Acute assessment of subjective appetite and implicated hormones after a hypnosis-induced hallucinated meal: a randomized cross-over pilot trial

Visual food hallucination modulates subjective appetite and implicated hormones in highly hypnotizable women: a randomized cross-over pilot trial

Iolanda Cioffi [1, 5], Roberto Gambino [1], Rosalba Rosato [2], Bice Properzi [3], Giuseppe Regaldo [4], Valentina Ponzo[1], Marianna Pellegrini [1], Franco Contaldo [5], Fabrizio Pasanisi [5], Ezio Ghigo [1], Simona Bo [1]

[1] Department of Medical Sciences, University of Turin, Turin, Italy; [2] Department of Psychology, University of Turin, Turin, Italy; [3] Unit of Internal Medicine, Hospital of Turin, Città della Salute e della Scienza, Turin, Italy; [4] Obstetric Department, Hospital of Ciriè, Turin, Italy; [5] Department of Medicine and Surgery, Federico II University Hospital, Naples, Italy.

Corresponding author:

Simona Bo, Department of Medical Sciences, University of Turin, Italy

c.so AM Dogliotti 14, 10126 Turin, Italy

Tel +390116336036

E-mail: simona.bo@unito.it

Acknowledgments

We gratefully acknowledge Emanuele Triberti for his help in running the study.

Author contributions: I.C. and S.B. designed research; R.G., B.P., V.P. and I.C. carried out the research; R.G. analyzed blood sample, R.R. performed statistical analysis; and I.C. and S.B. wrote the paper. F.C., F.P., M.P. and E.G. revised the paper and interpreted the results. I.C. and S.B. had primary responsibility for final content. All authors read and approved the final manuscript.

ClinicalTrials.gov Identifier: NCT03934580.

Abstract

Objective: In this pilot cross-over trial, we compared the effects of visual food hallucination by hypnosis with those of a real meal on subjective appetite and appetite-regulating hormones.

Methods: Eight healthy post-menopausal women were randomized to consume a hallucinated breakfast (HB) or a real breakfast (RB). Participants underwent appetite sensations measurements (each 30-min until 270-min) and blood sample collection (at 20, 60, 90, 180-min). A 3-day food-record was filled after each session.

Results: The repeated measures adjusted ANCOVA did not show any meal×time interactions between-meals on subjective appetite. As expected, significantly higher glucose ($p<0.001$), insulin ($p<0.001$), and lower free fatty acid ($p<0.001$) concentrations were found after the RB. Furthermore, RB significantly increased postprandial levels of glucagon-like-peptide-1 and peptide-YY at 20, 60, 90 and 180-min, whereas acylated-ghrelin and leptin levels did not differ between-meals. Postprandial neuropeptide-Y and orexin-A values significantly increased at different time-points after RB, but not following HB, while α-melanocyte-stimulating hormone levels enhanced after HB only. Energy intakes were significantly lower after HB on the test day only (HB=1146.6±343.8 *vs* RB=1634.7±274.2 kcal/d; $p=0.003$).

Conclusions: Food-hallucination under hypnosis might modulate subjective appetite, by affecting the brain appetite-peptides. Further studies are needed to verify these results in individuals with obesity.

Keywords: Hypnosis; Appetite; Orexin, Neuropeptide-Y; Obesity.

Introduction

A complex network of behavioral and metabolic pathways controls appetite [1]. These regulatory mechanisms are characterized by: 1) short-term or episodic signals, mainly inhibitory and usually generated in response to food ingestion, and 2) long-term or tonic signals, arising from tissue stores, such as adipose tissue, reflecting the state of depletion or repletion of energy reserves [2,3]. Those signals are highly integrated within complex brain mechanisms to control appetite [1].

The ingestion of a meal triggers the release of many gastrointestinal peptides such as cholecystokinin (CCK), peptide YY (PYY), glucagon-like-peptide-1 (GLP-1), with inhibitory actions on food intake. Levels of the orexigenic ghrelin decrease postprandially, in response to the macronutrient composition of meals [4]. Adiposity signals such as leptin and insulin cross the brain blood-barrier, bind to respective receptors on the pro-opiomelanocortin (POMC) neurons and stimulate the release of-α-melanocyte-stimulating hormone (α-MSH) that inhibits food intake [5]. Those peptides suppress the activity of orexigenic neurons expressing neuropeptide Y (NPY) and agouti-related neuropeptide (AgRP) [6], antagonizing the effects of α-MSH on melanocortin-4 receptor (MC4R) through the release of AgRP [5].

Feeding is also influenced by hedonic, reward-related factors, i.e. the motivation to eat determined by the rewarding effects of highly palatable food, controlled by different neural circuits and brain regions [7]. The activation of reward circuit, via dopamine and endocannabinoids increases the expression of hunger peptides like NPY and Orexin-A (OX-A), and blunts the signaling of satiety peptides (insulin, leptin and CCK) [1]. Dysregulation of the hedonic processes could cause food intake beyond needs [8], leading to obesity, which is characterized by the alterations of many homeostatic mechanisms involved in food control, as well as by changes in motivation and reward circuits [8,9].

