# The Effects of Oxytocin in Obese Adults

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**Title: A randomized, double-blind, placebo-controlled clinical trial of 8-week intranasal oxytocin administration in adults with obesity: Rationale, study design, and methods**

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**Abstract**

**Background:** Obesity affects more than one-third of adults in the U.S., and effective treatment options are urgently needed. Oxytocin administration induces weight loss in animal models of obesity via effects on caloric intake, energy expenditure, and fat metabolism. We study intranasal oxytocin, an investigational drug shown to reduce caloric intake in humans, as a potential novel treatment for obesity.

**Methods:** We report the rationale, design, methods, and biostatistical analysis plan of a randomized, double-blind, placebo-controlled clinical trial of intranasal oxytocin for weight loss (primary endpoint) in adults with obesity. Participants (aged 18–45 years) were randomly allocated (1:1) to oxytocin (four times daily over eight weeks) versus placebo. Randomization was stratified by biological sex and BMI (≥30 to <35 kg/m$^2$, ≥35 to <40 kg/m$^2$, ≥40 kg/m$^2$). We investigate the efficacy, safety, and mechanisms of oxytocin administration in reducing body weight. Secondary endpoints include changes in resting energy expenditure, body composition, caloric intake, metabolic profile, and brain activation via functional magnetic resonance imaging in response to food images and during an impulse control task. Safety and tolerability are assessed biweekly and at a 6-week follow-up after treatment completion.

**Results:** Sixty-one male and female participants aged 18–45 years were randomized (mean age 34 years, mean BMI 37 kg/m$^2$). The study sample is diverse with 38% identifying as non-White and 20% Hispanic.

**Conclusion:** Investigating intranasal oxytocin's efficacy, safety, and mechanisms as an anti-obesity medication will advance the search for optimal treatment strategies for obesity and its associated severe sequelae.

**Keywords:** Obesity; Oxytocin; Weight loss; Appetite regulation; Energy metabolism; Neuroimaging

## 1. Introduction

Obesity affects over 42% of the adult U.S. population [1] and is associated with increased risk of type 2 diabetes mellitus, hepatic steatosis, cardiovascular disease, cancer, and premature mortality [2]. More than 20% of U.S. health care costs are directly attributable to obesity [3]. Furthermore, obesity is a significant risk factor for severe COVID-19-associated illness, hospitalization, intensive care, and death [4]. Weight loss improves outcomes [5], but meaningful weight reduction is difficult to achieve and sustain. Available treatment options to supplement lifestyle modifications have long been limited to bariatric surgery, associated with peri-/post-operative complications [6], and weight-loss medications with modest efficacy and bothersome side effects. The established anti-obesity interventions often fail to yield sustained weight reduction, partly due to a physiological decrease in resting energy expenditure (REE) accompanying weight loss [7]. Clinical trials have revealed promising new drugs for weight management, such as the recently FDA-approved glucagon-like peptide-1 (GLP-1) receptor agonist semaglutide [8]. Nonetheless, gastrointestinal side effects are common, and most GLP-1 receptor agonists currently approved for weight loss can only be administrated as subcutaneous injections [9]. There is an imperative need for novel, effective, and tolerable therapeutic strategies for weight loss to reduce obesity-associated health risks and increase the life expectancy and wellbeing of individuals with obesity.

Oxytocin is a nine amino acid peptide hormone produced by neurons in the paraventricular and supraoptic nuclei of the hypothalamus, secreted directly into brain regions involved in energy homeostasis and appetite regulation, and released into the bloodstream by the posterior pituitary gland [10–12]. Oxytocin neurons project to anorexigenic arcuate nucleus proopiomelanocortin neurons critical for regulation of energy balance, as well as the ventral tegmental area (VTA) and nucleus accumbens responsible for reward-related eating behavior. Further, oxytocin has been shown to mediate anorexigenic actions of leptin and cholecystokinin (CCK). See Figure 1 for a working model of obesity-related pathophysiology and proposed neural and metabolic oxytocin effects. Animal studies have shown that oxytocin may drive weight loss and result in sustained weight reduction by different mechanisms [13–15]. In rodents and non-human primates with diet-induced obesity, oxytocin administration prevents the physiological decrease in REE occurring with weight loss [13,16–18]. Oxytocin induces lipolysis resulting in reduced adipocyte size and decreased subcutaneous, visceral, and liver fat while preserving lean mass [13,15,17,19,20]. Exogenous oxytocin increases fat utilization [21] and might even induce conversion of white adipose tissue into thermogenic “beige” adipocytes [15]. Acute oxytocin administration reduces caloric consumption [22,23], particularly of palatable foods [24,25], whereas oxytocin antagonists have the reverse effect [26]. Further, oxytocin reduces cortisol levels and inflammation, which may, in turn, reduce caloric intake and improve metabolism [10,12].

In humans, proof-of-concept investigations show that acute intranasal oxytocin administration reduces food intake in both healthy men and women and those with obesity [21,25,27,28], potentially through modulation of neural processing in brain regions related to reward, energy homeostasis, and cognitive control [29–31]. Perturbations in these neural pathways may underly the development of obesity [32,33]. Intranasal oxytocin is well-tolerated by humans with minimal adverse events [34]. A clinical trial in a small sample of non-diabetic individuals with obesity (n=9 in the oxytocin arm) reported a significant BMI reduction after 8-week treatment with 24 international units (IU) (4 times/day) of intranasal oxytocin compared to placebo [35]. While the

findings are promising, this pilot study was small and did not assess mechanisms or safety. More extensive clinical trials are needed.

## 2. Methods

### *2.1. Study rationale, preliminary data, and aims*

Given promising preclinical and preliminary clinical findings indicating beneficial effects of oxytocin on eating behavior, metabolism, and weight loss [12,35], investigation of the efficacy and safety of prolonged intranasal oxytocin administration as a potential weight-loss therapeutic in humans is warranted. Further, exploring mechanisms underlying the metabolic actions of oxytocin is of high interest. Our clinical trial addresses these key research questions by determining whether 8-week intranasal oxytocin promotes weight reduction in adults with obesity and investigating the neural and metabolic pathways leading to the proposed oxytocin effects on body weight.

