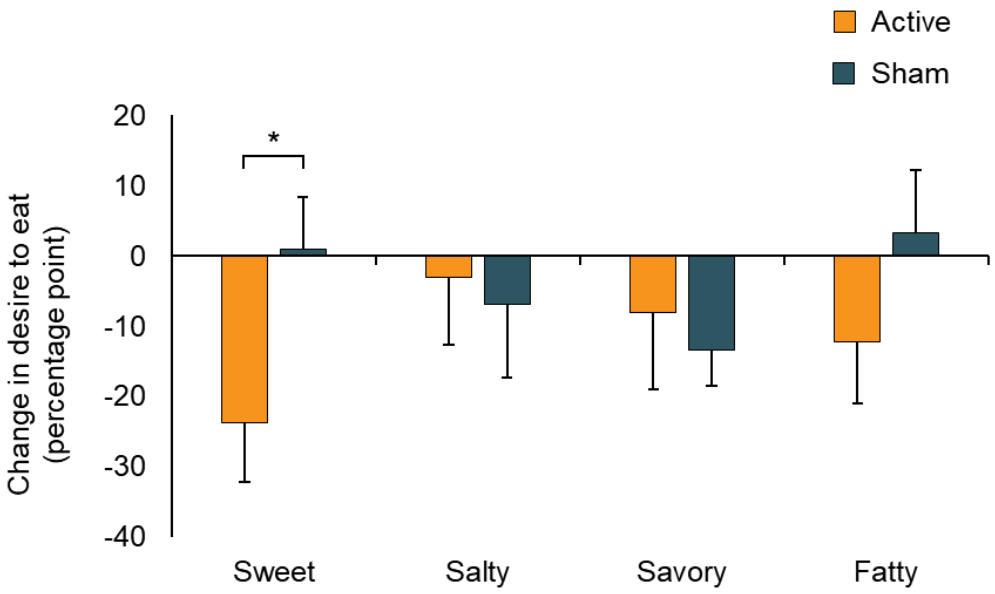

Figure 5. Change in desire to eat in tDCS groups relative to baseline. Data are estimated marginal means [SEE]. Responses were analyzed using the generalized estimating equation (GEE). The GEE analysis was performed on a gamma distribution with a log link function for sweet, savory, and fatty foods. *P = 0.039. tDCS = transcranial direct current stimulation.

4. Discussion

With this pilot, randomized clinical trial, we investigated the effect of 20 repeated, active tDCS sessions over the right DLPFC in conjunction with a prescribed diet in a 4-week intervention protocol, with the goal of increasing the capability of the participants to follow an HD aimed at weight loss. Our data suggest that active tDCS added to an HD can reduce the desire to eat sweet foods. Although the weight loss in subjects submitted to active tDCS was not statistically superior to those submitted to sham tDCS, the different patterns in the weight changes between groups suggest that there may be a plausible effect from this intervention as an adjunct modality to diet for weight loss.

Two studies have so far addressed whether tDCS could act as an adjunct therapy to restrictive diets in weight management using less intensive protocols [14,16]. Recently, 38 middle-aged women were randomized to eight repetitive anodal tDCS sessions over the left DLPFC in combination with a diet during a 4-week intervention (five sessions in the first week without diet, three sessions in the second week with diet, and HD only in weeks 3 and 4). This study found that the active group

reached greater weight loss than the sham group at week four, a mean net benefit of 1.03%, and a borderline reduction in desire to eat but no changes in hunger, fullness or food cravings [14]. Similarly, Heinitz, et al. (2017) studied a repetitive 4-week protocol with 12 anodal tDCS sessions (3 sessions/week) over the left DLPFC, along with an HD in overweight participants; the results showed a reduction in hunger, urge to eat, and a reduction in snack food intake but no changes in body weight [16].

Other protocols also analyzed the effect of repetitive tDCS sessions on body weight without a diet intervention [6,15]. In a study of 27 overweight, healthy subjects with high levels of food cravings, participants were assigned to five consecutive, active tDCS sessions versus sham (1 session/day) without diet intervention; comparison of both groups showed a reduction in cravings for sweet, high-fat and fast-foods but not in body weight [6]. On the other hand, another study of nine obese subjects in a double-blind, placebo-controlled, crossover study for three consecutive anodal tDCS sessions targeting the left DLPFC along with an ad libitum intake found that anodal tDCS caused a 0.9% weight reduction compared with cathodal stimulation, as well as a lower ad libitum energy, fat and soda intake [15]. However, although a recent study has shown that tDCS was effective in reducing weight in overweight middle-aged women [14], evidence that the reduction in craving-related symptoms and calorie intake from tDCS results in weight loss is still lacking.

We observed a reduced desire for sweet foods at the end of the intervention in the active group. This result is compatible with the existing evidence, which supports the efficacy of neuromodulation for reducing food cravings [20,21]. Among the studies with repeated tDCS sessions that used VAS to assess desire/urge to eat [16,22,23], two assessed this effect in obese subjects [16,23]. In the study of Heinitz et al. (2017), there was a significant reduction in the VAS ratings for urge to eat at the end of the intervention [16]. Only one previous study with healthy young males in a repetitive tDCS protocol (8 sessions in 8 days) assessed the desire for specific taste categories and found a significant reduction in the desire for nonspecific and savory foods [22]. Regarding hunger, in consonance with previous findings [4,22–25], our study did not find evidence of change in the overall subjective sensation of hunger, confirming previous observations that tDCS could reduce food cravings independent of hunger perception [22,25]. The reduction we observed in the desire to eat sweet foods may have led the active group to reduce their consumption of ultra-processed foods, and thus, this group reached a non-significant greater weight loss at the end of the 4-week

intervention. In the ELSA-Brazil cohort study authors suggest not only that the amount of ingested energy contributes to weight changes, but also that the nature of this energy is independent of the level of energy intake [26].

Regarding adherence to the HD, both groups were adherent to the prescribed energy and macronutrient goals, as assessed by a 3-day WDR. It has already been demonstrated that adherence to low-calorie diets is higher in the first few months, when patients are more motivated to see results [27]. We found no statistically significant reduction in food consumption between groups over time. Studies with a single tDCS session [10,11,13], as well as multiple sessions [15,16,22], could be an effective means of reducing food intake during an in-lab eating test. However, a recent meta-analysis showed that there was no significant effect size for tDCS on in-lab energy intake among the overweight population [21]. Unlike previous studies, which tested the effect of tDCS on food intake in a laboratory test, we measured the participants' usual dietary intake through a 3-day WDR, believing it to be a more appropriate way to measure consumption at home. Thus, differences between our results and those in the literature may be explained, in part, because of this difference in our design, which exposed subjects to a more realistic setting of tDCS. As a result, this design allows for an assessment of the effectiveness, rather than efficacy, of tDCS in weight loss and craving.

Possible confounding factors related to the outcomes were also analyzed. Participants maintained the same level of physical activity throughout the study, classified as sedentary, and their step count was lower than average for overweight or obese adults [28]. The resting metabolic rate did not change over time or between groups, so it was not a confounder of the study results. Previously, we demonstrated that the psychiatric profile (anxiety, depression, mood and attention) and quality of life were not different between groups at baseline or during the study and do not seem to represent a confounding factor for our findings [18].

The strengths of this study include the application of a protocol with a higher frequency of tDCS sessions (the largest number of sessions over a limited period, amounting to 20 sessions in a 4-week period) and well-controlled recruitment resulting in balanced randomization and equilibrated baseline characteristics between groups. Additionally, the dietary intervention was oriented by specialized dieticians who calculated the energy restriction according to a validated weight loss protocol [19]. Finally, although this protocol required frequent visits to the research clinic by the

participant, there was an 18% dropout rate, which is considered acceptable for a weight loss study.

