Weight loss induced by deep Transcranial Magnetic Stimulation in obesity:

a randomised, double-blind, sham-controlled study

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ABSTRACT

Aims: Obesity is a global health emergency. Metabolic and voluntary factors regulate appetite, and dysfunctional dopamine-modulated reward circuits are implicated in pathological eating behaviour. Deep Transcranial Magnetic Stimulation (dTMS) is already used to modulate cerebral dopamine activation in neuro-psychiatric disorders. We hypothesized that dTMS reduces food craving and causes weight loss via neuromodulation.

Materials and methods: This pilot study was designed as a randomised, double-blind, sham-controlled study. A total of 33 obese patients (9 males, 24 females, age 48.1±10.6 yrs, BMI 36.9±4.7 Kg/m^2) were randomised and completed the study: 13 obese subjects underwent a 5-week treatment with high-frequency (HF) dTMS (18 Hz), 10 were treated with low-frequency (LF) dTMS (1 Hz), and 10 were Sham-treated. Food craving, metabolic and neuro-endocrine parameters were evaluated at baseline, after the 5-week treatment, and at follow-up visits (1 month, 6 months, 1 year after the end of treatment).

Results: The mixed-model analysis for repeated measures showed a significant interaction of time and groups on body weight (p=0.001) and BMI (p=0.001), with a significant body weight (-7.83±2.28 Kg, p=0.0009) and BMI (-2.83±0.83, p=0.0009) decrease in HF *vs* sham. A decreasing trend in food craving in HF *vs* LF and Sham (p=0.073) was observed. A significant improvement of metabolic and physical activity parameters was found (p<0.05) in HF.

Conclusions: We demonstrated the safety and efficacy of dTMS, in addition to physical exercise and hypocaloric diet, in reducing body weight up to 1 year period in obesity. As a possible mechanism of the HF dTMS, we hypothesize a modulation of the dopaminergic pathway and stimulation of physical activity.

Trial registration number: The trial is registered with ClinicalTrials.gov, number NCT03009695.

INTRODUCTION

Obesity has reached epidemic proportions becoming a global health concern (1,2). State-of-the-art approaches to treat obesity include life-style interventions (diet and physical activity programs) supported by psychological and behavioural interventions to overcome the clinical problems of obese people to undergo dietary and exercise programs (e.g. the yo-yo effect) (3). In order to ameliorate patients' compliance, pharmacological treatment is considered part of a comprehensive strategy of obesity management (3). Currently, bariatric surgery represents the most effective treatment for morbid obesity in terms of long-term weight loss, nevertheless it is considered a major surgical intervention with significant risks of perioperative mortality (4). In addition, the emergence or re-emergence after bariatric surgery of a binge eating disorder, and the loss of eating control, result in reduced weight loss and/or increased weight regain (5).

Obesity is a heterogeneous condition not classified as an eating disorder, but which may be both a risk factor for, and a consequence of the latter. Considering several common behavioural and neurobiological mechanisms, there is increasing interest in the conceptualization of disordered eating as craving for food. Several brain regions appear to be involved in the mechanisms of food craving. Neuroimaging studies in obesity showed a consistent lower postprandial activation in the dorsolateral prefrontal cortex (DLPFC), a sub-region of the prefrontal cortex (PFC). This suggests a dysfunctional inhibitory control and decision-making ability over food consumption (6), implicating this brain region as a potential target for intervention in obesity.

Altered activities in the reward circuitry, similar to drug addiction, have also been reported in obese subjects (7). Several studies suggest that eating palatable food increases activation in reward regions and causes dopamine (DA) release in the dorsal striatum (8). On the other hand, a

reduced striatal DA D2 receptor availability (9), and an inferior striatal responsivity to the taste of high-calorie beverages (10) was observed in obese adults compared to lean adults. This led to the hypothesis that obese subjects have a lower sensitivity of DA-based regions and seek overeating to compensate for this deficiency (9).

A complex and highly coordinated system of peripheral appetite hormones and centrally mediated neuronal regulation is also involved in body weight homeostasis (11). Peptide hormones (e.g. leptin, ghrelin, insulin) act in the central nervous system by affecting brain pathways that regulate food intake. Specific peptide hormones receptors (e.g. leptin and insulin receptors) are expressed on dopaminergic neurons both in brain regions regulating "homeostatic hunger" (e.g. hypothalamus), and in the reward areas linked to "hedonic hunger" (e.g. substantia nigra, VTA), releasing signals to cortical, limbic, and striatal regions, involved in motivational and behavioural responses to the rewarding food stimuli (12).

A methodology that was proven to be effective in inducing long lasting changes in cortical excitability and DA release is repetitive Transcranial Magnetic Stimulation (rTMS) (13), a novel, non-invasive technique, based on the principle of electromagnetic induction (14). When applied at a low frequency (≤1Hz), TMS suppresses cortical excitability, while high-frequency TMS (≥5Hz) enhances cortical excitability (13). Repetitive TMS has been found to have therapeutic benefits for several neuropsychiatric disorders, and recently, has been proposed as a potential treatment in addiction disorders (15-17). To stimulate deep brain regions, Zangen et al developed the H-coil (18). Compared to conventional coils, H-coil contains an array of elements which are contoured to the shape of the skull, allowing deeper (up to 4.5-5.5 cm from the skull *vs* 1.5 cm of the standard coils) and larger volumes of brain stimulation, affecting both cortical and subcortical regions. Promising results have been obtained by the application of deep TMS (dTMS) in reducing nicotine dependence (19), alcohol craving (20,21) and cocaine abuse (22). Deep-TMS (H coil) can generate

an electric field which can penetrate the cortex up to 4 cm, noticeably increasing the penetration depth of the traditional TMS systems (23).

Consistent with the dysregulation of the PFC inhibitory control and brain reward system in obese subjects and with the dTMS modulatory effect on the reward system, we hypothesized a potential role of repetitive dTMS in reducing food craving. The present pilot study was designed to investigate the safety and the efficacy of a 5-week treatment with dTMS in reducing food craving and body weight in obese subjects, comparing high frequency (18 Hz) with low frequency (1 Hz) stimulation, and with Sham (primary outcome). Secondary outcomes taken into account were: 1) to identify chronic modifications of neuro-endocrine pathways related to food craving in response to dTMS; 2) to investigate chronic effects of dTMS treatment on metabolic parameters and body energy homeostasis.

MATERIALS AND METHODS

Trial design

Study setting

This study was performed at the Endocrinology and Metabolic Diseases Division, IRCCS Policlinico San Donato, San Donato Milanese (MI), Italy.