We demonstrated that a single dose of 24 IU intranasal oxytocin administered to fasting men across the weight spectrum reduced caloric intake at a test meal, increased fat utilization, and improved insulin sensitivity [21]. In a crossover functional magnetic resonance imaging (fMRI) study of men with overweight or obesity, we found that 24 IU intranasal oxytocin (versus placebo) reduced brain activation to high-calorie food images in the VTA, the origin of the mesolimbic dopaminergic reward system, and other hedonic (e.g., orbitofrontal cortex [OFC] and anterior insula) and homeostatic (e.g., hypothalamus) areas [30], and reduced functional connectivity between the VTA and reward-related brain regions (e.g., amygdala and insula) [29]. In contrast, oxytocin increased activation in cognitive control-related brain regions (e.g., dorsal anterior cingulate cortex [dACC] and dorsolateral prefrontal cortex [dlPFC]) [30] and improved impulse control, as assessed using a behavioral task previously linked to eating behavior [36].

Thus, we hypothesize that 8-week intranasal oxytocin will reduce food intake (by decreasing reward-related and homeostatic drive to eat and increasing eating-related impulse control) and

body fat and weight (via effects on eating behavior, REE, and fat metabolism) with minimal side effects. We propose intranasal oxytocin as a potential novel, effective, and safe therapy for weight loss in human obesity.

Our study was designed to investigate the following:

- Aim 1 – Determine oxytocin effects on weight, REE, and body composition: We hypothesize that 8-week intranasal oxytocin compared to placebo (a) reduces body weight, (b) increases REE (adjusted for lean/fat free mass), and (c) reduces total body, visceral, and liver fat in adults with obesity.
- Aim 2 – Determine oxytocin effects on caloric intake and underlying neural pathways (fMRI): We hypothesize that intranasal oxytocin compared to placebo reduces caloric intake at a test meal (independent of weight change), mediated by (a) reduced activation (in fasted and fed states) of reward-related food motivation (VTA, OFC, anterior insula) and homeostatic (hypothalamus) brain regions during a visual food stimuli paradigm and (b) increased impulse control indicated by increased activation (fasted state) of cognitive control-related brain regions (dlPFC, dACC, anterior portion of supplementary motor cortex/preSMA) during a validated task requiring the engagement of cognitive control to suppress impulsive responses.

The study is conducted under an Investigational New Drug application cleared by the U.S. Food and Drug Administration at a single study site, and the institutional review board approved the study protocol.

### *2.2. Study design, participants, and randomization*

We are conducting a randomized, double-blind, placebo-controlled study to investigate the effects of intranasal oxytocin (24 IU, 4 times/day, for 8 weeks) in adults with obesity (BMI $\geq$30 kg/m$^2$), aged 18–45 years. We randomized 61 individuals to achieve our target evaluable population of at least 50 participants who complete the 8-week treatment period. Participants were stratified by

biological sex (female/male) and obesity class (class 1: BMI 30.0–34.9 kg/m$^2$, class 2: BMI 35.0–39.9 kg/m$^2$, class 3: BMI ≥40.0 kg/m$^2$) and randomized 1:1 to receive oxytocin or placebo. The unblinded study pharmacist performed randomization. All other study staff and participants are blinded to treatment assignment. We recruited participants through Massachusetts General Hospital (MGH) and advertisements online/in the surrounding community. Table 1 summarizes the inclusion and exclusion criteria of the study.

### *2.3. Study and safety endpoints*

The primary endpoint is percent change in body weight from baseline following 8-week intranasal oxytocin compared to placebo. Key secondary endpoints include change from baseline to end of treatment in (a) REE (adjusted for lean mass), (b) body composition (total body, visceral, and liver fat mass), and (c) caloric intake (adjusted for weight) following 8-week intranasal oxytocin compared to placebo.

Other secondary endpoints include change in metabolic profile (e.g., insulin sensitivity, lipid panel, high-sensitivity C-reactive protein [hsCRP] at 8 weeks; fat metabolism at 2 weeks), behavior (e.g., eating behavior, reward-related food motivation, impulse control, social emotional functioning, symptoms of disordered eating, depression, and anxiety), and quality of life between baseline and 8-week intranasal oxytocin treatment compared to placebo (Table 2).

We systematically assess safety and treatment tolerability throughout the study (Table 2), with a final safety visit 6 weeks after treatment completion and additional visits if needed (e.g., early termination). We categorize severity of adverse events according to the Common Terminology Criteria for Adverse Events (CTCAE, v5.0) [37] and evaluate expectedness of adverse events and relationship to study drug and procedures.

### *2.4. Study procedures and assessments*

Study time points comprise the screening visit to determine eligibility, two baseline visits (the second one includes randomization and treatment initiation after baseline assessments are completed), on-treatment study time points (week 2, 4, 6, and 8 visits), and a final week 14 follow-up visit to assess sustained treatment effects and safety.

Study visits take place at the MGH Translational and Clinical Research Center and the Athinoula A. Martinos Center for Biomedical Imaging. We obtain and manage study data using a web-based secure Research Electronic Data Capture (REDCap) system [38].

See Figure 2 for an overview of the study design, including visits, endpoints, and assessment methods. Table 2 provides a detailed list of study procedures/assessments at each study visit.

*2.4.1. Diagnostic and screening measures*

We ask potential participants to complete a pre-screening questionnaire to establish preliminary study eligibility (Table 1). Following informed consent, we confirm eligibility at a screening visit, including review of medical history and physical examination, anthropometric measurements, pregnancy test in females, urine drug screen, blood tests (Table 2), electrocardiogram, and the Mini-International Neuropsychiatric Interview (M.I.N.I.) [39] to evaluate for current/past psychiatric conditions.

*2.4.2. Anthropometric measurements*

Anthropometric measurements are assessed by trained personnel using calibrated instruments. They include measurements of height (in triplicate using a stadiometer, at screening/baseline visits), weight, and waist/hip circumference (smallest, umbilicus, midpoint, iliac, broadest hip). BMI, waist-to-hip ratio, and percent ideal body weight are calculated (see Table 2 for formulas).

*2.4.3. Resting energy expenditure (REE)*

We perform indirect calorimetry (VMAX Encore 29 metabolic cart/Viasys Healthcare/Carefusion/San Diego/CA/USA) to assess fasting REE at baseline, week 6 and 8 visits.