This pilot study also has some limitations. First, the small sample size limited the statistical power to detect differences in weight loss with the intervention. Additionally, the energy restriction may not have been enough to identify a significant effect of tDCS on weight loss. On the other hand, it was expected that a more intensive weight loss diet would result in a higher dropout rate and non-adherence.

In conclusion, the results of this pilot study did not show a significant difference between 20 repetitive active tDCS sessions over the right DLPF to optimize weight loss and food intake in subjects with excessive weight compared to sham tDCS. Despite being statistically insignificant, the greater weight loss in the active tDCS group compared to the sham group suggests that a protocol with a greater number of subjects needs to be developed to determine whether neuromodulation with tDCS may be used as an adjunct modality for the treatment of obesity. Nevertheless, we were able to find an association between active treatment and the reduced desire to eat sweet foods. These findings ratify the perspective of this therapeutic modality as a potential strategy for the treatment of food cravings and metabolic disorders related to carbohydrate consumption.

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Data statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declaration of competing interest

The authors have no conflicts of interest to declare that are directly relevant to the contents of this manuscript. PS reports to be the founder of NEMO - Neuromodulation (www.nemo.med.br).

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clnesp.2020.10.005.

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APÊNDICE A – SUPPORT INFORMATION

Table S1. Major exclusion criteria

Exclusion Criteria
• Women who were pregnant, breastfeeding, trying to become pregnant, or who were not using contraception
• Women in menopausal transition or who have had early menopause
• People with a history of changes in cranial anatomy or metallic intracranial implants
• People with preexisting lesions where the tDCS electrodes are to be placed
• A history of any type of bariatric surgery
• A history or diagnosis of severe neuropsychiatric disease or any severe chronic or acute illness
• A history of substance use (medicines or herbal medicines) that could interfere with body weight
• A current or past history of alcohol or other drug abuse;
• People with any acute condition or exacerbation of a chronic condition that would interfere with the procedures.

tDCS: transcranial direct current stimulation

Table S2. Adjusted analysis of the effect of tDCS on energy and macronutrient intake by groups relative to baseline.

	Active			Sham			*P* value
	Baseline*	1st Fortnight	2nd Fortnight	Baseline*	1st Fortnight	2nd Fortnight	
Energy (kcal/day)	2679.9	1557.6 (1361.5 to 1753.8)	1591.4 (1355.9 to 1826.9)	2679.9	1561.3 (1320.4 to 1802.2)	1461.1 (1287.6 to 1634.7)	0.426
Energy (kcal/kg/day) †	29.9	18.3 (15.5 to 21.2)	18.9 (16.7 to 21.1)	29.9	17.7 (13.9 to 21.4)	16.5 (14.4 to 18.7)	0.362
Carbohydrate (g/day)	295.3	179.6 (148.7 to 210.5)	191.9 (155.5 to 228.3)	295.3	169.8 (150.3 to 189.4)	177.2 (151.5 to 202.9)	0.816
Protein (g/day) †	119.2	84.7 (77.3 to 92.2)	90.9 (78.7 to 103.0)	119.2	92.2 (71.8 to 112.7)	84.5 (72.4 to 96.5)	0.191
Total fat (g/day)	108.1	59.2 (48.1 to 70.2)	54.2 (45.2 to 63.2)	108.1	56.9 (45.7 to 68.2)	48.9 (41.8 to 55.9)	0.729
Total fiber (g/day) †	22.9	26.8 (23.0 to 30.6)	26.1 (21.8 to 30.5)	22.9	22.9 (18.8 to 27.0)	22.0 (17.7 to 26.2)	0.855

Data are presented as the estimated marginal mean (95% CI). Responses were analyzed using a generalized estimating equation (GEE). *Variables were adjusted for the baseline values of each outcome and for the number of tDCS sessions (fixed at 19.7 tDCS sessions). †GEE analysis was performed on a gamma distribution with a log link function. 1st Fortnight: period between the first and second weeks of intervention. 2nd Fortnight: period between the third and fourth weeks of intervention. tDCS, transcranial direct current stimulation.

Table S3. Adjusted analysis of the effect of tDCS on desire to eat by groups relative to baseline.

	Active		Sham		*P* value
	Baseline (n= 14)*	After intervention (n=10)†	Baseline (n= 14)	After intervention (n= 14)	
Subjective satiety and hunger VAS					
How is your hungry/satiety at this very moment? (-3= “extremely hungry” / +3= “extremely full”)	-1.0 (-1.2 to -0.8)	-0.8 (-1.1 to -0.6)	-1.0 (-1.2 to -0.8)	-1.4 (-1.8 to -0.9)	0.183
Desire to eat VAS					
Would you like to eat something sweet? (0 = “yes, very much” / 100 = “not at all”)	55.5 (45.6 to 65.4)^{Aa}	100.1 (74.1 to 126.0)^{Ab}	61.5 (54.8 to 68.3)^{Aa}	66.1 (47.8 to 84.5)^{Ba}	0.043
Would you like to eat something salty? (0 = “yes, very much” / 100 = “not at all”)	34.8 (30.5 to 39.0)	40.5 (24.7 to 56.2)	36.0 (31.1 to 40.9)	42.8 (24.9 to 60.7)	0.938
Would you like to eat something savory? (0 = “yes, very much” / 100 = “not at all”)	23.9 (21.4 to 26.4)	35.5 (16.9 to 54.2)	22.8 (20.3 to 25.3)	36.2 (27.1 to 45.3)	0.878
Would you like to eat something fatty? (0 = “yes, very much” / 100 = “not at all”)	65.1 (55.3 to 75.0)	92.2 (65.8 to 118.7)	66.0 (58.1 to 73.9)	69.5 (54.6 to 84.3)	0.239

Data are presented as estimated marginal mean (95% CI) and were adjusted for the baseline values of each outcome and for the number of tDCS sessions (fixed at 19.7 tDCS sessions). Responses were analyzed using a generalized estimating equation (GEE). For subjective satiety and hunger, desire for sweet, savory, and fatty foods, the GEE analysis was performed on a gamma distribution with a log link function. *For subjective satiety and hunger VAS, n= 13. †For desire to eat VAS, n=11. Means without a common lowercase letter differ in time, $P < 0.050$. Means without a common capital letter differ in group, $P < 0.050$. VAS, visual analogue scale.

Table S4. Effects of tDCS associated with a hypocaloric diet on body composition, resting metabolic rate and physical activity.

	Active		Sham		p value
	Baseline (n= 14)	After intervention (n= 10)*	Baseline (n= 14)	After intervention (n= 13)†	
Body size and composition					
Waist circumference (cm)	100.3 (94.4 to 106.2)	95.3 (89.8 to 100.8)	101.4 (97.4 to 105.3)	97.7 (94.2 to 101.3)	0.472
Waist-to-Hip (ratio)	0.91 (0.86 to 0.96)^{Aa}	0.88 (0.83 to 0.92)^{Ab}	0.91 (0.87 to 0.94)^{Aa}	0.90 (0.87 to 0.94)^{Aa}	0.049
Body fat (%)	37.2 (33.4 to 41.0)	34.8 (30.1 to 39.4)	36.6 (32.8 to 40.4)	34.7 (30.4 to 39.0)	0.651
Skeletal muscle mass (%)	35.3 (32.9 to 37.8)	36.7 (33.8 to 39.7)	35.8 (33.4 to 38.2)	36.9 (34.1 to 39.7)	0.648
Resting metabolic rate					
aRMR (kcal/day)	1676.9 (1531.5 to 1822.3)	1628.7 (1463.2 to 1794.1)	1745.1 (1593.0 to 1897.3)	1692.7 (1558.8 to 1826.6)	0.949
Physical activity					
Steps (steps/day)	5164.2 (3481.5 to 6846.8)	6918.3 (3892.5 to 9944.0)	5774.5 (4286.8 to 7262.3)	6241.5 (4651.8 to 7831.2)	0.385

Data are estimated marginal means (95% CI). Responses were analyzed using the generalized estimating equation (GEE). For body fat, skeletal muscle mass aRMR, and steps, GEE analysis was performed on a gamma distribution with a log link function. *Waist circumference and waist-to-hip ratio, n = 11; for body fat and skeletal muscle, n = 12. †Waist circumference, waist-to-hip ratio, body fat, and skeletal muscle, n = 14. Means without a common lowercase letter differ in time; $P < 0.01$. Means without a common capital letter differ by group; $P < 0.05$. aRMR; adjusted resting metabolic rate; tDCS, transcranial direct current stimulation.