A double-blind, sham-controlled, randomised clinical trial was designed to investigate the effects of a 5-weeks treatment with dTMS in reducing food craving and body weight in obese subjects, comparing high frequency (HF) with low frequency (LF) stimulation and with Sham. Additionally, we also explored the chronic effects on neuro-endocrine pathways related to appetite/satiety balance, metabolic parameters, and body energy homeostasis.

Randomisation and masking

Patients fulfilling all inclusion/exclusion criteria were randomised to one of three experimental groups. Deep TMS stimulation conditions could either be HF (18 Hz group), LF (1 Hz group) or Sham (sham group). The range of stimulatory (18 Hz) and inhibitory (1 Hz) frequencies were based on previous literature evidence in addiction disorders (19,22). Patients were randomised in a 1:1:1 allocation ratio. The study design is shown in the flow chart (Figure 1). Allocation in the three groups was performed according to a randomisation sequence generated by a computerized program. The randomisation code was only given to the treating investigator at the first treatment session by an independent investigator not involved with any other aspect of the trial. The independent investigator could be contacted at any time to unblind the randomization code in the case of serious adverse events. Participants and other investigators were unaware of the type of treatment assignment. The magnetic stimulation coil for active and sham treatments (dTMS sessions) was the same. Magnetic cards encoding for real or sham stimulation were used to activate the dTMS device or not, according to the randomization sequence. Both real and sham stimulation produced identical sounds and scalp sensations during the sessions.

Study approval

This study was conducted in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments; it received approval by the local Institutional Review Board (Ethics Committee of San Raffaele Hospital, Milan, Italy). All participants provided written informed consent before participating in any study procedures.

Study participants

Adult men and women (aged 22 to 65 years, inclusive), who referred to the Endocrinology and Metabolic Diseases outpatient clinic for overweight/obesity treatment from January 2017 until July 2017, were screened with a short interview to determine eligibility. Patient recruitment strategy

involved only direct interviews. No paper or web advertisement were utilized. Inclusion and exclusion criteria are shown in Table 1.

Intervention

Each patient received a total of 15 treatments, 3 times per week in 5 weeks (visit 1-15). Prior to stimulation, the enrolled obese subjects were either shown a series of palatable food images (*cue*) or not (*no cue).* Patients were not administered any drugs or psychological or psychiatric therapy during the study period (1 year duration). Deep TMS was the only treatment allowed. Participants could discontinue the study treatment for not more than three not consecutive dTMS sessions for a valid reason.

Follow-up visits were planned 1 month (FU1), 6 months (FU2), and 1 year (FU3) after the end of the treatment.

Repetitive Deep Transcranial Stimulation procedure (dTMS)

The dTMS was performed by a trained physician using a Magstim Rapid^{2}TMS (The Magstim Co. Ltd., Whitland, Carmarthenshire, United Kingdom) stimulator equipped with an H-shaped coil (H-ADD), specifically designed to bilaterally stimulate the PFC and the insula (18,24). This H-coil allows direct stimulation of deeper brain regions like insula (3 cm *vs* 1.5 cm from the skull). Details of stimulation procedure are reported in Supplementary Material.

Diet and Lifestyle Recommendations

During the entire study, all subjects were prescribed a hypocaloric diet. Details of the diet prescription are provided in Supplementary Material. Furthermore, the subjects were also instructed to have a moderate-intensity physical activity (e.g., 30 min walking every day) during the study.

Measurements

Evaluation of food craving

The Food Craving Questionnaire-Trait (FCQ-T), a self-report inventory, was used to assess food craving (25). It is a multidimensional questionnaire consisting of 39 items selected from the literature on addiction and eating disorders. The total score was considered for evaluation in this study. FCQ-T was administered at baseline, at the end of the 5-week treatment, and at FU1, FU2, and FU3 visits.

Anthropometric values and blood pressure

Anthropometric measurements were recorded at baseline, at the last dTMS session (visit 15), and at FU1, FU2, and FU3. They included: body weight and height, to calculate BMI (kg/m^2).

Systolic and diastolic blood pressure (SBP and DBP) were measured at each dTMS session, and at the FU visits.

Resting Energy Expenditure (REE) and Respiratory Quotient (RQ)

Metabolism analysis was performed by measuring the REE and the RQ with indirect calorimetry (26,27). Indirect calorimetry was performed at baseline visit, at visit 15, and at FU2 visit.

Details of indirect calorimetry procedure are reported in the Supplementary Material.

Activity Energy Expenditure (AEE)

During the entire 5-week treatment period, participants underwent an evaluation of Activity Energy Expenditure (AEE) through Actigraph technology (25). Physical activity was recorded with accelerometers during the initial 5 week-period. During the additional follow-up, it was monitored via phone calls. Details of Actigraph technology are provided into the Supplementary Material.

Laboratory measurements

Blood tests were carried out at the first and last dTMS sessions, and at FU1, FU2, FU3 visits. The metabolites assessment included: glucose (mg/dL), glycated haemoglobin (mmol/mol), cholesterol (mg/dL), triglycerides (mg/dL). The hormonal and neuroendocrine markers assessment included: insulin (μU/mL), leptin (ng/mL), total ghrelin (ng/mL), β-endorphins (ng/mL), epinephrine (pg/mL), and norepinephrine (ng/mL). Details of laboratory measurement procedures are reported in the Supplementary Material.

Statistical methods

Descriptive statistics (means, standard deviations, counts, and percentages) were used to describe the study populations. To evaluate the intervention effect on food craving, body weight, and neuro-endocrine parameters related to appetite/satiety balance, repeated-measures regression models (PROC MIXED; SAS Institute) with type of treatment (between-subject factor with 3 levels), time (within-subject factor with 5 levels), and the respective interaction as independent variables, were used. Parameters were reported as least-square means (±SE). Mixed modelling is a useful tool for analysing repeated measures over time, and a main advantage is its ability to retain cases with missing data points. A post-hoc t-test comparing the variation of the investigated variables at 1 year, when the interaction is significant, has been evaluated. Specifically we evaluated if there is a significant difference between treatments when comparing the change from baseline: (FU3 - Baseline) in group HF vs Sham, and in group LF vs Sham. A Bonferroni adjustment for multiple testing was considered as 17 variables were investigated.

The Kolmogorov-Smirnov test was used to check if the sample distributions were normal. Whenever the variables did not meet the normality assumptions, a log transformation was successful in normalizing the data.

Changes in median concentrations (from baseline to 5 weeks of treatment) of physical activity parameters in the three experimental groups were analysed by the non-parametric Kruskal–Wallis test. For body weight and BMI a simple ANOVA to evaluate the difference among groups when considering the change at 1 year to baseline, was performed.

Statistical analysis was done using SAS version 9.4 (SAS Institute, Cary, NC). A two-sided P value ≤ 0.05 was deemed to be statistically significant.