*2.4.4. Body composition*

We obtain body composition measurements at baseline and week 8 visits. We use dual-energy X-ray absorptiometry (Hologic Horizon A/Hologic Inc./Marlborough/MA/USA) to measure total fat mass (precision 3% [40]), total lean mass, estimated visceral adipose tissue mass, and trunk fat mass. On a 3T MRI system (Siemens Trio/Siemens Medical Systems/Erlangen/Germany), we perform single-axial slices through the abdomen at the level of L4 and left mid-thigh to determine fat depots and thigh muscle cross-sectional area. We determine abdominal visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT) cross-sectional areas ($cm^2$) and their sum, total abdominal adipose tissue (TAT), mid-thigh SAT and muscle area ($cm^2$) using VITRAK software (Merge/eFilm/Milwaukee/WI/USA). We assess liver fat via breath-hold single-voxel 1H-MR spectroscopy (1H-MRS) using a PRESS pulse sequence without water suppression: TE = 30 ms, 8 acquisitions, TR = 1500 ms, 1024 data points, receiver bandwidth 2000 Hz) [41] and placing a voxel of 20×20×20 mm (8 mL) within the right and left hepatic lobes avoiding vessels or artifact. We use LCModel software (v6.3 -0K/S. Provencher/Oakville/Ontario/Canada) to fit 1H-MRS data, expressed in lipid-water-ratio.

*2.4.5. Fat biopsy*

Interventional, musculoskeletal radiologists perform abdominal subcutaneous fat biopsies (2–3 core biopsies) at baseline and week 2 visits under sterile conditions and local lidocaine anesthesia using a 14-gauge Temno biopsy needle (CareFusion/San Diego/CA/USA). Biopsies are immediately transferred to dry ice, ethanol, or formalin solution and stored appropriately until

immunohistochemical and molecular analyses to investigate oxytocin effects on fat metabolism pathways involving white adipocyte “browning”, fatty acid oxidation, and lipolysis.

*2.4.6. Caloric intake*

A validated breakfast test meal [21] takes place after a minimum 10-hour overnight fast at baseline and week 6 visits (oxytocin administration 20–30 minutes before test meal at week 6 visit). Participants select a meal from a menu created by research dietitians using ProNutra™ software (VioCare/Princeton/NJ/USA). Metabolism and Nutrition Research staff prepare double portions with known nutrient content, and participants have 30 minutes to eat as much as they would like. We quantify caloric intake at test meals (i.e., total calories, carbohydrate/fat/protein content) using ProNutra™ software.

At neuroimaging visits (baseline 2 and week 4, study drug administration held until after study visit), we ask participants to eat a standardized meal (~500 kcal, 60% carbohydrates, 20% fat, 20% protein) over 15 minutes in its entirety to allow for assessment of food motivation neural circuitry and appetite hormones in the fed state [42–44]. Caloric intake is quantified using ProNutra™ software.

Self-report measures of caloric intake include 24-hour food recalls and 4-day food records collected from participants at baseline, on-treatment, and off-treatment follow-up visits. Records are analyzed for total calories, macro- and micronutrients by study dieticians using NDS-R software (Nutrition Coordinating Center/University of Minnesota/Minneapolis/MN/USA).

*2.4.7. Laboratory assessments*

Blood samples are obtained after a minimum 10-hour overnight fast at approximately 8–9 a.m. at all study visits. Additionally, postprandial blood samples are obtained at neuroimaging visits (baseline 2, week 4) at 30, 60, and 120 minutes after a standardized meal (see 2.4.6.). Whole blood samples are immediately processed as follows: addition of Pefabloc® or aprotinin (for

unstable appetite-regulating hormones only), centrifugation (4 °C, 2800xg, 20 minutes) to separate plasma/serum, dispensed into aliquots (acidification with HCl for unstable appetite-regulating hormones only), and storage at -80 °C until laboratory analysis or immediate measurement (e.g., for glucose and HbA1c). We will report assays and characteristics (detection limits, intra-/inter-assay coefficients of variation) in publications using data from this study. We plan to assess metabolic profile (e.g., insulin sensitivity, lipid panel, hsCRP, appetite-regulating hormones) and gonadal steroids. We collect 24-hour urine (e.g., cortisol) and stool (e.g., microbiome) samples, aliquoted and stored at -80 °C, and hair samples (e.g., cortisol; proximal 2 cm segment of approximately 3 mm diameter taken from posterior vertex position). See Table 2 for details.

*2.4.8. Neuroimaging*

MRI scanning takes place on an FDA-approved 3T Skyra scanner (Siemens/Erlangen/Germany). We perform whole-brain functional fMRI using a gradient-echo EPI pulse sequence (33 contiguous oblique-axial slices angled 30° from the AC-PC axis, 4 mm thick, TR/TE = 2000/30 ms, flip angle = 90°, FOV = 200x200 mm, 120 total images per run).

- Food cue paradigm [42] – before (fasted state) and after (fed state) a standardized meal (see 2.4.6.): Participants view 100 high-calorie food stimuli (50% sweet/50% savory), 100 low-calorie food stimuli, 100 non-food object stimuli, and 100 fixation stimuli in a block design (5 runs, 4 minutes/run, 3 seconds/stimulus, 5 images/block, 16 blocks/run, images presented only once). Participants also rate a selection of food stimuli on valence and caloric content. The primary variable of interest is brain activation to high-calorie food stimuli compared to non-food stimuli.
- Stop-Signal Task (SST) [45,46] – fasted state: Participants are instructed to manually respond to visual go signals (circles) as fast and accurately as possible (within 3 seconds) but withhold their response when a stop signal (X) appears (25% of all trials, randomly selected, stop-signal delay following staircase-tracking algorithm). Trials are presented in a block design [64

trials/block, 3 blocks] following task practice outside the scanner. The primary variable of interest is brain activation to successful compared to failed response inhibition.

In addition to functional tasks, we acquire structural T1-weighted MRI images (MPRAGE sequence) for co-registration between structural and functional datasets. On a subset of participants (for whom scanning time allows), the Multi-Source Interference Task (an fMRI task reliably activating the cognitive control network involved in inhibiting task interference) [47], multi-echo T2-weighted MRI images, diffusion-weighted images, and resting state fMRI EPI images are acquired for further exploratory investigation of cognitive control, hypothalamic gliosis, white matter tractography, and functional connectivity.

#### *2.4.9. Psychological, behavioral, and quality of life assessments*

We use self-report questionnaires and behavioral tasks to assess obesity-related psychological aspects and behaviors that might also be affected by oxytocin administration, including physical activity, appetite and eating behavior and attitudes [11], reward processing and food motivation [29,30,48], impulse control, socioemotional functioning [49,50], and mood and anxiety symptoms [51]. Quality of life will also be evaluated. See Table 2 for details.