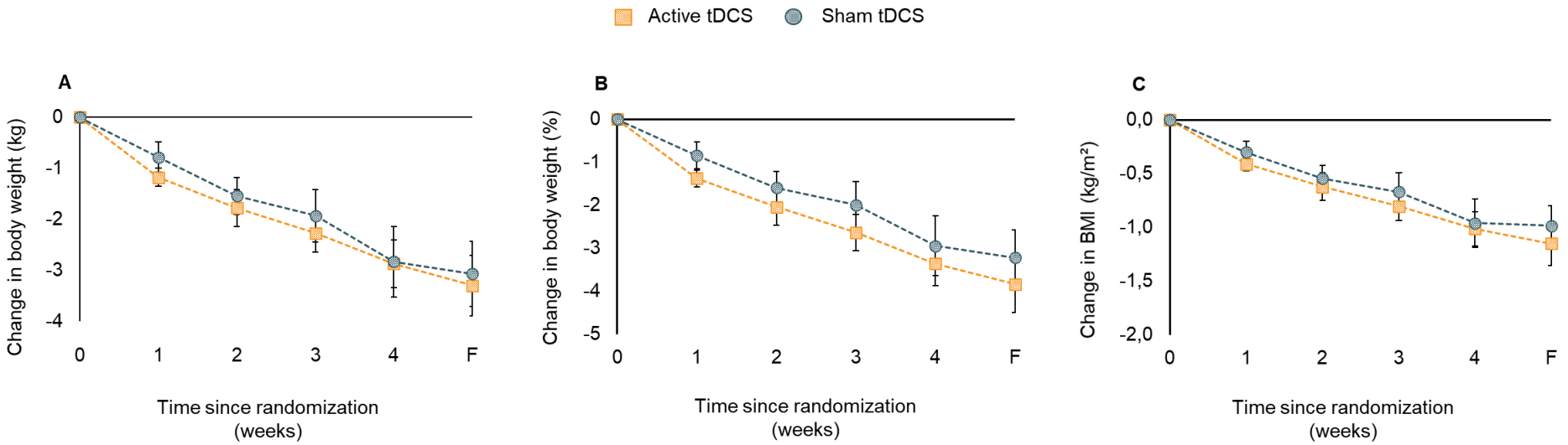

Figure S1. Per-protocol analysis of the effect of tDCS intervention on weight loss by groups relative to baseline.
Data are estimated means [SEE]. Responses were analyzed using a generalized estimating equation (GEE). (A) *P* = 0.628. (B) *P* = 0.871. (C) *P* = 0.715. BMI = body mass index. F = final evaluation assessed three days after the last tDCS session. tDCS = transcranial direct current stimulation.

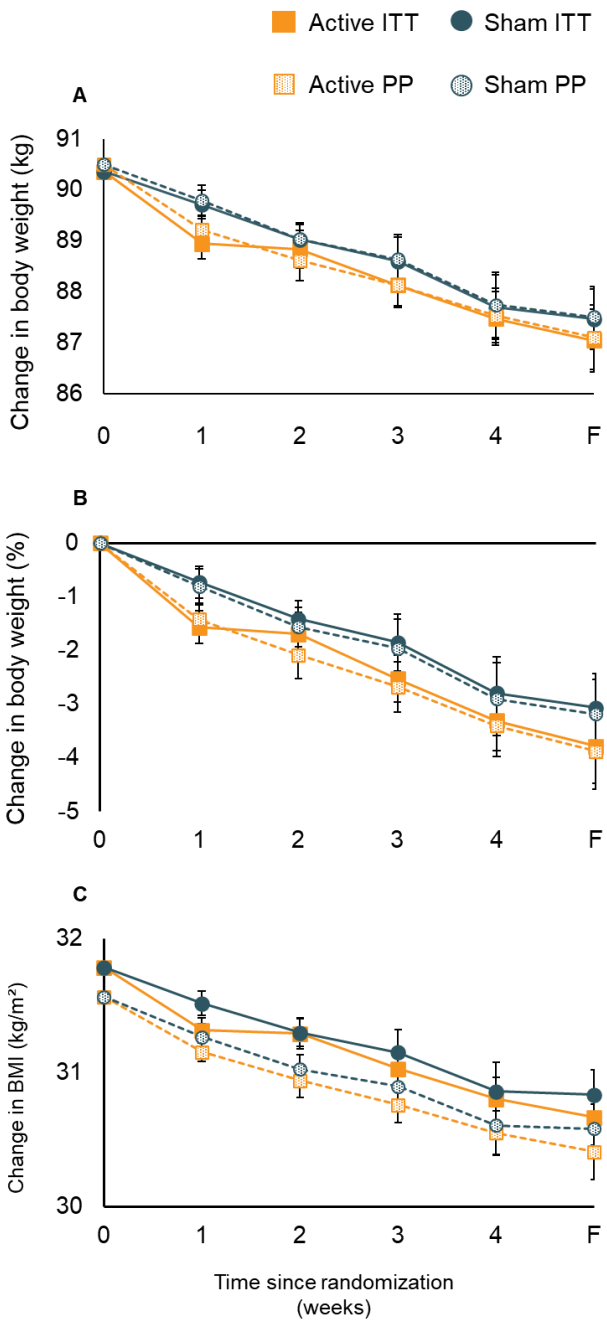

Figure S2. Adjusted analysis of the effect of tDCS on weight loss by groups relative to baseline.

Data are presented as the estimated means [SEE]. Responses were analyzed using a generalized estimating equation (GEE). ITT: n=28; closed symbols. PP: n=23; open symbols. (A) Adjusted for initial body weight and for the number of tDCS sessions; ITT, *P* = 0.424; PP, *P* = 0.881. (B) Adjusted for initial body weight and for the number of tDCS sessions; ITT, *P* = 0.428; PP, *P* = 0.871. (C) Adjusted for initial BMI and for the number of tDCS sessions; ITT, *P* = 0.391; PP, *P* = 0.829. BMI=body mass index. F = final evaluation assessed three days after the last tDCS session. ITT = intention-to-treat. PP = per-protocol. tDCS = transcranial direct current stimulation.

Effects of transcranial direct current stimulation associated with hypocaloric diet on glucose homeostasis in obesity

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7 ARTIGO IV

7.1 EFFECTS OF TRANSCRANIAL DIRECT CURRENT STIMULATION ASSOCIATED WITH HYPOCALORIC DIET ON GLUCOSE HOMEOSTASIS IN OBESITY[4]

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Keywords: obesity; transcranial direct current stimulation; tDCS; neuromodulation; glucose homeostasis.

Running title: tDCS and glycemic parameters in obesity

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Disclosure: The authors declared no conflict of interest.

Author Contribution: C.A. and F.G. designed the study. C.A., G.R.N., P.E.C., and A.F.O. conducted a literature search. C.A. and R.C.F. designed the diet and its prescription. P.N.M, conducted the randomization. F.G. provided the essential materials. G.R.N., P.E.C., and AFO executed the tDCS sessions. P.A.C.F. conducted the biochemical investigation. C.A., E.B., and F.G. conducted the statistical analyses and data interpretation. L.A.M. helped with analysis of data. C.A. and F.G. wrote the manuscript. C.A. and F.G are the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study Importance

What is already known about this subject?