RESULTS

Participant characteristics

Out of the 39 initially randomised patients (15 in HF, 12 in LF, 12 in Sham), 33 subjects completed the study as per protocol. Six patients dropped out from the study and were excluded from the statistical analysis. The mean age of the analysed sample group was 48.1±10.6 years and the mean BMI was 36.9±4.7 kg/m^2. Of the 33 patients, 13 obese subjects were allocated in HF group, 10 in LF group, and 10 in Sham group.

Thirty-one out of the 33 enrolled patients underwent FU1 visit; 24 underwent FU2; 17 underwent FU3 (Figure 1).

Baseline characteristics for the three groups are reported in Table 2. At baseline, no significant differences were observed for the examined parameters between the three groups ($p>0.05$).*Drop-out rate*

Out of the 39 enrolled patients, 6 patients dropped out from the study: 4 patients decided to withdraw from the study for personal reasons other than side effects (1 in HF, 1 in LF, 2 in Sham), 1 patient (in LF) accidentally fell reporting a shoulder fracture, only 1 patient (in HF) discontinued the treatment for a possible treatment side effect (high blood pressure). Dropout patients were excluded from the statistical analysis due to missing outcome data.

Food craving (FCQ)

Figure 2A illustrates the FCQ-T Score levels in the three treatment groups over time throughout the treatment period. The mixed-model analysis revealed a trend towards a significant

interaction between intervention group and time (p=0.073). At one year of follow-up there was a decrease in FCQ-T Score in HF patients (120.7 ±10 at baseline *vs* 81.8 ±12.7, after 1 year).

Body weight and BMI

Mixed-model analyses showed a significant interaction between intervention group and time for body weight and BMI (p=0.001) (Figure 2B, 2C).

At the end of the follow-up, there was a significant decrease in body weight and BMI in HF patients (103.6±4.2 kg at baseline *vs* 94.9±4.4 kg after 1 year, for body weight (difference -2.83, p=0.0009), and 36.8±1.0 at baseline *vs* 33.6±1.4 after 1 year, for BMI) (difference -7.83, p=0.0009).

Cohen's d, evaluated using a one way ANOVA model, was 0.21 (95%CI: 0.00-0.55) for weight and 0.22 (95%CI: 0.00-0.55) for BMI.

No significant differences between *cue/no cue* subgroups was found in both food craving and body weight variations (p>0.05).

Resting Energy Expenditure (REE) and Respiratory Quotient (RQ)

As to the metabolic parameters evaluated by indirect calorimetry (Table 3), the mixed-model analysis revealed a trend towards a significant interaction between intervention group and time on RQ (p=0.061) but no interaction was shown in REE (p=0.279).

Blood pressure

No significant differences over time between treatment groups were found in SBP (p=0.236). The mixed-model analysis revealed a trend interaction between intervention group and time in DBP (p=0.079) (Table 3).

Activity energy expenditure (AEE)

Out of the 33 enrolled patients, 28 (12 in HF, 8 in LF, 8 in Sham) underwent an evaluation of the AEE during the 5 weeks of treatment. A significant increase in AEE was found in HF compared to other groups (p=0.049). Consequently, in the same group, a trend to increase in TEE was observed (p=0.078). After 5 weeks of treatment, a trend to increase was observed in HF for: METs, steps, and travelled kilometres (Supplemental TableS1 and FigureS1).

Metabolic and neuro-endocrine assessments

Chronic variations of laboratory measurements are presented in Table 3.

As to neuroendocrine markers , a significant effect of interaction between intervention group and time on logarithmised leptin (Figure 2D) (p=0.002) and on logarithmised epinephrine (Supplemental FigureS2) (p=0.004) was found with a significant change in HF vs sham (difference=-1.11, p=0.0014 and 1.44, p=0.0020, respectively). The mixed-model analysis revealed a trend effect of time by group interaction on β-endorphin (p=0.078) (Supplemental FigureS3).

As to metabolic parameters, the same analysis revealed a borderline significant time by group interaction on logarithmised glycated haemoglobin (p=0.007) (Supplemental FigureS4).

Adverse events and safety

No serious or severe side effects were observed. Obese subjects who received HF dTMS experienced more frequent headaches (6/13) than LF (4/10) and Sham groups (3/10). This side effect resolved spontaneously within 1-2 days from the beginning of treatment. There were no significant differences among the groups in the frequency and intensity of other adverse events: drowsiness (HF: 2/13, LF: 4/10, Sham: 1/10), neck pain (HF: 2/13, LF: 1/10, Sham: 2/10), temporary hypertension (HF: 1/13, LF: 1/10, Sham: 1/10). Only 1 patient enrolled in HF discontinued the treatment for high blood pressure.

DISCUSSION

This is the first clinical pilot study utilizing dTMS in obesity that demonstrates a decrease in body weight with an indication for a long-lasting weight control effect (up to 1 year). This effect occurred in obese patients receiving a total or 15 sessions over 5 weeks of HF stimulation. Several mechanisms could be involved in the pronounced weight lowering effects produced by the HF stimulation.

The first mechanism is an effect on the PFC, which is centrally implicated in inhibitory control processes and linked to self-control in the dietary context. In fact, an impaired activation of PFC, specifically of the left DLPFC, has been reported in response to a meal in obese individuals (29). This suggests a weakened ability to control feeding behaviour. Our findings build on previous evidence that excitatory stimulation of DLPFC via rTMS enhances its inhibitory capacity and thereby, alters habits in both substance and food addicted subjects (29). Currently, excitatory rTMS, targeting the left DLPFC, has been found to be effective in reliably reducing food cravings in single format (30,31). However, changes in food intake have been inconsistent with a single session of rTMS. Application of multi-session rTMS to eating disorders has also yielded promising but ultimately controversial results, especially in relation to bulimia nervosa and binge eating disorder (32). In our study neuromodulation was specifically addressed to subjects with obesity for the first time, and was performed by dTMS (H coil), increasing the penetration depth of the traditional TMS systems used in the previous studies (23).

In fact, an additional pathway potentially explaining the therapeutic effect of HF dTMS in obesity is the modulation of the cortico-mesolimbic dopamine system, or "reward system", which is implicated in the regulation of hedonic eating behaviour (33). In fact, dopamine signalling is involved in the "wanting" or desire for certain types of food, which underlies food craving (34).

Considering its prominent role in interpreting internal and external cues, the insula is increasingly recognized as being a critical neural substrate for both drug and food addiction by mediating cue reactivity and processes related to decision-making (35). Therefore, modulating the insular cortex function was considered a novel therapeutic strategy to treat addiction. This was made possible by the advent of the H-coil, targeting deeper brain structures which were not previously feasible. A previous study targeting dTMS to the PFC and insula bilaterally demonstrated the efficacy of HF stimulation in reducing nicotine addiction (19). The activation of deeper brain regions can take place directly or indirectly, namely mediated by activation of DLPFC.