Hypnosis has been suggested as a potential tool for the management of obesity because it could help in controlling the compulsive and uncontrolled behaviors leading people to eat in less conscious ways [10,11]. Even if the effects of hypnosis on weight loss are controversial [12,13], it has been successfully used to modulate gastrointestinal motility and sensory functions [14–17]. Food imagery stimulated by hypnosis, especially of an appetizing meal, promotes gastric secretion and changes in motility, inducing variation in subjective appetite that are similar to those determined by a real meal [15,18]. Still, it has been described that some individuals, highly hypnotizable, are able to develop visual hypnotic hallucinations, such as food hallucination, showing a compelling experience of the reality of a given perception, even though the perceived stimulus is actually not present [19]. Thus, in this randomized cross-over study, we tested the hypothesis that visual hallucination of a breakfast meal (HB) would affect subjective appetite like eating a real breakfast (RB) in healthy subjects. Furthermore, different peptides involved in appetite control were assessed in the postprandial phase.

Methods

Participants

Participants were selected among attenders to advanced hypnosis courses in Turin, Italy, according to the following inclusion criteria: age<65 years, female gender, high hypnotizability and ability to develop hypnotic visual hallucination, BMI 20-27 kg/m^2, menopausal status (to avoid any interference between appetite and menstrual cycle). Exclusion criteria were: smoking, breakfast skipping, regular intake of any drug/supplement, presence of any pathological conditions, including mental and eating disorders, any alimentary restrictions or specific diets, allergy/food intolerance/dislike of the offered breakfast-meal, being a shift/night worker, unable to give informed consent.

The study protocol complied with the Helsinki Declaration, received the local Ethical Committee approval, and was registered at clinicaltrials.gov (NCT03934580). Informed consent was obtained from all individual participants included in the study.

Study design

Eight postmenopausal women were randomly assigned to the HB or RB test in a crossover design by using a web-based program to build the randomization list (**Figure 1**).

Outcomes

The primary outcome was evaluating changes in subjective appetite after HB when compared to RB. The secondary outcomes were analyzing changes in circulating concentrations of NPY, OX-A, α-MSH, GLP-1, PYY, acylated ghrelin (AG), leptin, insulin, glucose, and free fatty acids (FFA) after both meals.

Intervention

On the day before each test, participants were instructed to avoid alcohol and hard physical activity, had dinner no later than 8:00 p.m. and consumed a diet low in dietary fiber and fermentable food (i.e. avoiding wholegrain bread/pasta, breakfast cereals, kernels and pearled kernels, legumes, potatoes, nuts, fruit, and vegetables). In addition, participants were asked to fill-in a 24h-food record in order to check their compliance to the given recommendations.

On test day, at 8:30 a.m., fasting participants underwent baseline measurements of body weight and height, assessment of subjective appetite by visual analogue scale (VAS), blood sample collection and the insertion of a 16-G indwelling catheter into an antecubital vein of the forearm, subsequently kept patent by the slow infusion of 500 ml of saline solution until the last blood sample collection.

Thereafter, the breakfast meal was served or visually hallucinated. It consisted of 80g white bread, 30g ham and 30g cheese (380 kcal; 20.4 g proteins, 9.8 g fats, 52.5 g carbohydrates, 2.5 g fibers) and 250 ml still water, to be consumed within 15-min. Subjective sensations of hunger, satiety, fullness and propensity to eat were assessed each 30-min for 270-min; blood samples were collected at 20, 60, 90 and 180-min from the end of meal. In order to avoid blood drawing-related stress, all the blood samples were withdrawn from an extension line tubing. Participants remained

seated from the beginning of the test until the last blood sample collection and they could read, talk or listen to music while seated. Then, they could leave the room and move without carrying out exercise for additional 90-min, during which they continued to report every 30-min their subjective feelings of appetite.

Hypnotic condition and procedure

Hypnotic susceptibility is the ability of responsiveness to suggestions for changes in subjective experience and for alterations in perception, sensation, emotion, thought, or behavior [19]. To perform this experimental study, women were screened for high hypnotizability using the Harvard Group Scale of Hypnotic Ability, Form A [20] and the Waterloo-Group C Scale of Hypnotic Susceptibility [21]. Afterward, in those highly hypnotizable, a trained hypnotherapist (GR) assessed the individual capacity to experience vivid hallucinations and each assessment included a variety of visual hypnotically-suggested hallucinations [19].