- Transcranial direct current stimulation (tDCS) is a method for enhancing brain activity with potential impact in glucose metabolism.
- An increase in cerebral energy consumption induced by anodal tDCS was observed in healthy weight subjects after a single tDCS session, after two tDCS sessions 115 minutes apart, and after eight following days of tDCS.
- tDCS increased systemic glucose disposal for an hour at most during a hyperinsulinemic-euglycemic glucose clamp, after a single or double sessions.

What are the new findings in your manuscript?

- Four weeks of repetitive, active tDCS over the right dorsolateral prefrontal cortex improved fasting plasma glucose, fasting insulin, and Matsuda insulin sensitivity index in subjects with obesity on a low-calorie diet.

How might your results change the direction of research?

- Future studies are needed to investigate if tDCS could be used as a non-pharmacologic method to improve glucose homeostasis in individuals with overweight or obesity or even to explore this intervention modality in subjects with T2DM.

ABSTRACT

Objective: To test the effects of repetitive active tDCS over the right dorsolateral prefrontal cortex (rDLPFC) associated with a hypocaloric diet (HD) on glucose homeostasis in people with excessive weight.

Methods: Adults with overweight or obesity were selected in a randomized, double-blind pilot study to complete four weeks (20 sessions) of fixed-dose tDCS (2mA, 20 min) delivered over the rDLPFC and associated with a standard HD. Subjects were randomly assigned (1:1) and stratified by sex to the active tDCS group (active) or the sham tDCS group (sham). Changes in glucose homeostasis were assessed in a four-hour liquid meal tolerance test, performed before and after the intervention.

Results: 28 participants were randomized (79% obese; 37.6 [5.8] years). After the intervention, fasting plasma glucose (−7.8mg/dL [−14.0 to −1.6]) and insulin levels (–7.7μIU/mL [–13.9 to –1.6]) decreased in the active compared to the sham. Similarly, the MISI increases in the active (4.7$pmol^{-1}$×$mmol^{-1}$ [1.6 to 7.8]) compared to the sham (0.6$pmol^{-1}$×$mmol^{-1}$ [−1.4 to 3.2]).

Conclusions: Repetitive, active tDCS over the rDLPFC could be a promising non-invasive technique to improve glucose homeostasis in individuals with overweight or obesity on a low-calorie diet, highlighting the importance of investigating this intervention modality in subjects with T2DM.

INTRODUCTION

Overweight and obesity prevalence has increased worldwide. According to the Global Burden of Disease Obesity Collaborators (2017), in 2015, 603.7 million adults were estimated to be with obesity, with an overall prevalence of 12% (1). Obesity is associated with several health conditions, and is a major risk factor for the development of insulin resistance (2, 3). If obesity-related insulin resistance progresses to type 2 diabetes mellitus (T2DM), then pancreatic β-cells may not compensate insulin resistance (β-cell disfunction), leading to lower insulin secretion, incapacity to compensate glucose blood levels, and hyperglycemia (4).

The brain controls systemic glucose and energy metabolism. The central nervous system regulates peripheral glucose metabolism by stimulating hepatic glucose release or pancreatic insulin production (5). Then, insulin acts in the hypothalamus to regulate body weight. Therefore, if β-cell dysfunction reduces insulin release, it could decrease insulin action in this brain region and thus cause weight gain and greater insulin resistance (2).

Transcranial direct current stimulation (tDCS), a non-invasive neuromodulation technique, has been suggested as a new modality of treatment to improve systemic glucose metabolism. Studies suggest that neuromodulation increases cerebral energy consumption (by increasing brain levels of high-energy phosphate) and thus reduces cerebral energy levels and increases glucose transport across the blood-brain barrier (6, 7). Accordingly, the energy transport to peripheral areas is suppressed and, since glucose transport across the blood-brain barrier is mainly insulin-independent, glucose allocates in the brain.

Studies have already shown the tDCS ability to increase glucose systemic tolerance (8–11). However, these studies were performed in healthy subjects, who had no glucose metabolism malfunctions and were under eight days of neurostimulation protocol at most. However, these results seem inconsistent for subjects with obesity, who show lower neuroenergetic reactivity than healthy subjects and decreased systemic glucose uptake after a neurostimulation (11).

We recently conducted a more intensive and frequent tDCS protocol adjunct to a hypocaloric diet (HD) in individuals with obesity and we found that adding active tDCS to an HD can reduce craving for sweet foods (12). In this study, we extend our analysis from this four-week trial to determine whether 20 sessions of active stimulation over

the right dorsolateral prefrontal cortex (DLPFC), along with an HD, affect glucose homeostasis in individuals with overweight or obesity. Therefore, we investigated changes in fasting and in postprandial responses of plasma glucose and insulin and their regulation and control: insulin resistance (HOMA-IR), insulin sensitivity (MISI), insulin secretion (ISI), pancreatic β-cell function (LMTT-DI), and glycated albumin (GA).

METHODS

This double-blind, randomized, and sham-controlled pilot study was approved by the Research Ethics Committee of the Hospital de Clínicas de Porto Alegre, Brazil (protocol number: 42996915.0.0000.5327). The study was pre-registered on ClinicalTrials.gov (NCT02683902) and conducted in accordance with the Declaration of Helsinki. This study protocol was previously published in accordance with Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) guidelines (13). All Consolidated Standards of Reporting Trials (CONSORT) details and the main results of the trial on the primary endpoint can be found in the primary manuscript (12).

Participants

Inclusion criteria targeted adults aged 20–50 years with body mass index (BMI) between 25.0 and 35.0 kg/m² and stable body weight, who had not had nutritional counseling at least six months before the screening, and who were able to comply with the study protocol. Exclusion criteria: patients with any medical condition (including pregnancy, digestive disease, malignancies, or major neurological, psychiatric, or endocrine disease) that could prevent their inclusion in a weight loss intervention study; patients with metallic intracranial implants or preexisting lesions where tDCS electrodes would be placed; patients with current or past history of alcohol or other drugs abuse, including medicines or herbal medicines that could affect body weight, glycemic control, or metabolic status.

Study protocol

After selection, participants underwent a three-day baseline assessment. At the first baseline visit, a complete clinical (medical history and physical examination) and nutritional interview and an anthropometric evaluation were conducted. Body weight

was assessed in the morning, with the participant barefoot, wearing light clothing, fasting, and having avoided exercises in the last 24 hours (InBody 230, Biospace Co. Ltd, Seoul, South Korea). Height was measured using a wall-mounted stadiometer and recorded to the nearest 0.1 cm. BMI was calculated as weight (kg)/height (m^2).

To determine the participants' status of glucose tolerance, at the second baseline visit, 12-hour fasting blood samples were collected to analyze glycated hemoglobin (A1C) and to perform a 75-g OGTT, in which glucose was measured at 0 and 120 minutes. A1C levels were determined with a high-performance liquid chromatography method (VARIANT II TURBO A1c kit; Bio-Rad Laboratories, Hercules, CA, USA) certified by the National Glycohemoglobin Standardization Program, which corroborates with the International Federation of Clinical Chemistry. Glucose was determined by an enzymatic UV-hexokinase method (Cobas c702, Roche Diagnostic, Mannheim, Germany). These measures were only taken at the baseline at the Laboratory Diagnosis Division of the Hospital de Clínicas de Porto Alegre.