The number of human studies using rTMS to manipulate craving is growing (36). In our study, an enduring decreasing trend in food craving was observed in HF compared to other two groups, although the interaction effect between time and treatment was found more pronounced on body weight. Food craving is influenced not only by the intended experimental intervention, but also by environmental context, individual's psychological state (including mood, focus of attention, and expectancy), and treatment-seeking status (37). Furthermore, food craving evaluation was performed by using a self-administered questionnaire; although FCQ-T is considered a reliable tool to detect food craving, like almost all self-reported questionnaires, is a subjective measurement and could be influenced by several variables.

A more pronounced decrease of leptin levels was found in HF compared with other two groups. Leptin is a peptide hormone produced by adipocytes in proportion to their triglyceride content; leptin plasma levels link changes in fat stores to adaptive responses in the central control of energy balance, and correlate with adipose tissue amount. Leptin receptors are expressed on

dopaminergic neurons both in brain regions regulating "homeostatic hunger" (e.g. hypothalamus), and in areas of the reward network linked to "hedonic hunger" (e.g. substantia nigra, VTA). A neuroimaging study highlighted that higher plasma leptin levels correlate with hyper-responsiveness of reward brain areas to high-calorie food cues in obesity, suggesting that dysfunctional leptin signalling may lead to overconsumption of these foods (12). A potential relationship between plasma leptin concentrations and craving was recently reported also in cocaine-addicted subjects (38). The leptin reduction observed in HF indicates that dTMS could exert control on food craving also via the modulation of neuro-endocrine pathways.

In addition to dopamine, endogenous opioid compounds are also involved in the "reward system", mainly in the pleasurable feeling ("liking") associated with the food rewarding stimuli (30). Particularly, the β-endorphins secreted by the pro-opiomelanocortin (POMC) neurons in the hypothalamic arcuate nucleus, inhibit further POMC activation, leading to a decreased appetite and increased energy expenditure (33). In this study, a trend toward a significant interaction effect was found between time and treatment on β-endorphins level. The increase of β-endorphins during the one-year treatment with HF TMS are in line with our recent work, demonstrating that HF TMS acutely increases β-endorphins (39).

Finally, we found a significant and enduring effect of HF dTMS in increasing epinephrine. Few studies investigated the modulation produced by rTMS on the sympathetic nervous system. In animal behavioural models of depression, rTMS of the brain was found to significantly up-regulate β-adrenergic receptors in the frontal cortex, after only 10 days of treatment (40), suggesting a possible involvement of the adrenergic system in the mechanisms of action of the dTMS. A dysregulation of the autonomic nervous system also plays a role in the pathophysiology of obesity, being involved in the modulation of the appetite/satiety signal and energy expenditure (41). On one hand, the observed increase of epinephrine after 5 weeks of HF dTMS in obese patients could affect the food craving in obesity, although the underlying mechanisms of action need to be clarified. On

the other hand, the stimulation of the sympathetic system plays a role in increasing physical activity. Physical Activity is normally defective in obese subjects. Nonetheless a life-style intervention beyond dietary counselling is mandatory to achieve and maintain weight reduction. Voluntary contractions of skeletal muscle fibres are regulated by effective cortical areas, including motor areas and PFC. Sympathetic activation increases frequency, intensity and strength of skeletal muscle contractile activity (42). Intracerebral administration of DA agonists (43), or of DA antagonists (44) respectively activates and inhibits locomotor activity in rats. More recently, Beeler et al demonstrated in the D2R knockdown mouse model that low DA D2 receptor increases vulnerability to obesity via reduced physical activity rather than via increased appetitive motivation (45). Our present data demonstrate that HF dTMS increases locomotor activity over a 5-week period. Since several reports suggested that HF TMS increases the concentration of endogenous DA in the striatum (34), and in the Broadmann area 11 of the medial orbitofrontal cortex (45), it is conceivable that at least part of the weight-lowering effect of our treatment is related to direct activation of locomotor activity in the obese subjects. In addition, the observed increase of epinephrine after 5 weeks of HF dTMS suggests a role of stimulation-induced sympathetic system activation in increasing physical activity.

The reduction of glycosylated haemoglobin level during the one-year treatment in the HF group is rather interesting. At present, the most likely explanation for HbA1c reduction is related to the decrease of body weight. Future studies are needed to directly assess a potential role of TMS in the treatment of type 2 diabetes

Concerning the study limitations, the limited number of obese subjects enrolled did not allow us to adjust the analysis for possible confounding factors, for example for the cue/no-cue information. Another issue of this study is the loss at follow-up: the missing outcome data did not allow us to perform a proper Intention-To-Treat (ITT) analysis. Although we reached statistical

significance for the main outcomes, the effect size, when calculated for this study population, is small to moderate. Future multi-center studies are needed to confirm the findings of our pilot study.

In conclusion, this study indicates that HF dTMS over the lateral PFC and insula reduces body weight with significant and long-lasting effects via several mechanisms. It is conceivable that the main mechanism is the increased dopaminergic activity in the mesolimbic and mesostriatal pathways. Our data suggest a potential role for β-endorphins and epinephrine increase during high frequency TMS treatment to be additional mechanisms. Reduction of body weight is obtained via both a decrease of craving for food and an increase of physical activity. Future studies should determine whether this promising technique, associated with life style intervention, may become an established obesity treatment, alone or in combination with weight-lowering drugs or after failure of bariatric surgery.

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LL, AF, and IT contributed to designing the research study. LL, AF, and CM conducted experiments; specifically, LL provided research conduct oversight; AF contributed to performing dTMS after a specific training, and to providing medical supervision; CM and SM contributed to performing instrumental tests (indirect calorimetry, body plethysmography, accelerometer) and collecting blood samples. AF and MA contributed to acquiring data; FA and VM performed statistical analysis. AF, LL, FA and VM contributed to writing and editing the manuscript. LL confirms that he had full access to all the data in the study and has final responsibility for the decision to submit for publication.

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The authors have declared that no conflict of interest exists.

Prior Presentation

The preliminary results of this study were presented as an oral presentation at the 53rd European Association for the Study of Diabetes (EASD) Annual Meeting, Lisbon, Portugal, 11 – 15 September 2017.

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Legend for figures

Figure 1. The CONSORT diagram shows the flow of patients through each stage of the randomized, controlled trial.

Figure 2. Effects of dTMS on food craving, body weight, BMI, and leptin levels in the three groups during the treatment and follow-up period.

(A) The panel illustrates the food craving, evaluated by Food Cravings Questionnaire-Trait, in the three treatment groups over time throughout the treatment and follow-up period. A trend interaction between intervention group and time on food craving was revealed by the mixed-model analysis (p=0.073).