### *2.5. Biostatistical considerations*

#### *2.5.1. Sample size and statistical power*

Sixty-one participants were randomized to obtain evaluable data for at least 50 participants completing the 8-week treatment period. We conducted power and sample size calculations using 2-sample t-tests at a 2-tailed significance level of $\alpha = 0.05$. The primary endpoint of this study is percent change in body weight from baseline following 8-week intranasal oxytocin treatment compared to placebo; with 25 participants per arm, we have 80% power to detect a between-group (oxytocin versus placebo) mean difference in weight loss of ≥3.2 kg, based on a reported

standard deviation for the between-group difference in weight loss of approximately 4 kg in a pilot study of 8-week intranasal oxytocin administration in adults with obesity [35].

*2.5.2. Analysis of neuroimaging data*

FMRI data will be analyzed using Statistical Parametric Mapping software running under the MATLAB® environment (SPM12) [52]. Preprocessing steps will include realignment and geometric unwarping using magnitude and phase images from the field map, slice-time correction, echo planar (EPI) co-registration to the participant's segmented, skull-stripped, bias-corrected T1-weighted image, normalization to Montreal Neurological Institute space with resampling to 2 mm isotropic using 4$^{th}$ degree B-spline interpolation, and smoothing with a 6 mm isotropic full width at half-maximum Gaussian kernel. Each run of EPI data will be evaluated for motion: (a) the degree of linear movement will be assessed and classified (threshold = 3 mm linear movement in orthogonal planes), and (b) the ART toolbox (Artifact Detection Tools; www.nitrc.org/projects/artifact_detect) will be used to detect outlier volumes based on image intensity relative to the global mean image (threshold = 3.5xSD) and motion (threshold = 0.8 mm, measured as scan-to-scan movement). Individual runs will be excluded if they show either (a) >3 mm linear movement and >10% outliers, or (b) >20% outliers.

For subject-level analyses, a general linear model (GLM) either with regressors of high-calorie foods, low-calorie foods, and objects (food cue paradigm) or with regressors of successful stop trials and failed stop trials (SST) will be specified. Regressors will be placed at the onset of each condition within a block, convolved with the canonical hemodynamic response function. Global mean signal and motion outliers will be entered as nuisance regressors. Following GLM estimation, the primary contrast (high-calorie foods versus objects, successful versus failed stop trials) will be computed at the single-subject level to estimate condition effects at every voxel, separately for each session (fasted/fed). For subsequent group-level analysis, we will use a factorial design with baseline versus 4-week contrasts to examine changes in brain activation

following oxytocin treatment in a-priori defined regions of interest (ROIs, food cue paradigm: VTA, OFC, anterior insula; SST: dlPFC, dACC, preSMA). For mediation analyses, beta values will be extracted from subject-level contrast images for clusters within a-priori ROIs that meet family-wise error rate correction ($p < 0.05$) using Marsbar software [53]. We will also analyze activation in additional reward-related food motivation and impulse control-related brain regions and the whole brain (applying $\alpha$-error correction procedures). Moreover, we will examine activation in the hypothalamus as a brain region responsible for energy homeostasis.

*2.5.3. Statistical analysis plan*

Figure 3 shows our statistical analysis plan. We will perform statistical analyses using R statistical software, and appropriate R packages [54]. For continuous endpoints (see 2.3.), we will apply mixed model regression for repeated measurements (MMRM). We will consider the between-group (oxytocin versus placebo) difference in the least-squares mean change of each individual endpoint variable from baseline to each follow-up study time point of interest as the response in MMRM; the change from baseline to week 8 study time point (end of treatment) will be of primary interest (see 2.3.). Treatment group (oxytocin versus placebo), study time point (visit), and treatment group*study time point interaction will be included as fixed factors in MMRM. The baseline value of the endpoint variable and stratification factors (biological sex, BMI) will be entered as covariates. Additional covariates may be considered as appropriate. The intercept and time slope of individual participants will constitute random effects. Parameters of MMRM will be estimated via restricted maximum likelihood (REML). Depending on the distributional characteristics of the data, we may apply logarithmic transformations and/or robust statistical methods. We will adjust for multiple comparisons where appropriate via the approach of Hothorn, Bretz, and Westfall based on single-step simultaneous inference using a joint multivariate t-distribution [55]. We will use a modified intention-to-treat approach for efficacy analyses, including all randomized participants with available data for at least the second baseline visit

(randomization/initiation of treatment) and the first on-treatment study time point. We will apply a per-protocol approach for sensitivity analysis, including only randomized participants who complete the study and demonstrate ≥80% adherence to treatment. We will carefully examine patterns of any missing data regarding their relationships with the outcomes of interest and incorporate uncertainty due to missing data with multiple imputations where applicable.

We will conduct regression-based mediation analyses to explore mechanisms underlying the proposed effects of intranasal oxytocin treatment: We will investigate whether (a) the change in brain activation in key reward-related food motivation (VTA, OFC, anterior insula) and homeostatic (hypothalamus) regions during fasted and fed states as well as in cognitive control-related regions (dlPFC, dACC, preSMA) during the fasted state from baseline following 4-week oxytocin treatment mediates change in caloric intake at a test meal 6 weeks from baseline, (b) the change in caloric intake at 6 weeks from baseline mediates the percentage change in body weight at 8 weeks from baseline, and (c) the change in REE at 6 weeks from baseline mediates the percentage change in body weight at 8 weeks from baseline.

Safety and compliance endpoints (assessed at each study time point) will be reported and tested for between-group (oxytocin versus placebo) differences. Endpoints regarding the off-treatment follow-up study time point (week 14 visit) will be reported and analyzed using MMRM in relation to baseline and end of treatment (week 8 visit) time points where appropriate (separately from on-treatment analyses).