At the third baseline visit (one day before the first tDCS session), after a 12-hour fast, a liquid meal tolerance test (LMTT) was performed, in which both glucose and insulin were measured at −30, −15, 0, 30, 60, 120, and 240 min. Three blood samples (−30, −15, and 0) were drawn over 15 min to determine the mean glucose and insulin fasting values. Participants were then requested to drink a standardized liquid meal (400 ml Trophic 1.5, 600 kcal, 53% carbohydrates, 16% protein, 31% fat; Prodiet, Curitiba, Brazil) within 10 minutes. Additional blood samples were collected at 30, 60, 120, and 240 minutes after the meal. Postprandial responses for glucose and insulin were obtained by estimating the area under the curve (AUC) from the LMTT with the trapezoidal rule (14). A serum sample from the fasting state was obtained to measure glycated albumin (GA). Participants repeated the LMTT at the end of the study, a day after the last tDCS session.

Insulin resistance was calculated with the HOMA2-IR index (15). The LMTT Matsuda insulin sensitivity index (MISI) was calculated as 10,000/(G0×I0×Gm×Im)0.5, in which G0 and I0 were pre-meal values for glucose (G) and insulin (I), respectively, and Gm and Im were the mean post-meal values during the 120 min of the test (16). LMTT insulin secretion index (ISI) was calculated as: total AUC for plasma insulin from 0 to 120 min divided by the total AUC for plasma glucose from 0 to 120 min (16). Pancreatic β-cell function was determined by the estimated disposition index

(DI=MISI×ISI) (16). MISI derived from a LMTT provide reliable insulin sensitivity and secretion estimates in repeated tests administered on separate days (16, 17).

Glucose was determined by an enzymatic UV-hexokinase method (Cobas c702, Roche Diagnostic, Mannheim, Germany) and insulin was determined with a chemiluminescent microparticle immunoassay (ARCHITECT Ci4100; Abbott Diagnostics, Lake Forest, IL, USA). GA was measured by enzymatic assay (GlycoGap®, Diazyme, California, EUA) in a Cobas c702 automatic analyzer (Roche Diagnostic, Mannheim, Germany) (18). The GlycoGap® kit quantifies total glycated serum proteins (GSP, mmol/L) and accurately estimates GA levels with the following equation: GA% = ((GSP × 0.182) + 1.97 / total albumin in g/dL) / 100 (19). The GlycoGap® kit in our lab had a 3.5% intra-assay variation coefficient. Complete details are provided elsewhere (13).

After the three-day baseline assessments, a dietitian prescribed HD to each participant, who received individual counseling to improve their dietary habits during a four-week intervention. The HD was prescribed a day before the first tDCS session to reduce at least 3% of participants' initial body weight in four weeks. Energy deficit was calculated individually (20) and macronutrients were equally balanced for each group (45 – 50% carbohydrates; 15 – 25% protein; ≤ 30% fat, mainly unsaturated fats; and ≥ 20 g/day fiber) (21). Diet composition was analyzed (U.S. Department of Agriculture Table (22)) with a NutriBase 17 Pro Edition software (version 17.2, CyberSoft, Inc., Phoenix, Arizona).

Participants were randomly assigned to one of two types of intervention: active tDCS + HD or sham tDCS + HD. They were randomized in blocks in a 1:1 ratio and stratified by sex before the first tDCS session, with trained technicians. All participants and investigators were blinded to the different interventions. Only trained technicians who knew the randomization allocations conducted tDCS sessions.

Twenty-minute daily tDCS sessions were conducted with a Chattanooga Ionto™ Dual Channel Electrophoresis System device (DJO Global, Guildford, UK) during the five weekdays (including holidays) for four weeks, with 20 sessions in total. For active tDCS, a direct current of 2 mA (30 s fade in/out) was performed with the anode electrode over the right DLPFC (F4) and with the cathode electrode over the left DLPFC (F3). Electrodes were sized 35 mm2, and a surface sponge was soaked with a standard saline solution (NaCl 0.9%). For sham tDCS, electrodes were placed identically to those in the active group, but the current was ramped up to 2 mA and

then lowered in the first and last 30 seconds of the 20-minute session. As a result, participants in the sham group were not stimulated for 19 minutes of the session.

Participants were asked to fast for three hours before each stimulation session, which was conducted at the same time of day for each participant. If a scheduled tDCS session was missed for any reason, it was rescheduled for the following week. Investigators did not administer or attend tDCS sessions to keep the blindness.

Statistical Procedures

Effects of tDCS over time were analyzed by the generalized estimated equation (GEE) using the tDCS groups (active vs. sham condition) and time (baseline and after tDCS); the interaction effect between groups and time was called “group-by-time.” Variables with non-Gaussian distribution were performed on gamma distribution with log link function.

The intention-to-treat (ITT) analyses were performed at all stages of data analysis, thus including all participants who underwent at least one tDCS session. A sensitivity analysis was also performed, in which weight loss and carbohydrate intake were included as adjustment covariates, determined by multiple regression. Results of weight loss and carbohydrate intake analysis can be found in our previous study (12). Outcomes are presented as p values for the group-by-time interaction and the estimated marginal means, with 95% confidence intervals for each tDCS group at each time point. A $p<0.050$ was considered statistically significant. All statistical tests were performed using the IBM SPSS Statistics software program, version 19.0 (IBM, Armonk, NY, USA).

RESULTS

Participant characteristics, participant flow diagram, and adverse events were previously reported (12). In short, 28 overweight participants were enrolled and included in the ITT analysis, and 23 participants completed all 20 planned tDCS sessions. Among those who did not complete the entire protocol, one withdrew after four sessions, another after five sessions, and three others after eight sessions. They had a higher BMI at baseline compared to the participants who completed the study. The 28 participants (14 men; 75% white) were aged 37.6 ± 5.8 years (mean ± standard

deviation), had a mean BMI of 31.5 ± 2.4 kg/m2, a mean fasting glucose of 93.2 ± 10.4 mg/dl, and a mean A1C of 5.4 ± 0.5%. Participants who received active or sham tDCS had similar demographic, clinical, and anthropometric baseline characteristics, including BMI, waist circumference, total body fat, and skeletal muscle mass (12). The baseline glycemic profile of participants was similar between groups (Table 1). At the end of the study, the groups did not differ regarding changes in body weight, waist circumference, total body fat, and skeletal muscle mass (Table 2) (12). Only one subject showed an OGTT compatible with T2DM. He did not have a previous history of the disease.

Table 1. Baseline glycemic and metabolic profile according to randomization.

	Active tDCS (n = 14)	**Sham tDCS (n = 14)**	**P value**
FPG, *mg/dl*	93.0 (89.0 – 97.0)	89.0 (86.0 – 92.0)	0.107
2-h PG during 75-g OGTT, mg*/dl*	120.3 ± 38.5	101.1 ± 24.1	0.127
A1c, ***%***	5.6 ± 0.4	5.3 ± 0.5	0.155
T2DM	1 (7.1)	0	

Baseline characteristics were summarized by means (SDs) for normally distributed continuous variables (Student t-test), medians (IQR) for non-normally distributed continuous variables (Mann-Whitney Test), and frequencies (%) for categorical variables (Pearson Chi-square). A1c: glycated hemoglobin. FPG: fasting plasma glucose. OGTT: oral glucose tolerance test. T2DM: type 2 diabetes mellitus.

Table 2 and Figure 1 show the fasting responses of tDCS combined with an HD over plasma glucose and insulin and GA assessed during an LMTT. Although fasting plasma glucose (FPG) had a group-by-time interaction of borderline significance (p = 0.067) in the crude analysis (Table 2), the adjustment for relative weight loss (%) and for carbohydrate intake significantly decreased (p = 0.045) in the FPG of the active group at the end of the study (–7.8 mg/dL [95% CI: –13.9 to –1.6]; p = 0.013) compared to the sham group (–0.9 mg/dL [95% CI: –4.0 to 2.2]; p = 0.561) (Figure 1A). In the crude analysis, fasting insulin remained stable over time between groups (p = 0.138) (Table 2). However, after adjusted analysis, fasting insulin levels were significantly lower (p = 0.020) in the active tDCS group (–7.7 µIU/mL [95% CI: –13.9 to –1.6]; p = 0.013) than in the sham group (–1.3 µIU/mL [95% CI: –3.3 to 0.7]; p = 0.191) at the end of the study (Figure 1B). No significant differences were found between groups

regarding tDCS effects over GA or HOMA2-IR responses (Table 2 and Figures 1C and 1D).