(B) (C) The panels illustrate, respectively, the body weight and BMI in the three treatment groups over time throughout the treatment and follow-up period. At baseline, no significant differences were observed between the three groups for both body weight and BMI (p>0.05). Mixed-model analyses showed a significant interaction between intervention group and time for body weight and BMI (p=0.001).

(D) The panel illustrates the leptin levels in the three treatment groups over time throughout the treatment and follow-up period. Mixed-model analysis showed a significant interaction between intervention group and time for leptin (p=0.002). Leptin is expressed as logarithmised values.

✚ The figure shows if there is a significant difference between groups when comparing the change from baseline: (FU3-baseline) in group HF vs Sham, (FU3-baseline) in group LF vs Sham.

Abbreviations: FCQ-T=Food Cravings Questionnaire-Trait; WK5=Week 5; FU=Follow-Up.

Table 1. Inclusion and Exclusion Criteria of participants.

Inclusion Criteria	Exclusion Criteria
Age 22-65 years	Personal or a family history of seizures
BMI 30-45 Kg/m^2	Psychotic and/or organic brain disorders
Willingness to reduce body weight	Implanted metal devices
	Fasting blood glucose level >150 mg/dl
	Abuse of substances other than nicotine
	Weight variation (>3%) within three months prior the screening visit
	Current or recent (within 6 months prior the screening visit) treatment with anti-obesity medications or other medications for weight reduction
	Medications associated with lowered seizure threshold
	Type 1 diabetes or insulin-treated type 2 diabetes

Abbreviations: BMI=Body Mass Index.

Table 2. Baseline characteristics of participants according to the study group.

	HF	LF	Sham
	n=13	n=10	n=10
Female sex, n (%)	8 (33.33%)	7 (29.17%)	9 (37.50%)
Age (years)	47.46±10.13	46.50±11.73	50.60±10.52
FCQ-T score	120.69±38.05	115.50±44.99	106.7±32.23
BMI	36.78±5.24	37.51±5.92	36.33±2.12
Body weight (kg)	103.61±17.21	102.61±17.35	97.38±8.19
SBP (mmHg)	122.55±13.34	118.33±19.36	124.00±8.43
DBP (mmHg)	80.33±8.21	75.00±15.00	76.50±11.07
Glucose (mg/dL)	95 [77-110]	87 [82-119]	82.5 [81-88]
Insulin (µU/mL)	17.18 [10.47-40.39]	22.81 [13.66-30.35]	13.07 [9.48-13.33]
Glycated haemoglobin (mmol/mol)	37 [34-40.50]	37 [33-38]	32.5 [32-34.5]
Triglycerides (mg/dL)	124 [92-184]	155 [110-281]	108.5 [94-136]
Cholesterol (mg/dL)	203.62±37.99	205.9±56.57	191.60±18.36
Leptin (ng/mL)	72.49 [45.49-87.52]	72.44 [20.9-127.59]	59.47 [22.3-77.95]
Epinephrine (pg/mL)	525.8 [129.33-888.90]	740.23 [600.36-927.68]	498.2 [206.4-897.9]
Ghrelin (ng/mL)	6.5 [4.76-10.37]	10.65 [9.10-19]	5.68 [3.4-9.66]
Norepinephrine (ng/mL)	4.24±1.72	4.81±2.55	3.40±2.44
β-Endorphin (ng/mL)	0.45 [0.33-0.52]	0.48 [0.34-0.59]	0.48 [0.43-0.52]
RQ	0.88±0.05	0.88±0.06	0.86±0.06
REE	0.96±0.13	0.93±0.12	0.92±0.12
TEE (Kcal/day)	2006.00 [1865.50-2315.00]	2107.50 [1946.00-2241.00]	1890.50 [1778.00-1921.00]
AEE (Kcal/day)	273.50 [153.00-284.00]	224.50 [169.00-298.00]	242.50 [156.00-315.50]
METs	1.55 [1.40-1.75]	1.45 [1.30-1.75]	1.60 [1.45-1.85]
Steps (steps/day)	6743.50 [3543.50-7547.50]	5013.50 [3829.00-8070.50]	6004.50 [3934.00-8055.50]
Distance (Km/day)	4.25 [2.55-4.95]	3.60 [2.70-5.35]	4.25 [2.65-5.40]

Table 2. Data are mean ± SD or number (%) or median [Q1 – Q3].

Abbreviations: HF=High Frequency; LF=Low Frequency; SD=Standard Deviation; FCQ-T=Food Cravings Questionnaire-Trait; BMI=Body Mass Index; SBP=Systolic Blood Pressure; DBP=Diastolic Blood Pressure; RQ=Respiratory Quotient; REE=Resting Energy Expenditure; TEE=Total Energy Expenditure; AEE=Activity Energy Expenditure; METs=Metabolic Equivalent of Tasks; SD=Standard Deviation.

BMI	Baseline	WK5	FU1	FU2	FU3	p-value time*group	post-hoc	STD effect
HF	36.78±1.32	35.23±1.32	34.51±1.33	34.98±1.34	33.58±1.39		-2.83±0.83 p=0.0009	-3.41
LF	37.51±1.51	36.71±1.51	36.32±1.51	37.51±1.53	37.99±1.53	**0.001(*)**	0.85±0.79 p=0.2862	1.08
Sham	36.33±1.51	35.95±1.51	35.78±1.51	36.07±1.53	35.97±1.55		REF	

FCQ-T Score	Baseline	WK5	FU1	FU2	FU3	p-value time*group	post-hoc	STD effect
HF	120.69±9.99	64.86±10.87	65.71±10.17	78.89±10.61	81.83±12.72		-26.32±15.97	-1.65
LF	115.5±11.39	71.36±11.71	71.25±11.71	92.75±12.85	106.5±12.39	**0.073**	3.54±15.34	0.23
Sham	106.7±11.39	74.83±11.71	87.61±11.71	104.45±12.87	94.16±13.49		REF	

Body weight	Baseline	WK5	FU1	FU2	FU3	p-value time*group	post-hoc	STD effect
HF	103.60±4.23	99.26±4.23	97.22±4.24	98.59±4.26	94.89±4.38		-7.83±2.28 p=0.0009	-3.43
LF	102.61±4.82	100.40±4.82	99.32±4.83	102.51±4.87	103.92±4.87	**0.001(*)**	2.20±2.19 p=0.3152	1.00
Sham	97.38±4.82	96.40±4.82	95.97±4.82	96.86±4.87	96.49±4.93		REF	