The achievement of the hypnotic state is easily verified by the hypnotherapist, through the detection of different bodily changes, such as slow heart rate, deep breath and/or muscle relaxation. Going through the hypnotic phenomena, individuals are immersed in a virtual reality, perceiving all feelings and actions as real, without being able to distinguish those from real life and remembering all sensations/actions as lived [19]. Before the hypnotic induction, the real meal was showed to participants to obtain a visual hallucination identical to this meal. Since all subjects were highly hypnotizable, the induction phase was fast. After a few minutes, participants were asked to open their eyes, remaining in a hypnotic state, to see and handle a virtual meal with the recommendation to finish the meal within 15-min. All the participants succeeded in hallucinating their meal, by experiencing all sensory aspects of the eating process, including appearance, aroma and taste of the virtual meal, as reported after chewing and swallowing it. Overall, the hypnotic procedure lasted about 20-min: 1-3-min to go into the hypnotic state, ~15-min to hallucinate and virtually eat the meal and ~2-min to exit from the hypnotic state. Finally, to prevent any confounding factors on appetite sensation, the hypnotherapist was instructed to avoid any adjectives like “delicious or tasty” for describing the virtual meal and not to ask questions about perceived appetite after eating.

Measurements

VAS was used to assess subjective hunger, satiety, fullness, and prospective food consumption before and after the meals. The scales are 100 mm length with words anchored at each end, expressing the most positive and negative rating [22]. All participants completed a 24-h food record on the day before each test and then a 3-day food record starting from the day of each test up to 2 consecutive days. They were instructed to estimate food portions by using household measurement tools. A dietitian reviewed the food diaries and calculated energy and macronutrient intakes by using the WINFOOD database (3.4 version; Medimatica, Teramo, Italy).

Blood specimens, collected in tubes containing EDTA and Pefabloc (SIGMA, Italy) as a protease inhibitor, were immediately centrifuged, and aliquots of plasma were stored at -80°C until the analysis. Serum glucose was measured by enzymatic colorimetric assay (Menarini Diagnostics, Florence, Italy) and serum insulin by immunoradiometric assay (Beckman Coulter, Immunotech, Prague, Czech Republic) with intra-assay coefficients of variation (CVs) ≤3.99% and inter-assay CVs ≤4.8%. FFA concentrations were measured by a fluorometric assay (Sigma-Aldrich, St. Louis, MO, USA). AG was measured by an acetylcholinesterase-labelled antibody-based sandwich enzyme immunoassay (Bertin-Pharma, France) (intra-assay and inter-assay CVs: 2.6-4.8% and 5.0-7.0%, respectively). Leptin was determined by sandwich enzyme-linked immunosorbent assays (BioVendor, Brno, Czech Republic) (intra-and inter-assay CVs: 4.2% and 6.7%, respectively). α-MSH was assayed by competitive enzyme-linked immunosorbent assays (LSBio, Seattle, WA, USA) (intra-and inter-assay CVs: <4.2% and <7.6%, respectively). Serum GLP-1 levels were analysed by sandwich ELISA assay (DRG Instruments GmbH, Marburg, Germany) (intra and interassay CVs: 3.7% and 7.4%, respectively). Serum PYY and NPY levels were determined by sandwich ELISA assay (EMD Millipore Corporation, st. Louis,Missouri, USA) (intra and interassay CVs: 2.3% and 6.2% for PYY, and 3.3% and 7.8% for NPY, respectively). Serum OX-A was analysed by a competitive binding enzyme-linked immunosorbent assay technology (Abbexa Ltd, Cambridge, UK) (intra and interassay CVs 4.6% and 6.9%, respectively).

Blinding

Due to the nature of the intervention, blinding participants and health professionals was not possible. The laboratory personnel who performed the biochemical analyses was blinded to the group assignment.

Sample size and statistical analyses

Based on Flint [22] a difference of 10 mm in subjective appetite by VAS would be detected with $\alpha=0.05$ and a power=80% using 8 subjects in a paired design.

The net area-under-the curve (AUC) values were calculated by the trapezoidal method. All dependent variables were controlled for normal distribution using the Shapiro-Wilk test.

A repeated-measures ANCOVA analysis was used to examine the effect of meal and time and the meal×time interaction term on the postprandial response of subjective appetite, metabolic and hormonal responses, where subject was modelled as a random variable and corresponding baseline value, age, BMI and meal sequence (A/B or B/A) were modelled as covariates. Tukey-adjusted post hoc pairwise comparisons were performed. Data are presented in the text as mean ± standard deviation (SD), while in the figures as mean ± standard error (SE).

The Student's t-test for paired data or the Wilcoxon matched paired test (not-normally distributed variables) were used to investigate within-subject differences of the AUCs after HB and RB (SAS 9.4, SAS Inc., Cary, North Carolina).

Results

Eight women with a mean age of 53.3±2.4 years and an average BMI of 22.5±3.3 kg/m^2 participated and completed the trial. All women complied well with the recommended low-fiber diet, as reported by food records (data not shown).

Appetite scores

Subjective feelings of hunger, satiety, fullness and prospective food consumption are presented in **Figure 2**. The repeated measures ANCOVA, adjusted for the baseline values, age and BMI, did not show any significant meal×time interactions for feelings of hunger (p=0.14), satiety (p=0.33), fullness (p=0.77) and prospective food consumption (p=0.71) between-meals. The corresponding AUC for hunger sensation was lower, though not significantly different, after HB when compared to RB (HB 5841±2186 *vs* RB 8003±4848mm×min; p=0.40). No difference was observed for satiety, fullness and prospective food consumption AUCs (HB 19500±3269 *vs* RB 17848±3762 mm×min, p=0.18; HB 18634±3241 *vs* RB 18660±2638 mm×min, p=0.83; HB 7719±2692 *vs* RB 9384±4094 mm×min, p=0.78; respectively).