### *2.6. Study drug, study drug administration, and treatment adherence*

The study drug, 24 IU of intranasal oxytocin (Syntocinon® Nasal Spray, Mylan, Switzerland) or placebo (containing the same inactive ingredients as Syntocinon®), is applied as 3 sprays per nostril, 4 times per day (20–30 minutes before main meals and at bedtime), for an 8-week treatment period. We used the same intranasal oxytocin dose (24 IU) in our prior studies of men showing significant effects on food reward, homeostatic, and cognitive control-related

neurocircuits, impulsivity, and caloric intake [21,29,30,56]. Further, this oxytocin regimen (versus placebo) significantly reduced weight in a small study of 20 adults with obesity [35]. In the latter pilot study, as well as in a review article of 38 randomized controlled trials (n = 926 participants) administering up to 40 IU of intranasal oxytocin (4 times/day) over up to 13 weeks [57], no serious adverse events were reported and there were no differences in adverse events between oxytocin and placebo groups.

To assess treatment adherence, we ask participants to complete and return a drug diary at each visit during the 8-week treatment (Table 2). Adherence is determined per the drug diary reflecting ≥80% study drug administration. Drug accountability is assessed by study staff throughout the study, and subjects are asked to return all used and unused study drug at each visit.

### *2.7. Study monitoring, quality assurance, and protection of human subjects*

Source documents are monitored and reviewed by study staff throughout the study, and medical and research records are maintained appropriately per regulatory and institutional requirements for the protection of confidentiality of participants. The study is conducted in full conformity with federal regulations and institutional requirements (Declaration of Helsinki; regulations for the protection of human patients of research; Good Clinical Practice), including periodic and expedited safety reporting. An independent data and safety monitoring board consisting of a biostatistician and two endocrinologists met before study initiation and regularly thereafter to monitor progress and safety. Our study is registered on ClinicalTrials.gov (NCT03043053).

## 3. Results

Table 3 presents characteristics of the randomized study sample, which has almost equal biological sex distribution and racial (38% non-White) and ethnic (20% Hispanic/Latino) diversity.

## 4. Discussion

We have carefully designed and powered this randomized controlled double-blind trial to determine the efficacy, safety, and underlying mechanisms of 8-week oxytocin administration versus placebo as a novel weight-loss therapeutic in a diverse population of adults with obesity. Our study builds on solid evidence from animal models [10–12] and a preliminary clinical trial [35]. The innovative approach uses metabolic, neuroimaging, and behavioral assessments to provide important information about oxytocin mechanisms and actions.

# Disclosures


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### Conflict of Interest Statement

E.A.L. served on the scientific advisory board and has a financial interest in OXT Therapeutics, Inc. E.A.L. also received funding for an investigator-initiated study by Tonix Pharmaceuticals. All other authors declare no conflicts of interest.

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# Tables

**Table 1. Inclusion and exclusion criteria.**

| Inclusion criteria | Exclusion criteria |
|---|---|
| • 18–45 years old<br>• BMI ≥30 kg/m$^2$<br>• Willingness to maintain current diet and lifestyle for the duration of study participation (e.g., agree not to start any special diets or lifestyle changes intended to modify weight) | ***Weight/diet***<br>• Weight change >5 kg within 3 months prior to randomization<br>• Nonstandard diet (e.g., Paleo, Atkins, raw diet, macrobiotic diet)<br>• Significant change in physical activity or diet within 1 month of randomization<br><br>***Medical/psychiatric conditions***<br>• History of any of the following medical conditions:<br>  ○ Cardiovascular disease, prolonged QT on EKG at screening visit<br>  ○ Clinically significant gastrointestinal disorder<br>  ○ Bariatric surgery (except for participants with a history of laparoscopic adjustable gastric band surgery)<br>  ○ Bleeding disorder<br>  ○ Untreated thyroid disease<br>  ○ Malignancy within 5 years of screening visit (except for basal cell carcinoma or squamous cell carcinoma)<br>  ○ Epilepsy<br>  ○ Any significant illness or condition that the investigator determines could interfere with study participation and impact data collection or participant safety<br>• History of an eating disorder or psychosis<br>• Current psychiatric disorder (unless on stable dose of anti-depressant or anti-anxiety treatment for ≥6 months with no active symptoms that would qualify for an active psychiatric diagnosis as assessed by the Mini-International Neuropsychiatric Interview)<br>• Substance use disorder active within the last 6 months or clinical suspicion of ongoing substance use disorder at screening visit (based on history, Mini-International Neuropsychiatric Interview, and/or lab results)<br>• Current smoking or tobacco use<br>• Allergy to lidocaine<br><br>***Medications***<br>• Medication changes within 4 weeks of enrollment<br>• Oral contraceptives<br>• Use of prescription or over-the-counter drugs or dietary/herbal supplements affecting metabolism, blood glucose, or appetite (metformin allowed if on stable dose with stable weight for ≥3 months)<br><br>***Screening labs***<br>• Hematocrit >2% below normal<br>• Fasting glucose >125 mg/dL or hemoglobin A1c ≥7%<br>• Liver transaminases (ALT, AST) >2.5 times upper limit of normal<br>• Creatinine >1.5 mg/dL<br>• Hyponatremia<br><br>***Further exclusion criteria***<br>• Contraindications to MRI and/or DXA<br>• Pregnancy or breastfeeding<br>• Unwilling to use a medically acceptable form of contraception throughout the study period (female of child-bearing potential only)<br>• Participation in any clinical study involving an investigational drug, device, or biologic within 1 month of randomization |

Abbreviations: BMI, body mass index; EKG, electrocardiogram; ALT, alanine transaminase; AST, aspartate transaminase; MRI, magnetic resonance imaging; DXA, dual-energy X-ray absorptiometry.