Glucose §	Baseline	WK5	FU1	FU2	FU3	p-value time*group	post-hoc	STD effect
HF	4.53±0.05	4.51±0.05	4.52±0.05	4.52±0.06	4.56±0.06		0.04±0.07	0.57
LF	4.61±0.06	4.53±0.06	4.61±0.06	4.55±0.06	4.67±0.06	**0.745**	0.06±0.07	0.86
Sham	4.47±0.06	4.45±0.06	4.46±0.06	4.44±0.07	4.47±0.07		REF	

Insulin §	Baseline	WK5	FU1	FU2	FU3	p-value time*group	post-hoc	STD effect
HF	2.90±0.20	2.51±0.20	2.61±0.20	2.78±0.21	3.08±0.24		0.43±0.28	1.54
LF	3.12±0.22	2.79±0.22	2.82±0.23	2.98±0.24	3.10±0.24	**0.761**	0.24±0.27	0.88
Sham	2.70±0.23	2.46±0.22	2.47±0.23	2.43±0.26	2.45±0.26		REF	

Glycated haemoglobin §	Baseline	WK5	FU1	FU2	FU3	p-value time*group	post-hoc	STD effect
HF	3.64±0.05	3.57±0.05	3.54±0.05	3.56±0.05	3.55±0.05		-0.03±0.06	-0.05
LF	3.58±0.06	3.59±0.06	3.59±0.06	3.59±0.06	3.73±0.06	**0.007**	0.21±0.06	3.5
Sham	3.55±0.05	3.51±0.05	3.54±0.06	3.49±0.07	3.49±0.06		REF	

Triglycerides §	Baseline	WK5	FU1	FU2	FU3	p-value time*group	post-hoc	STD effect
HF	4.89±0.15	4.81±0.15	4.88±0.15	4.95±0.16	4.99±0.19		0.02±0.24	0.08
LF	5.04±0.17	4.80±0.17	4.65±0.17	4.79±0.18	4.98±0.18	**0.572**	-0.13±0.23	-0.56
Sham	4.70±0.17	4.72±0.17	4.68±0.17	4.77±0.19	4.78±0.20		REF	

Cholesterol	Baseline	WK5	FU1	FU2	FU3	p-value time*group	post-hoc	STD effect
HF	203.6±104.2	193.5±104.2	200.8±106.0	214.6±112.7	202.4±131.3		-3.26±16.25	-0.20
LF	205.9±118.8	178.7±118.8	192.4±121.5	193.7±128.4	196.7±128.4	**0.753**	-11.26±15.59	-0.73
Sham	191.6±118.8	176.4±118.8	185.9±121.5	182.3±133.2	193.6±139.5		REF	

ST	Baseline	WK5	FU1	FU2	FU3	p-value time*group	post-hoc	STD effect
HF	122.0±3.74	117.31±3.67	117.22±3.75	123.35±4.71	120.77±5.03		3.78±7.06	0.53
LF	118.07±4.30	112.23±4.30	114.7±4.90	129.55±5.22	127.23±4.90	**0.2359**	14.17±6.97	2.03
Sham	124±4.17	112.53±4.33	116.28±4.68	116.23±5.23	118.99±5.23		REF	

DBP	Baseline	WK5	FU1	FU2	FU3	p-value time*group	post-hoc	STD effect
HF	80.52±2.82	76.15±2.76	78.94±2.82	82.44±3.56	80.35±3.81		-1.85±5.39	-0.34
LF	74.80±3.24	70.38±3.24	71.37±3.70	82.45±3.95	83.96±3.70	**0.0794**	7.48±5.32	1.41
Sham	76.5±3.14	76.63±3.24	77.60±3.52	72.44±3.94	78.18±3.95		REF	

Leptin §	Baseline	WK5	FU1	FU2	FU3	p-value time*group	post-hoc	STD effect
HF	4.13±0.21	3.55±0.21	3.33±0.22	3.73±0.23	2.95±0.28		-1.11±0.33 p=0.0014	-3.36
LF	3.99±0.24	3.78±0.24	3.52±0.24	4.18±0.27	4.30±0.26	**0.002(*)**	0.36±0.31 p=0.2440	1.16
Sham	4.02±0.24	3.86±0.24	3.93±0.25	3.94±0.32	3.96±0.28		REF	

Ghrelin §	Baseline	WK5	FU1	FU2	FU3	p-value time*group	post-hoc	STD effect
HF	1.97±0.24	2.10±0.26	2.28±0.27	2.50±0.28	2.17±0.35		-0.08±0.56	-0.14
LF	2.42±0.26	1.97±0.26	2.44±0.27	1.59±0.33	2.12±0.30	**0.286**	0.57±0.54	1.06
Sham	1.83±0.26	2.03±0.26	2.38±0.27	2.43±0.35	2.10±0.35		REF	

Epinephrine §	Baseline	WK5	FU1	FU2	FU3	p-value time*group	post-hoc	STD effect
HF	5.99±0.29	6.16±0.29	6.44±0.30	6.09±0.32	6.66±0.38		1.44±0.45 p=0.0020	3.2
LF	6.52±0.33	6.37±0.33	6.44±0.33	6.69±0.35	5.37±0.35	**0.004**	-0.37±0.41 p=0.3780	-0.90
Sham	6.14±0.33	6.43±0.33	6.50±0.34	6.23±0.41	5.37±0.38		REF	

Norepinephrine	Baseline	WK5	FU1	FU2	FU3	p-value time*group	post-hoc	STD effect
HF	4.24±0.88	3.44±0.88	3.56±0.93	2.78±1.04	3.02±1.34		-0.64±1.83	-0.35
LF	4.81±1.01	4.20±1.01	5.31±1.04	3.78±1.13	1.72±1.27	**0.178**	-2.51±1.77	-1.42
Sham	3.40±1.01	3.66±1.04	6.91±1.09	4.02±1.39	2.82±1.28		REF	

β-Endorphin §	Baseline	WK5	FU1	FU2	FU3	p-value time*group	post-hoc	STD effect
HF	-0.85±0.13	-0.90±0.13	-0.70±0.13	-0.66±0.15	-0.10±0.18		0.56±0.22	2.54
LF	-0.76±0.15	-0.89±0.15	-0.87±0.15	-0.57±0.16	-0.56±0.16	**0.078**	-0.00±0.20	0.00
Sham	-0.73±0.15	-0.77±0.15	-0.48±0.15	-0.54±0.17	-0.54±0.17		REF	

RQ	Baseline	WK5	FU2	p-value time*group	post-hoc	STD effect
HF	0.88±0.02	0.82±0.02	0.90±0.02		0.01±0.04	0.25
LF	0.88±0.018	0.82±0.02	0.90±0.02	**0.061**	0.01±0.04	0.25
Sham	0.86±0.02	0.88±0.02	0.87±0.02		REF	

REE	Baseline	WK5	FU2	p-value time*group	post-hoc	STD effect
HF	0.96±0.03	0.85±0.03	0.94±0.04		0.02±0.06	0.33
LF	0.93±0.04	0.88±0.04	0.90±0.04	**0.279**	0.12±0.06	2
Sham	0.92±0.04	0.92±0.04	0.89±0.05		REF	

Table 3. A mixed-modelling approach was used to assess the effect of treatment on dependent variable over time [5-week (WK5), 1 month (FU1), 6 months (FU2), and 1 year (FU3)]. Data are expressed with LS means ± SD. P-values for the interaction of time with treatment groups (**time*group**), and for the difference between HF or LF vs Sham after 1 year compared to baseline **(post-hoc)** are reported only when the interaction was statistically significant.
Considering a Bonferroni correction a p-value <0.0029 was deemed to be statistically significant (*).
Standardised mean difference scores for all comparisons are reported **(STD effect)**.