Metabolic variables and hormones

Changes in blood glucose, insulin, FFA and leptin are shown in **Figure 3**. Repeated measures ANCOVA demonstrated a consistent meal×time interaction on glucose (p<0.001), insulin (p<0.001) and FFA (p=0.01) postprandially. The RB resulted in significantly higher glucose concentrations than HB (p<0.001) and post-hoc comparisons showed significant differences at time points 20 and 60-min (p<0.001 and p=0.002, respectively). Likewise, postprandial insulin response increased significantly after RB (p<0.001) at time 20, 60 and 90-min (p<0.001) compared to HB. Conversely, postprandial FFA concentration was reduced after RB (p<0.001), resulting significantly different at time 60, 90 and 180-min (p<0.001). Accordingly, the AUCs for glucose (HB 10637±1210 *vs* RB 12623±1059 mg/dL×min; p<0.001) and insulin (HB 766±196 *vs* RB 3908±1661 µU/mL×min; p<0.001) were increased, those for FFA decreased after RB (HB 77±20.0 *vs* RB 35±8.0 mmol/L×min; p<0.001).

Repeated measures ANCOVA did not show a meal×time interaction for leptin concentration between meals. After RB, a slight increase in leptin levels was observed at 180-min, not significantly different from corresponding values after HB. No difference in leptin AUCs was found between meals (HB 552812±416648 *vs* RB 725309±643110 pg/mL; p=0.67).

Peptides involved in appetite regulation

AG, GLP-1 and PYY concentrations are reported in **Figure 4**. The adjusted repeated measures ANCOVA showed a significant effect of time and meal on GLP-1 (p<0.001) and PYY (p<0.001), but not on AG levels. Compared with HB, the RB increased postprandial response for both GLP-1 at time points 20, 60, 90 and 180-min (p=0.0006, p=0.005, p=0.02, p=0.006) and PYY circulating concentrations at the same time points (p<0.001). Postprandial AG concentrations, though lower after RB, did not significantly differ between meals. RB resulted in increased AUCs for GLP-1 (HB 342±29 *vs* RB 447±106 pmol/L×min; p=0.025) and PYY (HB 5474±2284 *vs* RB 8732±2402 pg/mL×min; p=0.002), but not for AG (HB 15002±8077 *vs* RB 11976±16784 pg/mL×min; p=0.16).

A significant meal×time interaction was observed for postprandial NPY ($p<0.001$), OX-A ($p<0.001$) and α-MSH ($p=0.05$) levels (**Figure 5**). Overall, the RB resulted in increased NPY concentrations ($p<0.001$), with higher values at 90-min ($p=0.04$) and-180 min ($p<0.001$). Similarly, OX-A decreased and then increased after RB when compared to HB, with significant differences at time points 60-min ($p=0.04$) and 180-min ($p=0.002$). The overall α-MSH responses differed between meals ($p=0.01$) and were significantly higher for HB at 180-min ($p=0.01$). NPY AUC was lower after HB (HB 3468±592 *vs* RB 4564±964 pg/mL×min; $p=0.04$), while no differences were found for OX-A (HB 5003±2094 *vs* RB 4992±1003 pg/mL×min; $p=0.99$) and α-MSH AUCs (HB 1019±646 *vs* RB 911±410 ng/mL×min; $p=0.78$).

Self-reporting energy intake

Data collected by 3-d food diaries, including breakfast, did not significantly differ between meals (HB=1541±325 *vs* RB=1674±267 kcal/day; $p=0.19$), showing similar macronutrient distributions (proteins 19%, lipids 33-34%, carbohydrates 49% of total energy, fiber 13-14 g/day). However, when considering the calories consumed on the test day only, we found significantly lower energy intakes after HB (HB=1146.6±343.8 *vs* RB=1634.7±274.2 kcal/day; $p=0.003$), whereas no between-meals differences were observed for the following 2 days.

Discussion

This randomized cross-over pilot trial showed that hypnotic visual hallucination of a meal was able to acutely affect subjective appetite like eating a real food. Metabolic variables and gastrointestinal peptides changed with the real meal only, while hallucination seemed to affect the response of brain peptides, by keeping low the orexigenic NPY, OX-A and increasing the anorexigenic α-MSH circulating levels.

Modulation of subjective appetite by hypnosis

Hypnosis, employed as an anti-stress and relaxation technique, has been successfully used to treat many conditions related to chronic stress [14,17]. Imagining eating food, above all an appetizing meal, affected hunger and induced electrogastrogram changes similar to those determined by a real meal [18], leading to increased gastric emptying, acid and gastrin secretion, pancreatic secretion and release of pancreatic polypeptide [23]. Hypnotic hallucinations determine changes in the brain electrical activity and blood flow and is accompanied by measurable changes in both perceptual and attentional function of the brain specific-regions processing these activities [24].