**Table 2. Study procedures at individual study time points.**

| Assessments | Screen | BL | | ON-T (oxytocin versus placebo) | | | | OFF-T |
|---|---|---|---|---|---|---|---|---|
| | | BL1 | BL2 | W2 | W4 | W6 | W8 | W14 |
| **Screening and safety** | | | | | | | | |
| • Medical history and physical exam, MMSE | X | X | X | X | X | X | X | X |
| • Screening/safety labs (CBC, TSH, urine drug screen (at screen only); CMP, HbA1c) | X | | X | X | X | | X | X |
| • EKG | X | | | X | | | X | |
| • M.I.N.I. | X | | | | | | | |
| • Adverse events monitoring, review of concomitant medication, pregnancy test (urine/serum hCG) in females | X | X | X | X | X | X | X | X |
| **Anthropometric measurements** (height, weight, %IBW[a], BMI[b], WHR[c]) | X | X | X | X | X | X | X | X |
| **Energy expenditure, body composition, and fat metabolism** | | | | | | | | |
| • Indirect calorimetry (REE) | | X | | | | X | X | |
| • DXA (total fat mass, lean body mass) | | X | | | | | X | |
| • MRI (abdominal and thigh measures) | | X | | | | | X | |
| • 1H-MRS (liver fat) | | X | | | | | X | |
| • Fat biopsy | | X | | X | | | | |
| **Caloric intake** | | | | | | | | |
| • Breakfast test meal (experimental assessment of caloric intake) | | X | | | | X | | |
| • 4-day food record (typical eating) | | X | X | | X | X | X | X |
| • 24-hr food recall (caloric intake immediately preceding visit) | | X | X | | X | X | | |
| **Laboratory assessments** | | | | | | | | |
| • Blood for metabolic profile (e.g., insulin sensitivity, lipid panel, hsCRP, appetite-regulating hormones), gonadal steroids, leukocyte RNA, DNA (optional) | | | X | X | X | X | X | X |
| • 24h urine (e.g., cortisol, creatinine) | | X | | | | | X | |
| • Stool (e.g., microbiome; optional) | | | X | | X | | X | |
| • Hair (e.g., cortisol; optional) | | | X | | | | X | |
| **Neuroimaging** | | | | | | | | |
| • Food cue task (fasting and post-meal): VTA, OFC, anterior insula, hypothalamus | | | X | | X | | | |
| • Impulse control tasks (Stop-Signal Task; Multi-Source Interference Task [optional]) (fasting): dlPFC, dACC, preSMA | | | X | | X | | | |
| • Handedness questionnaire | | X | | | | | | |
| **Psychological, behavioral, and quality of life assessments** | | | | | | | | |
| • Physical activity (PPAQ[d]) | X | | | | X | X | | X |
| • Appetite (VAS[e]), eating behavior and attitudes (EDEQ[f], DEBQ[g], EADES[h], TFEQ[i]) | | X | X | | X | X | X | X |
| • Reward-related food motivation (BIS/BAS[j], CTT[k], PFS[l]) and reward-related behaviors and choices | | | X | | X | | | X |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| (Probabilistic Reward Task and Social Reward Task, TEPS[m]; optional) | | | | | | | |
| • Impulse control and compulsivity (Delay Discounting Task, Reversal Learning Task, BIS-11[n], BOCS[o], SRS[p]; optional) | | X | | X | | | |
| • Social-emotional functioning (DAPP-BQ[q], ISEL[r], LSAS[s], RMET[t], SAAM[u], TAS-20[v]) | X | | | | | X | |
| • Mood and anxiety symptoms (BDI-II, STAI-trait) | | X | | X | | X | X |
| • Quality of life (SF-36) | | X | | X | | X | X |
| **Adherence** | | | | | | | |
| • Study drug diary | | | X | X | X | X | |

Footnotes (including brief definition and reference) and abbreviations:

[a]Ideal body weight is determined using elbow breadth and frame size and the 1983 Metropolitan Life Insurance Height/Weight Tables [58].

[b]Body mass index is calculated as weight in kg / (height in m)$^2$.

[c]Waist-to-hip ratio is Iliac circumference over broadest hip circumference.

[d]Paffenbarger Physical Activity Questionnaire (interview conducted by trained research coordinators) measuring physical activity [59].

[e]Visual Analogue Scales (self-report) asking for subjective appetite [60].

[f]Eating Disorder Examination Questionnaire (self-report) screening for eating disorders [61].

[g]Dutch Eating Behavior Questionnaire (self-report) measuring restrained, emotional, and external eating [62].

[h]Eating and Appraisal Due to Emotions and Stress (self-report) questionnaire assessing stress coping in relation to eating [63].

[i]Three Factor Eating Questionnaire (self-report) measuring cognitive restraint of eating, disinhibition, and hunger [64].

[j]Behavioral Inhibition System/Behavioral Activation System (self-report) assessing motivation regarding aversive/appetitive stimuli [65].

[k]Cookie Taste Test (behavioral task, post standardized meal and snack) assessing reward-related food motivation beyond satiety [24,66].

[l]Power of Food Scale (self-report) measuring appetite for palatable foods [67].

[m]Temporal Experience of Pleasure Scale (self-report) measuring anticipatory and consummatory facets of pleasure [68].

[n]Barratt Impulsiveness Scale (self-report) assessing attentional, motor, and non-planning impulsiveness [69].

[o]Brief Obsessive-Compulsive Scale (self-report) measuring the presence of obsessive-compulsive symptoms [70].

[p]Self-Regulation Scale (self-report) capturing attentional control in goal pursuit [71].

[q]Dimensional Assessment of Personality Pathology-Basic Questionnaire (self-report) assessing suspiciousness and secure attachment [72].

[r]Interpersonal Support Evaluation List (self-report) assessing perceived availability of social support [73].

[s]Liebowitz Social Adjustment Scale (self-report) measuring fear and avoidance of social situations [74].

[t]Reading the Mind in the Eyes Test (behavioral task) assessing theory of mind [75].

[u]State Adult Attachment Measure (self-report) capturing attachment insecurity [76].

[v]Toronto Alexithymia Scale (self-report) measuring the ability to describe and identify emotions [77].

BL, baseline visit; T, Treatment; W, week of visit; HR, heart rate; MMSE, Mini-Mental Status Exam; CBC, complete blood count; TSH, thyroid stimulating hormone; CMP, comprehensive metabolic panel (including liver [alanine/aspartate transaminase] and renal [creatinine] function tests, electrolytes [potassium, sodium], and glucose); HbA1c, hemoglobin A1c; EKG, electrocardiogram; M.I.N.I., Mini-International Neuropsychiatric Interview; hCG, human chorionic gonadotropin; REE, resting energy expenditure; DXA, dual-energy X-ray absorptiometry; MRI, magnetic resonance imaging; 1H-MRS, magnetic resonance spectroscopy; hsCRP, high-sensitivity C-reactive protein; VTA, ventral tegmental area; OFC, orbitofrontal cortex; dlPFC, dorsolateral prefrontal cortex; dACC, dorsal anterior cingulate cortex; preSMA, anterior portion of the supplementary motor cortex; BDI, Beck Depression Inventory; STAI, State Trait Anxiety Inventory; SF-36, 36-Item Short Form Survey.