§ log-transformed variable

Abbreviations: HF=High Frequency; LF=Low Frequency; WK5=Week 5; FU=Follow-Up; FCQ-T=Food Cravings Questionnaire-Trait; BMI=Body Mass Index; SBP=Systolic Blood Pressure; DBP=Diastolic Blood Pressure; RQ=Respiratory Quotient; REE=Resting Energy Expenditure.

Figure 1

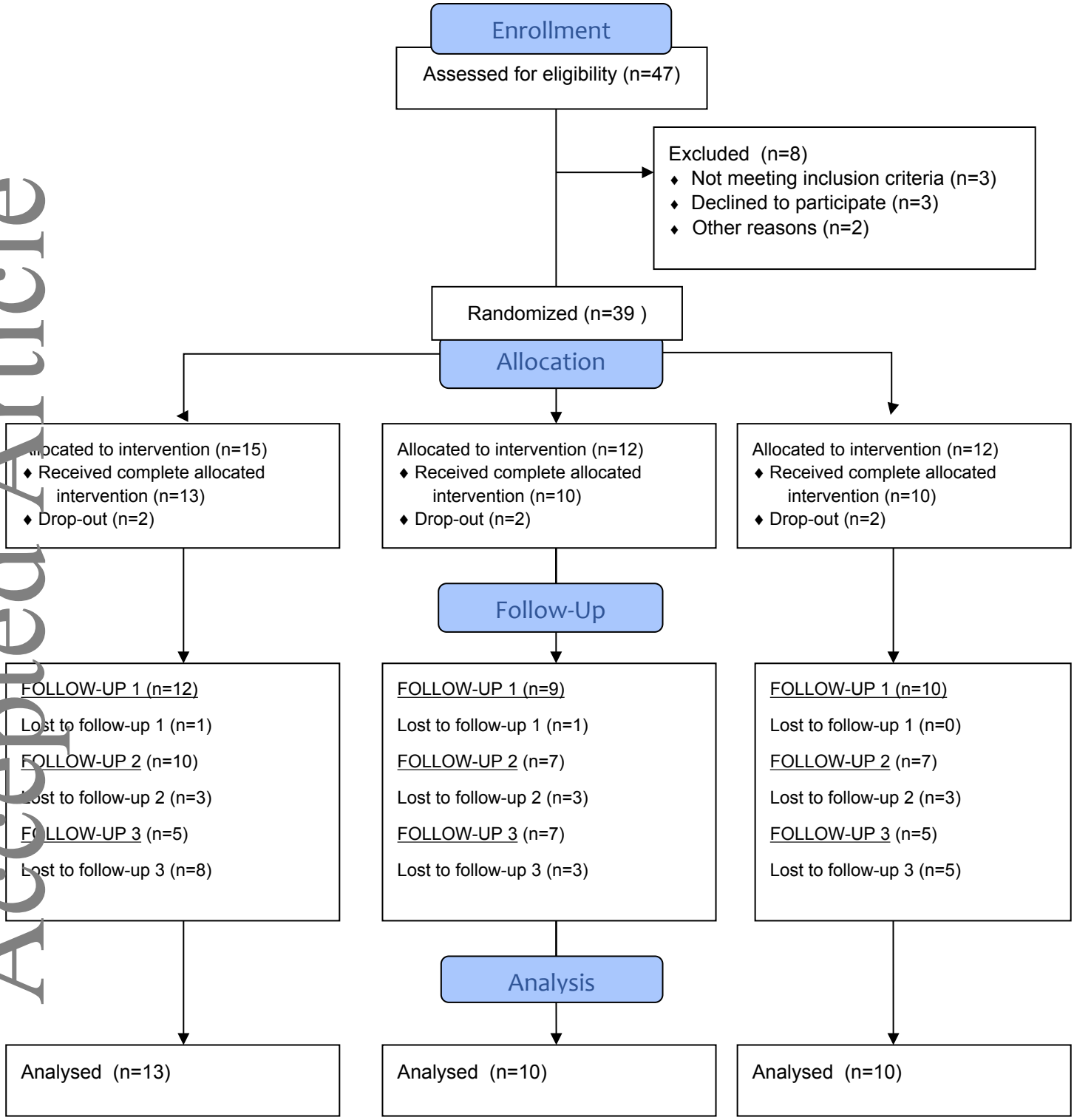
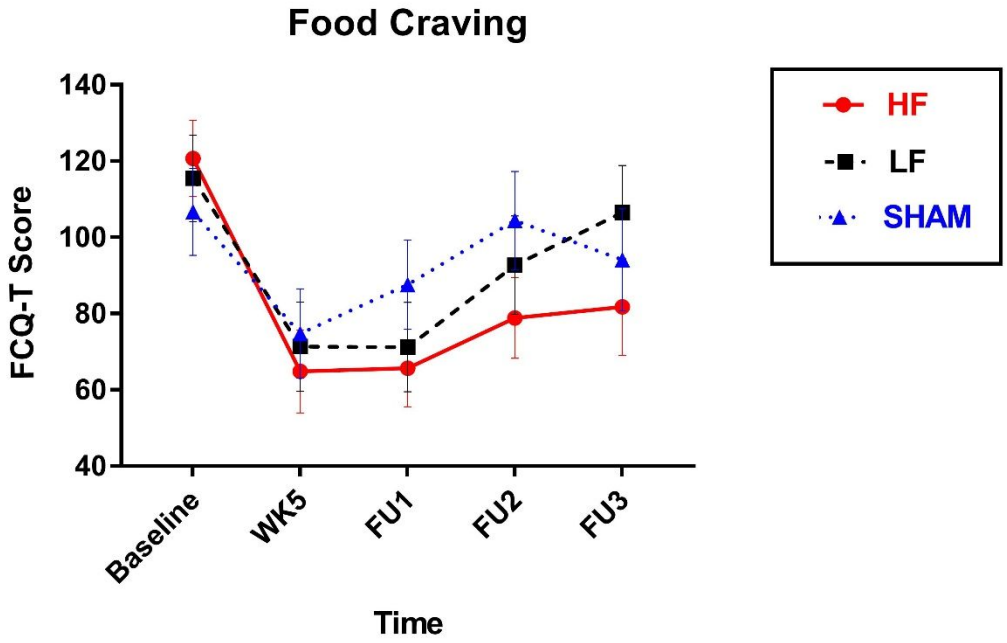
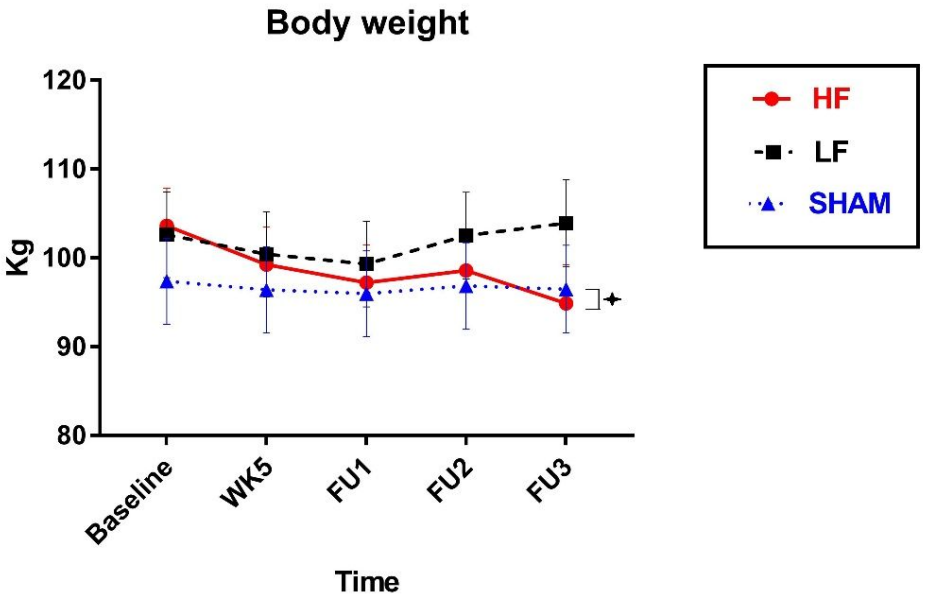