Herein, we found that the hallucination of a meal affects appetite sensation like a real meal, reporting similar feelings of hunger and satiety, even if, during HB, participants did not eat at all. To justify these findings, we assessed several peptides implicated in feeding behavior, that, at the best of our knowledge, have never been studied after visual food hallucinations.

Neuropeptides

Central nervous system control of feeding behavior is complex and include multiple neural circuits influenced by many peptides, hormones, neurotransmitters, and hedonic, reward-related factors [25]. The endocannabinoid system controls the activity of neurons producing OX-A, a neuropeptide responsible for the control of energy homeostasis, sleep and generating signals stimulating food search [26]. OX-A acts through activation of the OX-A receptor type 1 (OX-1R) and affects the cannabinoid receptor type 1 (CB1R) function by down-regulation of POMC synthesis and α-MSH release [27]. The orexigenic neurons are activated by low glucose concentrations, thus enhancing food-motivated behavior in condition of metabolic need [25] and undergo the effects of a bimodal excitatory or inhibitory regulation by hypothalamic leptin levels [28]. Accordingly, in our patients, OX-A concentrations decreased up to 60-min after the real meal, concurrently with the increase in glucose and insulin concentrations and then rose again. Indeed, after the HB, OX-A values did not change, even if the levels of ghrelin, which is a direct activator of orexigenic neurons [29], increased and leptin levels, an antagonist of orexin-induced activity [30], decreased thus suggesting the complexity in the interplay between different peripheral and central energy balance signals. Similarly, the levels of the orexigenic NPY, one of the most abundant brain peptides, playing an important role in energy homeostasis [31], did not change after HB, despite ghrelin increment, the decrease in leptin and insulin activating NPY/AgRP neurons, and PYY reduction which inhibits those neurons [32]. A feedback between OX-A and NPY neurons have been described: OX-A increases NPY tone, while NPY treatment leads to the inhibition of orexigenic neurons [33]. Accordingly, we observed increased OX-A and NPY after RB, but flat values of both peptides after HB.

On the opposite, the anorexigenic α-MSH plays a crucial role in controlling food intake and body weight by increasing satiety [25]. Both OX-A and NPY differently act as inhibitors of α-MSH secretion, while leptin and insulin promote the release of α–MSH [5,25]. In our participants, although both leptin and insulin levels were low after HB, α-MSH serum levels resulted significantly higher at 180-min when compared to RB, probably due to the lack of the inhibitory feedback physiologically exerted by both OX-A and NPY peptides, whose levels did not increase. Therefore, it could be hypothesized that food hallucination inhibited the secretion or prevented the increase of OX-A and NPY, thus affecting perceived appetite sensations.

Brain-Gut peptides

GLP-1 is involved in peripheral and central pathways modulating appetite [34] and induces satiety by affecting both homeostatic and reward mechanisms and slowing gastric emptying [35]. After a meal, the plasma concentrations of GLP-1 rise, but the values represent a small percentage of the GLP-1 secreted, because most newly released GLP-1 is degraded and inactivated [34]. Accordingly, GLP-1 levels increased and remained significantly higher after RB than after HB. Similarly, the concentrations of PYY, another intestinal peptide with inhibitory effects on food control [36], rose and remained significantly higher after the RB. As previously observed, its levels are found to rise 1-2 h

postprandially and high concentrations are maintained up for 6-h [37]. These findings outline the importance of the nutrient contact with the secretory intestinal cells in the secretion and modulation of the peripheral concentrations of these peptides, since only after RB, GLP-1 and PYY concentrations changed.

AG, the gastric orexigenic peptide, is the main signal for meal initiation which regulates insulin secretion and is inhibited by circulating nutrients, insulin and GLP-1 [38]. As expected, in our patients, ghrelin values decreased after RB, but not after HB, even if no significant differences were found, and the increase in insulin concentrations after RB preceded the reduction in ghrelin values, suggesting an inhibitory role.

Metabolic variables and hormones

Glucose trigger insulin secretion, and we found increased glucose and insulin values and reduced FFA concentrations after RB, but not following HB.

Leptin is produced primarily by white adipocytes in proportion to the size of body fat storage and is involved in the regulation of energy balance [39]. Leptin secretion acts on hypothalamic neurons promoting satiety through the activation of POMC and the inhibition of NPY/AgRP neurons [40]. Its expression and secretion can be stimulated in anabolic states by insulin and cell glucose uptake, while conditions of energy mobilization, such as the release of FFA, inhibits its secretion [41]. In our participants, leptin concentration progressively declined after the HB meal, consistent with the increase of circulating FFA due to the prolonged fasting. Conversely, low leptin levels did not act as stimulus for the increase of orexigenic hormones, such as NPY and OX-A [42].