**Table 3. Characteristics of the randomized participants at baseline (BL1 visit).**

| Characteristic | Mean (SD) or N (%) |
|---|---|
| ***Demographics*** | |
| N randomized | 61 |
| Biological sex: F \| M | 33 (54.10%) \| 28 (45.90%) |
| Age (years) | 33.58 (6.22) |
| *Race* | |
| • Asian | 6 (9.84%) |
| • Black | 13 (21.31%) |
| • White | 38 (62.30%) |
| • Other/More than one race | 3 (4.92%) |
| • Asked but unknown | 1 (1.64%) |
| *Ethnicity* | |
| • Hispanic/Latino ethnic group | 12 (19.67%) |
| ***Clinical characteristics*** | |
| BMI ($kg/m^2$) [F \| M] | 37.02 (4.93) [37.77 (4.92) \| 36.13 (4.87)] |
| BMI distribution ($kg/m^2$) | |
| • ≥30 to <35 [F \| M] | 24 (39.34%) [8 \| 16] |
| • ≥35 to <40 [F \| M] | 21 (34.43%) [15 \| 6] |
| • ≥40 [F \| M] | 16 (26.23%) [10 \| 6] |

Abbreviations: F, female; M, male; BMI, body mass index.

## Figures

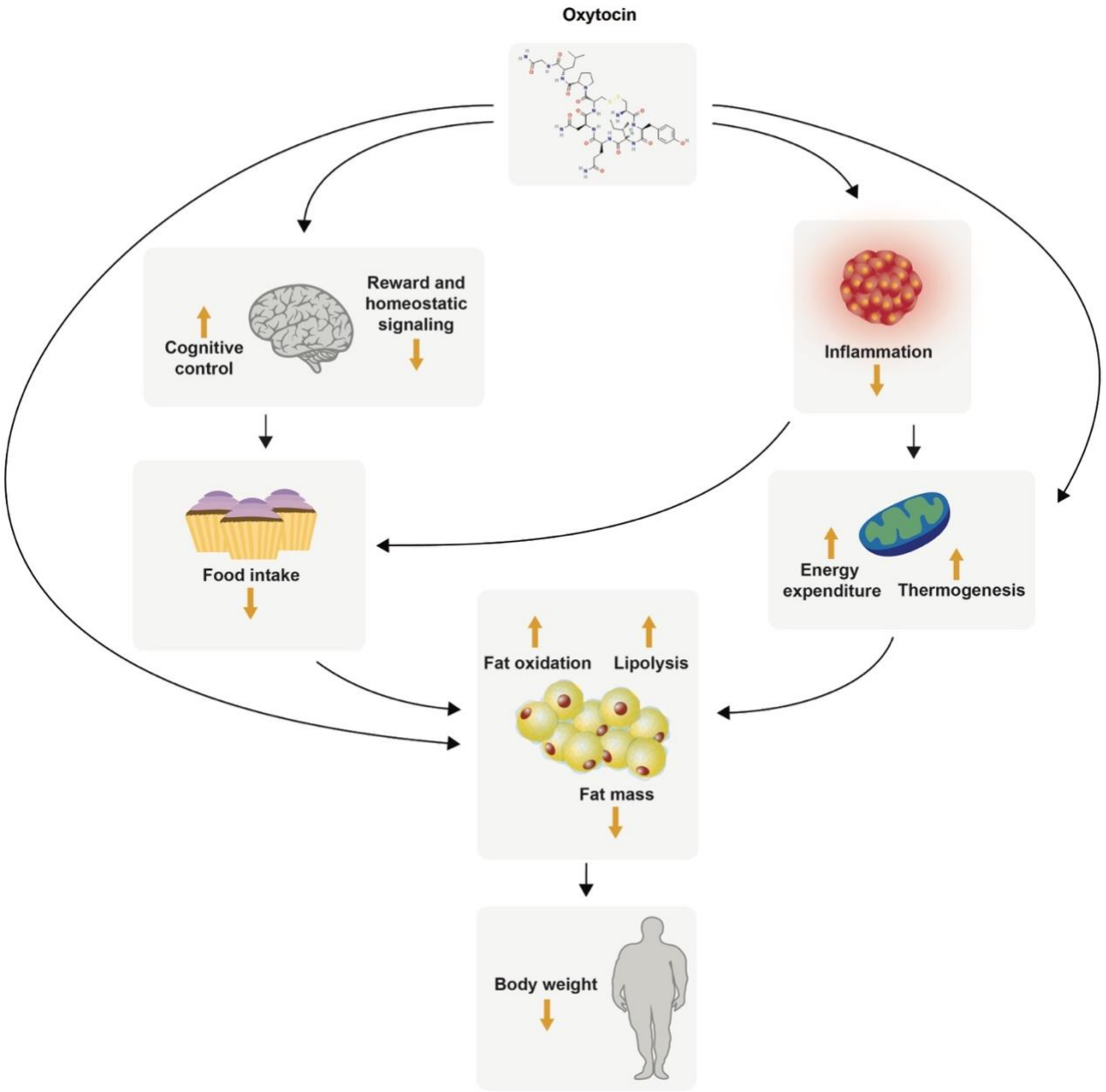
Oxytocin
Reward and homeostatic signaling
Cognitive control
Inflammation
Food intake
Energy expenditure
Thermogenesis
Fat oxidation
Lipolysis
Fat mass
Body weight

**Figure 1. Proposed model of oxytocin effects on eating behavior and metabolism leading to weight loss.**

Intranasal oxytocin may reduce body weight via multiple complementary mechanisms. Oxytocin may suppress caloric intake via reduced activation of reward and homeostatic brain regions and increased activation of cognitive control brain regions. Oxytocin reduces inflammation, which in turn may reduce appetite and increase energy expenditure. Oxytocin increases thermogenesis and energy expenditure. These effects lead to lipolysis, fat oxidation, and reduced fat mass, ultimately resulting in weight loss.