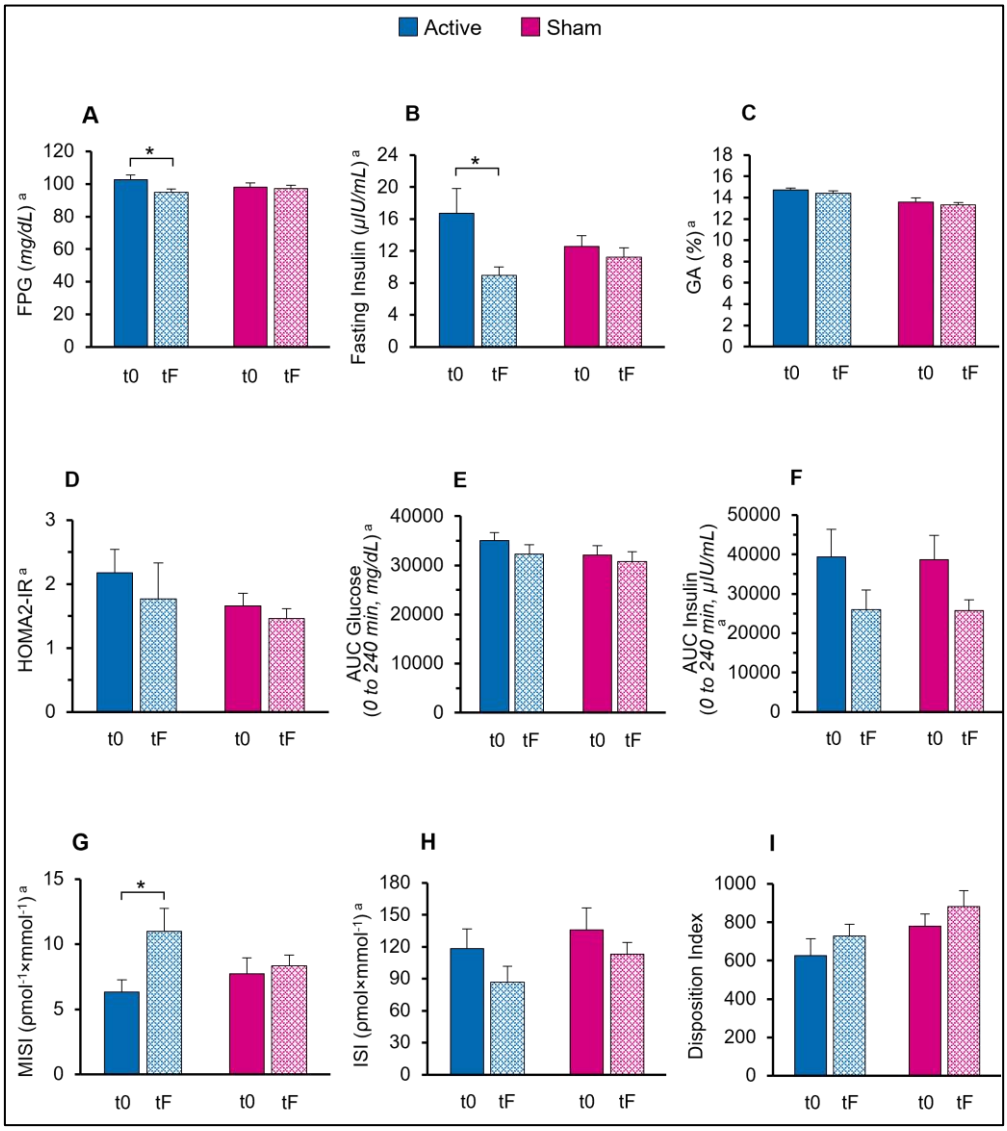

Figure 1. Adjusted analysis of the effects of tDCS with a hypocaloric diet on glycemic profile during a liquid meal tolerance test (LMTT) according to the intervention. Data are estimated marginal means. The generalized estimating equation (GEE) tested p value for interaction (tDCS by time) at t0 (baseline) and tF (after the intervention ended). Means without a common lowercase letter differ in time (t0 vs. tF), with $p < 0.05$. Capital letters represents comparisons between groups (active vs. sham). *GEE analysis was performed on gamma distribution with log link function. AUC: area under the curve. FPG: fasting plasma glucose. GA: glycated albumin. HOMA2-IR: Homeostatic Model Assessment of Insulin Resistance. ISI: Insulin Secretion Index. MISI: Matsuda Insulin Sensitivity Index. tDCS: transcranial direct current stimulation.

AUC was calculated to analyze if, at the end of the study, active tDCS stimulation could change the postprandial response of glucose and insulin after liquid meal intake during LMTT. Regarding these postprandial responses, at the end of the

study, changes in glucose and insulin AUC were the same between groups for crude (Table 2) or adjusted analysis (Figures 1D and 1E).

In the crude analysis, the active group significantly improved their insulin sensitivity indexes by 4.6 $pmol^{-1} \times mmol^{-1}$ (95% CI: 1.7 to 7.4; p = 0.002), whereas the sham group improved 1.1 $pmol^{-1} \times mmol^{-1}$ (95% CI: −1.1 to 3.2; p = 0.326) at the end of the study (Table 2). These differences remained significant after adjustment for relative weight loss (%) and carbohydrate intake (Figure 1G; p = 0.024). ISI and DI groups were similar throughout the study (Table 2), even after adjustments were made (ISI: p = 0.488; DI; p = 0.995) (Figures 1H and 1I).

DISCUSSION

In this double-blind, randomized, and sham-controlled pilot study, we aimed to analyze if 20 repeated active tDCS sessions with an HD could affect glucose homeostasis in individuals with overweight or obesity during a four-week intervention. According to our results, subjects in the active group improved regarding fasting plasma glucose, fasting insulin, and Matsuda insulin sensitivity index (MISI) after adjustment for weight loss (%) and carbohydrate consumption.

The observed reductions in fasting glucose and MISI corroborate with previous data, showing a relationship between tDCS and glucose homeostasis (6, 9, 10). In a crossover study of 15 healthy volunteers, a single active tDCS increased systemic glucose disposal in gold-standard experimental conditions, using a hyperinsulinemic-euglycemic glucose clamp (6). The same study found similar results for two tDCS sessions (with 115 min intervals between each session) and observed that glucose peripheral disposal was better for an hour at most after double tDCS sessions (10). In a different protocol, under physiological conditions, 14 healthy men with normal weight were submitted to eight consecutive days of tDCS sessions and showed lower blood glucose concentrations, which were measured immediately after the tDCS sessions, on the first and eighth day (9). Unlike previous studies that measured glucose profile immediately before or after the tDCS session, in our experiment, the two blood samples were collected one day before the 20 daily tDCS sessions started and one day after they ended. Therefore, we observed that FPG and MISI decreased under physiological conditions. Since this tDCS protocol was performed with a hypocaloric diet — and assuming that weight loss or excessive carbohydrate intake could affect glucose

homeostasis — we emphasize that our analysis considered and was adjusted in different models for changes in weight and carbohydrate intake of the active group and the sham group.