Our results suggested that the “brain area” may have a crucial role in the regulation of subjective appetite. Accordingly, genome-wide association studies found that an overwhelming majority of genes associated with BMI are expressed in the brain, many of them in the hypothalamus [43].

Short-term effect on food intake

Data from 3-day food records did not reveal any difference on mean energy and macronutrient intakes following both meals. By analyzing those results separately, we found that the calories consumed on the HB day were significantly lower than those consumed during the RB-day, whereas no differences were reported on the following 2 days, suggesting no compensatory overeating in the short-term.

Clinical implications

Only about 10% of individuals can develop visual hallucination by using hypnosis. Nevertheless, our results may be useful to explore the development of alternative strategies to control appetite. Since appetite is central to energy homeostasis and imbalance, the real challenge would be designing new approaches for modulating appetite regulation to promote weight loss and long-term maintenance.

The use of hypnotically induced imagery might potentially affect physiologic processes regulated by brain and implicated in weight control. Intriguingly, an aberrant activation of OX-A–mediated endocannabinoid signaling at POMC neurons, triggered by deficits in leptin signaling, has been reported in obesity, creating a vicious circle leading to inhibition of α-MSH synthesis, hyperphagia, body weight increase, and hepatic steatosis [27]. Similarly, NPY receptor has been reported to play a relevant role in several dysmetabolic and chronic diseases, by promoting insulin resistance, impaired glucose metabolism, adipose tissue formation, atherosclerosis acceleration, and cardiac dysfunction [31,44]. Furthermore, NPY levels have been reported to be modulated by different macronutrient composition and types of diet, and obesity is characterized by increased sensitivity of the NPY system [8].

Limitations

This pilot study has been performed in a small group of selected women who were highly hypnotizable, healthy and with a normal BMI, thus limiting its generalization to other groups and, in particular, to patients with overweight/obesity. Indeed, because of sex difference, the influence of estrogens, weight and age [45] in the regulation of peptide hormones, we have tried to reduce all sources of bias by studying a highly selected group. Subjective appetite was self-reported, but its measurement by VAS has been shown to be reproducible and reliable [22]. The choice of reporting, but not weighing, participants' food intake on the following days of the tests could be considered as a limitation [2], although food intake assessment was not the main objective of this study. Indeed, food hallucination is likely to affect short-term food intake, but this should be suitably explored. Concentrations of some peptides, such as OX-A, NPY and α-MSH, greatly varied among studies, with a wide variability in the magnitude order [45], due to the lack of international standardization. The plasma concentrations of OX-A, NPY and α-MSH are only an estimate of synthesis of these peptides in the brain, since these levels are much lower than those measured in cerebral spinal fluid, and whether peripheral circulating levels are able to exert clinically relevant effects is at present uncertain [28,33]. We have determined acute changes in the circulating levels of peptides at 180-min after the meals but did not evaluate long-term changes; indeed, it would be hard to obtain more than 3-h immobility from our participants without giving rise to restlessness or stress potentially altering the trial results.

Finally, this is an explorative study that assessed the acute effects of a single visual food hallucination on appetite regulation. However, the long-term effects of repeated visual food hallucination episodes on appetite ratings, appetite-regulating hormones, food intake and body weight have not been evaluated yet.

Conclusions

Our pilot trial suggests the possibility to modulate brain peptides implicated in the appetite regulation by hypnosis. Further studies are required to investigate the long-term changes on appetite and the implicated hormones in larger

samples of healthy individuals and patients with obesity, in order to identify potential alternative strategies for the control of body weight.

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

Ethical approval: "All procedures performed in the study were in accordance with the ethical standards of the institutional and/or national research committee (Ethical Committee of Turin University, reference number:181321) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards."

References

1. Hopkins M, Blundell J, Halford J, King N, Finlayson G. The Regulation of Food Intake in Humans. In: Feingold KR, Anawalt B, Boyce A, Chrousos G, Dungan K, Grossman A, et al., editors. Endotext [Internet]. South Dartmouth (MA): MDText.com, Inc.; 2000.
2. Blundell J, de Graaf C, Hulshof T, Jebb S, Livingstone B, Lluch A, et al. Appetite control: methodological aspects of the evaluation of foods. Obesity Reviews. 2010;11:251–70.
3. Halford JC, Blundell JE. Separate systems for serotonin and leptin in appetite control. Ann Med. 2000;32:222–32.
4. Zanchi D, Depoorter A, Egloff L, Haller S, Mählmann L, Lang UE, et al. The impact of gut hormones on the neural circuit of appetite and satiety: A systematic review. Neurosci Biobehav Rev. 2017;80:457–75.
5. Coll AP. Effects of pro-opiomelanocortin (POMC) on food intake and body weight: mechanisms and therapeutic potential? Clin Sci. 2007;113:171–82.
6. Williams DL. Neural integration of satiation and food reward: role of GLP-1 and orexin pathways. Physiol Behav. 2014;136:194–9.
7. Yeomans MR, Blundell JE, Leshem M. Palatability: response to nutritional need or need-free stimulation of appetite? Br J Nutr. 2004;Suppl 1:S3-14.
8. Gumbs MCR, van den Heuvel JK, la Fleur SE. The effect of obesogenic diets on brain Neuropeptide Y. Physiol Behav. 2016 01;162:161–73.
9. Steinert RE, Feinle-Bisset C, Asarian L, Horowitz M, Beglinger C, Geary N. Ghrelin, CCK, GLP-1, and PYY(3-36): Secretory Controls and Physiological Roles in Eating and Glycemia in Health, Obesity, and After RYGB. Physiol Rev. 2017;97:411–63.
10. Kirsch I. Hypnotic enhancement of cognitive-behavioral weight loss treatments--another meta-reanalysis. J Consult Clin Psychol. 1996;64:517–9.
11. Pittler MH, Ernst E. Complementary therapies for reducing body weight: a systematic review. Int J Obes (Lond). 2005;29:1030–8.