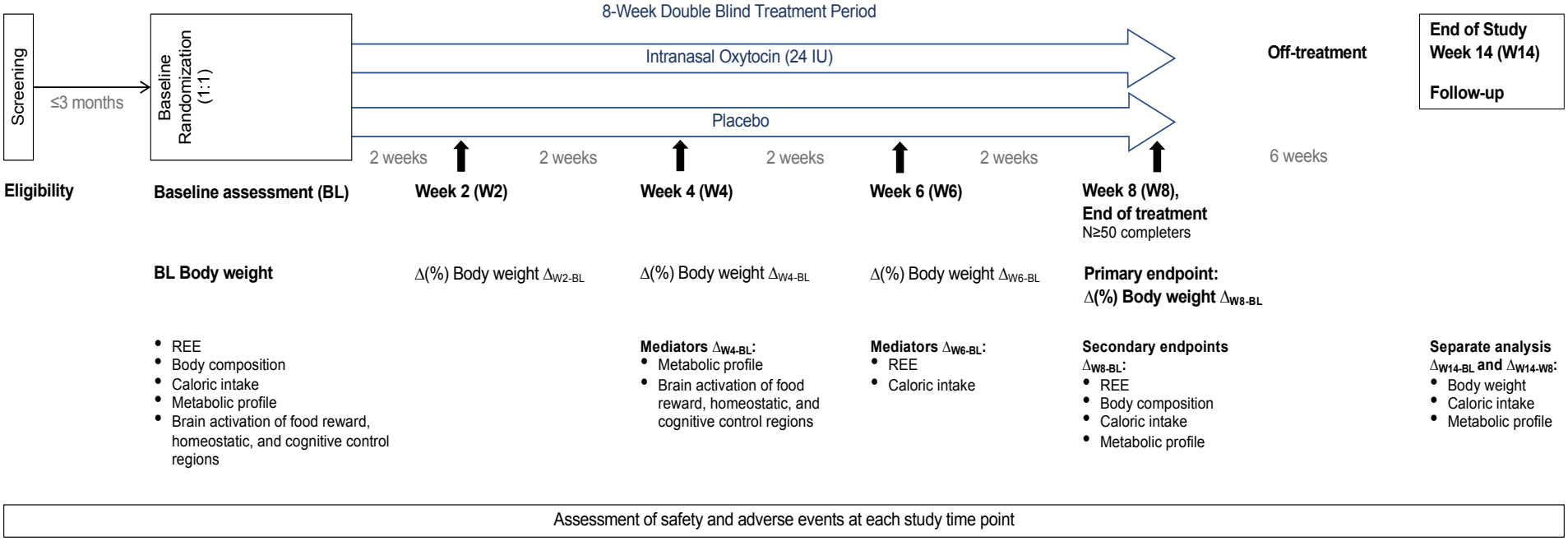


**Figure 2. Study design.**

Abbreviations: IU, international unit; REE, resting energy expenditure; BL, baseline visit; W, week of visit.

*Order of analyses*

## Randomized, double-blind, placebo-controlled clinical trial

**Participants:** F/M, 18-45 years, BMI ≥30kg/$m^2$
**Randomization at BL:** 1 : 1 oxytocin : placebo
**Stratification:** Biological sex, BMI

### 1 – Endpoints

| Variable | Use/Objective | Variable type | Statistical model | Methods for missing data |
|---|---|---|---|---|
| **Change(%) in body weight ($\Delta_{W8\text{-}BL}$)** | **Primary endpoint** | **Continuous** | Mixed-model regression for repeated measurements (MMRM), adjustment for multiple comparisons as appropriate via approach of Hothorn, Bretz, and Westfall | Modified intention-to-treat approach (mITT), Multiple imputation (MI) where applicable |
| REE (adjusted for lean mass) ($\Delta_{W8\text{-}BL}$) | Key secondary endpoint | Continuous | | |
| Total body, visceral, and liver fat mass ($\Delta_{W8\text{-}BL}$) | Key secondary endpoint | Continuous | | |
| Caloric intake ($\Delta_{W6\text{-}BL}$, $\Delta_{W8\text{-}BL}$) | Key secondary endpoint | Continuous | | |
| Metabolic profile ($\Delta_{W8\text{-}BL}$) | Secondary endpoint | Continuous | | |
| Psychological, behavioral and quality of life assessments | Secondary endpoint | Continuous | | |
| Vital signs, QTc on EKG, %IBW, BMI, waist-to-hip ratio | Vital signs, EKG, anthropometrics | Continuous | Descriptive statistics | |
| Age, biological sex, race, ethnicity, coexisting conditions, medication | Demographic and clinical variables | Continuous/ Categorical | | |

### 2 – Mediators

| Mediator variable | Dependent variable | Independent variable | Statistical model |
|---|---|---|---|
| Brain activation ($\Delta_{W4\text{-}BL}$): food reward, homeostatic and cognitive control regions | Caloric intake ($\Delta_{W6\text{-}BL}$) | Treatment (oxytocin vs. placebo) | Regression-based mediation analysis |
| Caloric intake ($\Delta_{W6\text{-}BL}$) | Change(%) in body weight ($\Delta_{W8\text{-}BL}$) | Treatment | |
| REE ($\Delta_{W6\text{-}BL}$) | Change(%) in body weight ($\Delta_{W8\text{-}BL}$) | Treatment | |

### 3 – Safety and Compliance (all study time points)

| Variable | Use/Objective | Variable type | Statistical model |
|---|---|---|---|
| N (%) of in-trial period treatment-emergent adverse events (AE), AE-related discontinuations | Safety endpoint | Categorical/ Count | $\chi^2$ test (oxytocin vs. placebo) for severity categories (mild, moderate, severe) |
| N (%) of total expected study drug doses administered per subject | Treatment adherence | Count | Descriptive statistics: N (%) with compliance ≥ 80% |

### 4 – Off-treatment follow-up (W14)

| Variable | Use/Objective | Variable type | Statistical model |
|---|---|---|---|
| Body weight, anthropometrics, caloric intake, metabolic profile, psychological, behavioral and quality of life assessments, safety | Follow-up (sustained effects) and safety | Continuous | Descriptive statistics, MMRM (separate analysis) |

**Figure 3. Proposed statistical analysis plan.**

Abbreviations: F, female; M, male; BMI, body mass index; BL, baseline visit; W, week of visit; REE, resting energy expenditure; QTc, QT corrected; EKG, electrocardiogram; IBW, ideal body weight.

For study endpoints, main contrasts are stated (e.g., $\Delta_{W8\text{-}BL}$); further contrasts may be investigated as appropriate and according to assessment time points of endpoint variables (Table 2).

## Declaration of interests

☐ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☒The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

E.A.L. served on the scientific advisory board and has a financial interest in OXT Therapeutics, Inc.

E.A.L. also received funding for an investigator-initiated study by Tonix Pharmaceuticals.

All other authors declare no conflicts of interest.