How tDCS regulates cerebral function and metabolism is still unknown. However, regarding tDCS effects on glucose homeostasis, one of the potential mechanisms that could explain why brain stimulation reduces FPG and MISI is the increase of cerebral energy (ATP) consumption by tDCS. According to previous studies, tDCS increases cerebral energy consumption and temporarily decreases brain ATP concentrations within 65 minutes after stimulation (6). This contributes to the physiological activation of the hypothalamic ATP-sensitive potassium (KATP) channels, which, in turn, increase systemic glucose uptake by inhibiting hepatic gluconeogenesis (6, 9, 10).

Studies have shown that tDCS could generate neuroplasticity directly in the cortical area of application, but might also affect distant brain areas, such as the hypothalamic area (23–25). Accordingly, different studies suggest that anodal tDCS over cortical areas can down-regulate the hypothalamus-pituitary-adrenal (HPA) axis activity, thus resulting in a reduction of blood or salivary cortisol levels compared to sham stimulation (6, 10, 26). Furthermore, the hypothalamus is essential to regulate blood glucose levels since it can sense, integrate, and respond to changes in circulating signals (27). Therefore, considering that hypothalamic regions are affected by active tDCS over the DLPFC, we can assume that the opening of KATP channels in the hypothalamus mediates a mechanism that can increase peripheral glucose disposal.

Another possible cause is the mechanism called “energy on demand” or “Selfish Brain Theory” (7, 10). The brain is a heavy energy consumer with a daily average uptake of up to 20% of total serum glucose (7). Therefore, the brain could supply itself with energy by allocating intrinsic energy resources from the outermost areas of the body (7). In other words, if the brain consumes greater energy, more glucose is transported across the blood-brain barrier. An increase in cerebral energy consumption induced by anodal tDCS was observed previously after a single 20-minute tDCS stimulation session (6), after two tDCS sessions 115 minutes apart (10), and after eight following days of tDCS (9). On the other hand, in individuals with obesity tDCS delayed the reactivity of cerebral energy consumption (11). For these individuals, tDCS

Table 2. Crude analysis of the effects of tDCS with a hypocaloric diet on glycemic profile during LMTT according to intervention.

	Active tDCS		Sham tDCS		p value
	Baseline (n= 14)	**After intervention (n=10)**	**Baseline (n= 14)**	**After intervention (n= 13)**	
Body weight (kg)*	88.8 (82.3 to 95.2)	84.3 (75.6 to 93.0)	92.0 (86.3 to 97.6)	89.6 (84.2 to 95.1)	0.786
FPG (mg/dL)	102.8 (96.6 to 108.9)	96.0 (92.0 to 100.0)	97.1 (91.8 to 102.4)	96.5 (92.3 to 100.7)	0.067
Fasting insulin (μIU/mL)	17.9 (11.1 to 24.6)	10.0 (6.5 to 13.6)	13.5 (10.1 to 17.0)	11.1 (8.8 to 13.4)	0.138
GA (%)	14.4 (14.0 to 14.8)	14.4 (14.0 to 14.9)	13.6 (12.9 to 14.3)	13.3 (12.9 to 13.7)	0.526
AUC glucose $_{0-240\ min}$ (mg/dL × min)	36350.0 (32420.4 to 40279.6)	32382.0 (28886.4 to 35877.6)	31676.8 (28171.7 to 35181.9)	30724.2 (26943.6 to 34504.8)	0.298
AUC insulin $_{0-240\ min}$ (μIU/mL × min)	42364.8 (26940.0 to 57789.6)	27441.2 (18698.4 to 36138.9)	43396.3 (28110.7 to 58681.9)	25346.5 (18993.8 to 31699.2)	0.628
HOMA2-IR	2.3 (1.5 to 3.2)	2.0 (0.6 to 3.4)	1.8 (1.3 to 2.2)	1.5 (1.2 to 1.8)	0.817
MISI ($pmol^{-1}\times mmol^{-1}$)	6.3 (4.4 to 8.2)	10.8 (7.2 to 14.5)	7.7 (5.0 to 10.3)	8.7 (6.8 to 10.7)	**0.039**
ISI ($\rho mol\times mmol^{-1}$)	128.6 (83.8 to 173.3)	93.5 (56.2 to 130.8)	147.2 (100.9 to 193.5)	112.1 (86.7 to 137.4)	0.797
DI $_{0-120\ min}$	642.7 (484.2 to 801.1)	725.9 (597.6 to 854.3)	796.5 (678.2 to 914.7)	883.5 (715.2 to 1051.7)	0.973

Data were summarized by means (95% CI). Responses were analyzed using generalized estimated equation (GEE) on gamma distribution with log link function. *These data were published previously but are demonstrated here for a better understanding of the results (12, 34). AUC: area under the curve. DI: disposition index. FPG: fasting plasma glucose. GA: glycated albumin. ISI: insulin secretion index. MISI: Matsuda insulin sensitivity index.

regularly increased energy consumption by 40 minutes later than normal-weight men (11). However, in our study, glycemic homeostasis was analyzed a day after the last tDCS session; therefore, our results may have been unaffected by this delay in brain reactivity of cerebral energy consumption caused by tDCS

To date, studies that showed improved glucose homeostasis explain their results with insulin-independent mechanisms described above (6, 9–11), given that fasting plasma insulin was stable during or after the tDCS sessions (9). Nonetheless, our data showed that fasting insulin significantly decreased and insulin sensitivity (MISI) improved after the 4-week intervention for the active tDCS group, which cannot be explained by changes in body weight or carbohydrate consumption. Insulin sensitivity reflects the ability to circulate insulin to enhance glucose disposal and inhibit hepatic glucose output (16). Obesity is essentially associated with lower insulin sensitivity, i.e., with greater insulin resistance (2). Therefore, individuals with obesity are expected to have higher insulin levels and poorer peripheral glucose uptake than lean individuals. In this sense, a crossover design study with a single 20-minute tDCS session showed that individuals with obesity had higher insulin concentrations and lower systemic glucose disposal caused by tDCS than normal-weight volunteers after a hyperinsulinemic-euglycemic clamp (11). These findings lead us to think that the repetitive and intense nature of our protocol may have induced brain neuroplasticity, which explains our results.

The effects of tDCS are long-lasting, and a stronger and longer stimulation emphasizes their efficacy (28, 29). Until now, studies have shown the immediate aftereffects of tDCS on glucose homeostasis, i.e., lower blood glucose concentrations or increased systemic glucose uptake a few minutes or hours after stimulation. In fact, our data suggest that a repetitive four-week protocol with 20 intense (2 mA) tDCS sessions could prolong the aftereffects, as observed in our study. Aftereffects of longer-lasting stimulation can last from minutes to more than 24 hours after intervention and cause a continuous remodeling of brain function called synaptic plasticity (28, 29). Evidence suggests several other mechanisms that change neural excitability and that may contribute to this plasticity, including temporary or lasting changes in Ca2+ channels dynamics, neurotransmitter release, protein expression, and gene activity. Possible changes in gene and protein expression could prolong tDCS effects for hours, days, and even months (28).

The brain-derived neurotrophic factor (BDNF) is highly concentrated in the hippocampus and the cerebral cortex and is considered a key molecule for neuroplasticity, enhancing the growth and distinction of new neurons and synapses (30). A recent meta-analysis showed that tDCS modulates and increases BDNF in healthy and diseased rodents after tDCS sessions (31). Results from in vitro studies showed that anodal tDCS modulates both gene and protein expression levels of BDNF (32). From a clinical perspective, these results may affect the management of conditions with reduced cortical BDNF levels. Studies with rodents support an endocrine role of BDNF in modulating food intake and glycemia (33). In nonfunctional leptin receptor mice, continuous treatment with BDNF over several days decreased food intake, lowered blood glucose, and increased whole-body glucose turnover (33). Similarly, lower BDNF serum levels harm glucose metabolism and are associated with obesity and diabetes complications in adults (33). Thus, the repetitive four-week tDCS sessions may have caused brain neuroplasticity in our volunteers, increasing their BDNF levels and improving the glycemic parameters observed.