12. Bo S, Rahimi F, Goitre I, Properzi B, Ponzo V, Regaldo G, et al. Effects of Self-Conditioning Techniques (Self-Hypnosis) in Promoting Weight Loss in Patients with Severe Obesity: A Randomized Controlled Trial. Obesity. 2018;26:1422–9.
13. Entwistle PA, Webb RJ, Abayomi JC, Johnson B, Sparkes AC, Davies IG. Unconscious Agendas in the Etiology of Refractory Obesity and the Role of Hypnosis in Their Identification and Resolution: *A New Paradigm for Weight-Management Programs or a Paradigm Revisited?* International Journal of Clinical and Experimental Hypnosis. 2014;62:330–59.
14. Chiarioni G, Vantini I, De Iorio F, Benini L. Prokinetic effect of gut-oriented hypnosis on gastric emptying. Aliment Pharmacol Ther. 2006;23:1241–9.
15. Klein KB, Spiegel D. Modulation of gastric acid secretion by hypnosis. Gastroenterology. 1989;96:1383–7.
16. Lea R, Houghton LA, Calvert EL, Larder S, Gonsalkorale WM, Whelan V, et al. Gut-focused hypnotherapy normalizes disordered rectal sensitivity in patients with irritable bowel syndrome. Aliment Pharmacol Ther. 2003;17:635–42.
17. Whorwell PJ, Prior A, Faragher EB. Controlled trial of hypnotherapy in the treatment of severe refractory irritable-bowel syndrome. Lancet. 1984;2:1232–4.
18. Enck P, Hefner J, Herbert BM, Mazurak N, Weimer K, Muth ER, et al. Sensitivity and Specificity of Hypnosis Effects on Gastric Myoelectrical Activity. Gray M, editor. PLoS ONE. 2013;8:e83486.
19. Giuseppe Regaldo. Manuale di ipnosi medica rapida. 2014.
20. Shor RE, Orne EC. Norms on the Harvard group scale of hypnotic susceptibility, form A. Int J Clin Exp Hypn. 1963;11:39–47.
21. Bowers KS. Waterloo-Stanford Group Scale of Hypnotic Susceptibility, Form C: manual and response booklet. Int J Clin Exp Hypn. 1998;46:250–68.
22. Flint A, Raben A, Blundell JE, Astrup A. Reproducibility, power and validity of visual analogue scales in assessment of appetite sensations in single test meal studies. Int J Obes Relat Metab Disord. 2000;24:38–48.
23. Katschinski M, Dahmen G, Reinshagen M, Beglinger C, Koop H, Nustede R, et al. Cephalic stimulation of gastrointestinal secretory and motor responses in humans. Gastroenterology. 1992;103:383–91.
24. Spiegel D. Negative and Positive Visual Hypnotic Hallucinations: Attending Inside and Out. International Journal of Clinical and Experimental Hypnosis. 2003;51:130–46.
25. Ferrario CR, Labouèbe G, Liu S, Nieh EH, Routh VH, Xu S, et al. Homeostasis Meets Motivation in the Battle to Control Food Intake. J Neurosci. 2016;36:11469–81.
26. Sakurai T. Roles of orexins in the regulation of body weight homeostasis. Obes Res Clin Pract. 2014;8:e414-420.

27. Morello G, Imperatore R, Palomba L, Finelli C, Labruna G, Pasanisi F, et al. Orexin-A represses satiety-inducing POMC neurons and contributes to obesity via stimulation of endocannabinoid signaling. Proc Natl Acad Sci USA. 2016;113:4759–64.

28. Imperatore R, Palomba L, Cristino L. Role of Orexin-A in Hypertension and Obesity. Curr Hypertens Rep. 2017;19:34.

29. Goforth PB, Myers MG. Roles for Orexin/Hypocretin in the Control of Energy Balance and Metabolism. Curr Top Behav Neurosci. 2017;33:137–56.

30. Nixon JP, Kotz CM, Novak CM, Billington CJ, Teske JA. Neuropeptides Controlling Energy Balance: Orexins and Neuromedins. In: Joost H-G, editor. Appetite Control. Berlin, Heidelberg: Springer Berlin Heidelberg; 2012. pp. 77–109.