Figure 2A



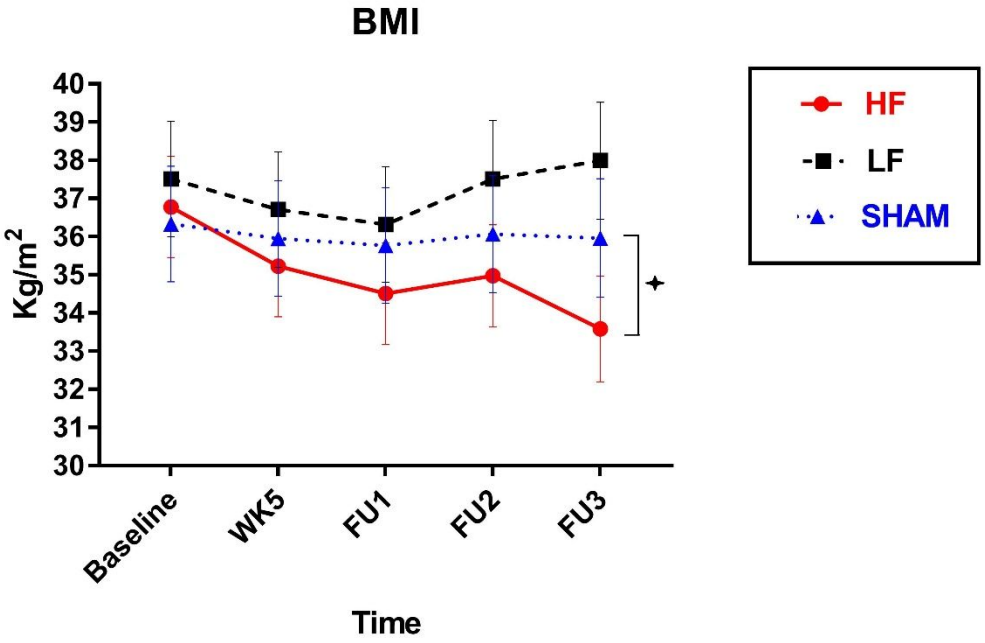
Effect of time within group: p=0.073

Figure 2B



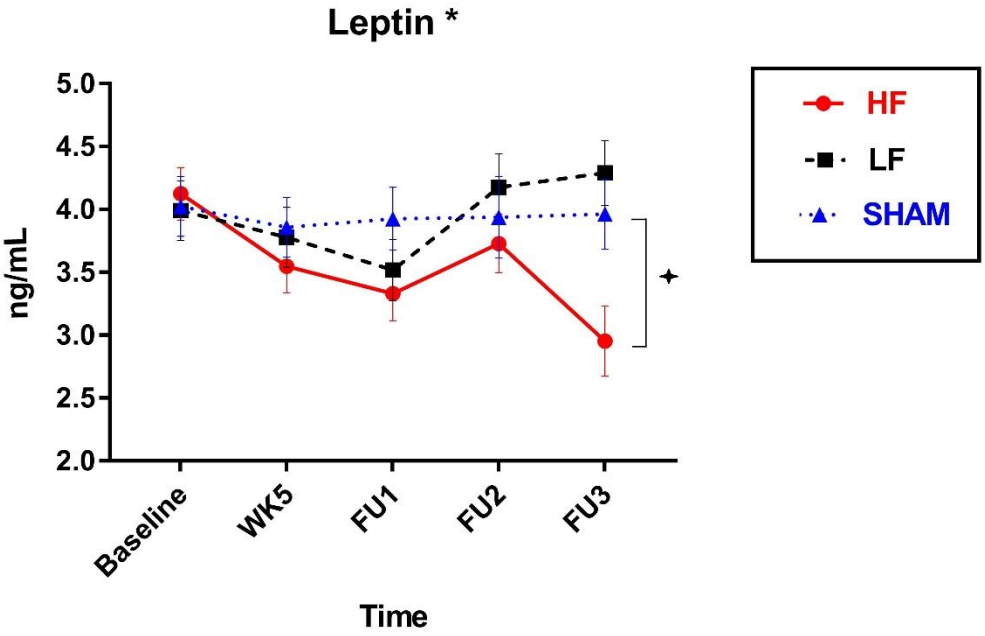
Effect of time within group: p=0.001

Figure 2C



Effect of time within group: p=0.001

Figure 2D



Effect of time within group: p=0.002

* log value