This study's strengths include a well-controlled recruitment, resulting in balanced randomization and baseline characteristics between groups, and the application of a protocol with recurrent tDCS sessions. Moreover, as demonstrated in our previous publications (12, 13, 34), this study was conducted in a very well-controlled environment. Participant groups have comparable baseline characteristics for sex, age, ethnicity, weight, habitual diet, physical activity, resting metabolic rate, anthropometric characteristics, and glycemic profile. At the end of the study, the groups were statistically equal regarding changes in body weight, waist circumference, total body fat, and skeletal muscle mass (12). In the same way, the intake of different macronutrients, as well as sugar, did not change over time between groups (12). Thus, even though participants in the active group had reduced their craving for sweets (12), differences in the amount of sugar intake did not happen (data not shown). Therefore, it is not possible to assume that the improvement found in glycemic profile and homeostasis was related to the effect of active tDCS on BMI, body composition, or the consumption of sweet or sugar-rich foods.

This study also has limitations. Since the study population was classified as normoglycemic, intervention effects on the glycemic profile were more difficult to identify. However, we found that FPG and fasting insulin significantly decreased, and

insulin sensitivity estimated by MISI increased. Therefore, the effects of repetitive tDCS on glycemic homeostasis in subjects with diabetes must be studied.

In conclusion, our results showed a significant improvement of glucose homeostasis after 20 repetitive tDCS sessions over the right DLPFC in individuals with overweight or obesity compared to sham tDCS. These findings show that this therapeutic modality could be a potential strategy to treat insulin resistance or diabetes in people with obesity.

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Data Availability Statment: Some or all datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

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CAPÍTULO III: CONSIDERAÇÕES FINAIS

8 CONSIDERAÇÕES FINAIS

A obesidade é uma doença crônica que exerce alto impacto na saúde pública e no bem-estar e qualidade de vida dos indivíduos afetados. Considerando que sua prevalência vem crescendo de forma exponencial nas últimas décadas, mesmo em países subdesenvolvidos, e que seu tratamento, apesar de multidisciplinar, continua produzindo resultados insatisfatórios, observa-se uma busca contínua por novas intervenções terapêuticas que sejam eficazes, baratas e com poucos efeitos colaterais. Uma nova abordagem, neste sentido, parece emergir do campo das neurociências. Mais especificamente, as técnicas de neuromodulção não-invasivas têm se mostrado eficazes na redução do desejo e do consumo de alimentos específicos através da manipulação do CPFdl por ETCC.

Neste trabalho, nós demonstramos que um protocolo com sessões diárias de ETCC sobre o CPFDL direito, com duração de quarto semanas, quando utilizado de forma conjunta com uma dieta de baixas calorias, é capaz de reduzir o desejo pelo consumo de doces, reduzir os níveis de glicose e insulina de jejum circulantes, bem como os índices de sensibilidade à insulina de indivíduos com sobrepeso ou obesidade. No entanto, apesar de clinicamente relevante, a perda de peso do grupo ETCC ativo não foi estatisticamente diferente da obtida pelo grupo placebo, de modo que não foi possível demonstrar que a ETCC associada à dieta hipocalórica possa otimizar o processo de emagrecimento nestes indivíduos.

O primeiro objetivo desta tese foi descrever o protocolo de pesquisa de forma criteriosa e completa, para que fosse possível avaliar os desfechos de interesse e garantir a segurança e viabilidade da técnica de neuroestimulação, tendo em vista o maior número de sessões propostas em relação à existente na literatura até aquele momento. Para este protocolo, a escolha por estimular o lado direito do CPFdl baseou-se em estudos anteriores que encontraram efeito significativo na redução da ingestão alimentar e do desejo de comer em indivíduos com peso saudável ou com excesso de peso.

O segundo objetivo foi avaliar se este protocolo poderia modificar, melhorando ou piorando, aspectos relacionados ao humor, qualidade do sono, depressão, ansiedade e qualidade de vida. Os resultados não demonstraram alterações significativas nos escores dos questionários de depressão, ansiedade, humor,

qualidade do sono ou qualidade de vida, mesmo após ajustes estatísticos para o uso de medicamentos antidepressivos ou alterações do peso corporal. Sendo assim, foi possível concluir que o uso diário da ETCC combinado a dieta não está associado ao comprometimento da saúde mental ou da qualidade de vida nesta população, podendo ser usada com segurança.

O terceiro objetivo foi avaliar se as sessões diárias de ETCC combinadas a uma dieta hipocalórica poderiam influenciar as escolhas alimentares, através da supressão do desejo de comer alimentos específicos, e se isso poderia levar o indivíduo a perder mais peso do que os indivíduos em dieta, mas que recebiam ETCC falsa (placebo). Ao final do protocolo de quatro semanas foi possível verificar que os indivíduos relatam menor desejo de comer doces; no entanto, esta modificação comportamental não foi traduzida em um menor consumo real de açúcares ou carboidratos ou em maior perda de peso. Sendo assim, estes dados ratificam a perspectiva dessa modalidade terapêutica como uma estratégia potencial para o tratamento de desejos alimentares. É importante destacar, porém, que os indivíduos tratados com ETCC ativa apresentaram uma redução de peso clinicamente relevante em comparação com o grupo placebo, com uma diferença média de 2,2 kg entre os grupos, sugerindo que este protocolo deveria ser repetido, considerando um número maior de indivíduos avaliados.

Por fim, o quarto objetivo desta tese foi avaliar os efeitos deste protocolo sobre parâmetros laboratoriais associados à homeostase da glicose. Para isso, investigamos as alterações da glicose, insulina e albumina glicada de jejum, de glicose e insulina pós-prandiais, e de aspectos relacionados à sua regulação e controle, como índices de resistência, sensibilidade, secreção de insulina e função de células β do pâncreas. Com base nos resultados, foi possível observar que quatro semanas de ETCC ativo, comparado ao placebo, sobre o CPFdl direito melhorou os índices de glicose e de insulina de jejum, bem como o índice de sensibilidade à insulina de Matsuda (MISI) nos participantes com excesso de peso e em dieta hipocalórica. Apesar dos resultados promissores, estudos futuros são necessários para investigar se a ETCC poderia ser utilizada como uma técnica não farmacológica adjuvante à dieta para melhor controle da homeostase glicêmica nestes indivíduos, ou até mesmo, para explorar esta modalidade de intervenção em indivíduos com DM2. Portanto, ensaios clínicos randomizados mais robustos são necessários a fim de confirmar os achados deste estudo para que esta técnica possa ser utilizada na prática clínica.

9 PERSPECTIVAS FUTURAS

Acreditamos que estes resultados poderão contribuir para o desenvolvimento e aprimoramento da pesquisa na área de neuromodulação e obesidade. Portanto, como perspectivas futuras podemos incluir:

- Análise sorológica das concentrações do fator neurotrófico derivado do cérebro (BDNF), para verificar a hipótese de ocorrência de neuroplasticidade em nossa amostra.
- Análises bioquímicas para verificar alterações no peptídeo-C, a fim de estimar a sensibilidade à insulna e a função das células β-pancreáticas, bem como alterações nos hormônios associados à regulação da fome e saciedade, como grelina, PYY, leptina e GLP-1.
- Análise do consumo de padrões alimentares entre os grupos ao longo do tempo, por meio dos registros alimentares de 3 dias, focados no consumo de alimentos hiperpalatáveis versus *in natura* ou minimamente processados, com o objetivo de verificar se as escolhas alimentares dos participantes foram influenciadas pela redução do desejo de comer doces relatadas pelo grupo ativo.
- Implementar novos estudos nesta área, incluindo um maior número de indivíduos, pertencentes à classificação de obesidade I e II e com a implementação de um déficit calórico mais acentuado (5%).