31. Yi M, Li H, Wu Z, Yan J, Liu Q, Ou C, et al. A Promising Therapeutic Target for Metabolic Diseases: Neuropeptide Y Receptors in Humans. Cell Physiol Biochem. 2018;45:88–107.

32. Muroi Y, Ishii T. A novel neuropeptide Y neuronal pathway linking energy state and reproductive behavior. Neuropeptides. 2016;59:1–8.

33. Mercer RE, Chee MJS, Colmers WF. The role of NPY in hypothalamic mediated food intake. Front Neuroendocrinol. 2011;32:398–415.

34. Holst JJ. Incretin hormones and the satiation signal. Int J Obes (Lond). 2013;37:1161–8.

35. van Bloemendaal L, Ten Kulve JS, la Fleur SE, Ijzerman RG, Diamant M. Effects of glucagon-like peptide 1 on appetite and body weight: focus on the CNS. J Endocrinol. 2014;221:T1-16.

36. Stadlbauer U, Woods SC, Langhans W, Meyer U. PYY3-36: Beyond food intake. Front Neuroendocrinol. 2015;38:1–11.

37. Gibbons C, Caudwell P, Finlayson G, Webb D-L, Hellström PM, Näslund E, et al. Comparison of Postprandial Profiles of Ghrelin, Active GLP-1, and Total PYY to Meals Varying in Fat and Carbohydrate and Their Association with Hunger and the Phases of Satiety. The Journal of Clinical Endocrinology & Metabolism. 2013;98:E847–55.

38. Broglio F, Arvat E, Benso A, Gottero C, Prodam F, Granata R, et al. Ghrelin: much more than a natural growth hormone secretagogue. Isr Med Assoc J. 2002;4:607–13.

39. Harris RBS. Direct and indirect effects of leptin on adipocyte metabolism. Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease. 2014;1842:414–23.

40. Andermann ML, Lowell BB. Toward a Wiring Diagram Understanding of Appetite Control. Neuron. 2017;95:757–78.

41. Oswal A, Yeo G. Leptin and the Control of Body Weight: A Review of Its Diverse Central Targets, Signaling Mechanisms, and Role in the Pathogenesis of Obesity. Obesity. 2010;18:221–9.

42. Cristino L, Busetto G, Imperatore R, Ferrandino I, Palomba L, Silvestri C, et al. Obesity-driven synaptic remodeling affects endocannabinoid control of orexinergic neurons. Proceedings of the National Academy of Sciences. 2013;110:E2229–38.

43. Yeo GSH. Genetics of obesity: can an old dog teach us new tricks? Diabetologia. 2017;60(5):778–83.

44. Zhu P, Sun W, Zhang C, Song Z, Lin S. The role of neuropeptide Y in the pathophysiology of atherosclerotic cardiovascular disease. International Journal of Cardiology. 2016;220:235–41.

45. Cintron D, Beckman JP, Bailey KR, Lahr BD, Jayachandran M, Miller VM. Plasma orexin A levels in recently menopausal women during and 3 years following use of hormone therapy. Maturitas. 2017;99:59–65.

Figure captions

Fig. 1

Session protocol of the study. VAS=Visual Analogue Scale to measure the subjective appetite.

Fig. 2

Unadjusted mean ratings of hunger (a), satiety (b), fullness (c) and prospective food consumption (d) for 270-min following the two test meals (HB= Hallucinated Breakfast; RB= Real Breakfast) are expressed as mean ± SE (n=8). Repeated-measures ANCOVA analysis was used to examine the effect of meal, time and meal × time interaction. Post hoc pairwise comparisons were performed.

Fig. 3

Unadjusted mean values of plasma glucose (a), insulin (b), free fatty acid (FFA) (c) and leptin (d) for 180-min following two the test meals (HB= Hallucinated Breakfast; RB= Real Breakfast) are expressed as mean ± SE (n=8). Repeated-measures ANCOVA analysis was used to examine the effect of meal, time and meal × time interaction. Post hoc pairwise comparisons were performed. §$p<0.01$; *$p<0.05$

Fig. 4

Unadjusted mean values of acylated-ghrelin (a), PYY (b) and GLP-1 (c) for 180-min following the two test meals (HB= Hallucinated Breakfast; RB= Real Breakfast) are expressed as mean ± SE (n=8). Repeated-measures ANCOVA analysis was used to examine the effect of meal, time and meal × time interaction. Post hoc pairwise comparisons were performed. §$p<0.01$; *$p<0.05$

Fig. 5

Unadjusted mean values of NPY (a), α-MSH (b) and Orexin-A (c) for 180-min following the two test meals (HB= Hallucinated Breakfast; RB= Real Breakfast) are expressed as mean ± SE (n=8). Repeated-measures ANCOVA analysis was used to examine the effect of meal, time and meal × time interaction. Post hoc pairwise comparisons were performed. §$p<0.01$; *$p<0.05$.