Chronic CNS oxytocin signaling preferentially induces fat loss in high-fat diet-fed rats by enhancing satiety responses and increasing lipid utilization.

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Blevins JE, Thompson BW, Anekinda VT, Ho JM, Graham JL, Roberts ZS, Hwang BH, Ogimoto K, Wolden-Hanson T, Nelson J, Kaiyala KJ, Havel PJ, Bales KL, Morton GJ, Schwartz MW, Baskin DG. Chronic CNS oxytocin signaling preferentially induces fat loss in high-fat diet-fed rats by enhancing satiety responses and increasing lipid utilization. Am J Physiol Regul Integr Comp Physiol 310: R640–R658, 2016. First published January 20, 2016; doi:10.1152/ajpregu.00220.2015.—Based largely on a number of short-term administration studies, growing evidence suggests that central oxytocin is important in the regulation of energy balance. The goal of the current work is to determine whether long-term third ventricular (3V) infusion of oxytocin into the central nervous system (CNS) is effective for obesity prevention and/or treatment in rat models. We found that chronic 3V oxytocin infusion between 21 and 26 days by osmotic minipumps both reduced weight gain associated with the progression of high-fat diet (HFD)-induced obesity and elicited a sustained reduction of fat mass with no decrease of lean mass in rats with established diet-induced obesity. We further demonstrated that these chronic oxytocin effects result from 1) maintenance of energy expenditure at preintervention levels despite ongoing weight loss, 2) a reduction in respiratory quotient, consistent with increased fat oxidation, and 3) an enhanced satiety response to cholecystokinin-8 and associated decrease of meal size. These weight-reducing effects persisted for approximately 10 days after termination of 3V oxytocin administration and occurred independently of whether sucrose was added to the HFD. We conclude that long-term 3V administration of oxytocin to rats can both prevent and treat diet-induced obesity.

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PUBLISHED DATA suggest that in addition to its well-recognized peripheral effects on uterine contraction during parturition and milk ejection during lactation (33), the nonapeptide oxytocin plays an important role in the regulation of energy homeostasis (23, 53, 59, 103, 104). Transgenic mice with deficient oxytocin (18) or oxytocin receptor (OTR) signaling (93) exhibit adult-onset obesity, and copy number variations associated with the OTR gene (OXTR) are linked with an early-onset obesity phenotype in humans (96). Furthermore, impaired oxytocin release within the hypothalamic paraventricular nucleus (PVN) is evident in diet-induced obese (DIO) mice (103), which could lead to defects in peripheral release of oxytocin, and potentially explain the decreased circulating levels in DIO mice (103, 104), genetically obese rodents (32, 73), as well as obese humans and individuals with Type 2 diabetes (74). Moreover, the pathogenesis of Prader-Willi syndrome, a rare human genetic disorder characterized by hyperphagia and severe obesity, is linked to a reduced size and number of PVN oxytocin neurons (91). Importantly, both acute and chronic administration of oxytocin is sufficient to bypass impaired leptin signaling to reduce weight gain or body weight in both DIO (23, 53, 59, 103, 104) and genetically obese rodent models (1, 42, 47, 54, 59, 73) as well as weight loss in DIO rhesus monkeys (10) and humans (105). While collectively these findings are indicative of an important physiological role for oxytocin in energy homeostasis, the mechanisms underlying this function have not been fully elucidated.

The effects of central nervous system (CNS) administration of oxytocin to reduce body weight gain over 7–15 days in DIO mice and rats are known (23, 103, 104); however, its effectiveness to elicit sustained weight loss during chronic administration into the CNS beyond 15 days has not been examined. This is a key unanswered question because the potential efficacy of oxytocin therapy as a treatment for obesity cannot be assessed without understanding the effects of chronic administration. It has been established that the anorectic effects of other peptides, such as exendin-4, peptide YY(3–36), and melanotan II (MTII), dissipate with chronic administration (52, 72, 75, 85). In the case of oxytocin, it is not known whether chronic CNS administration can prevent weight gain associated with the progression of diet-induced obesity or whether weight loss under oxytocin administration is specific for fat while sparing lean mass. Information about these critical questions is a prerequisite for understanding the mechanisms of oxytocin’s effects on energy homeostasis and its potential for therapeutic use to treat obesity. The present article reports studies and data on rats that focus on these key issues.

including sugars (10, 51, 61), but also high-fat diets (HFDs) (23, 53, 59, 103, 104). Conversely, defective oxytocin signaling is linked to increased intake of fat (103, 104) and carbohydrates, including sugar (2, 38, 51, 61, 68) suggesting a physiological role for oxytocin to limit consumption of fat and sugar. However, existing data have failed to establish the extent to which exogenous oxytocin may preferentially reduce consumption of specific macronutrients. We therefore also sought to better understand whether oxytocin-mediated weight loss is dependent on the content of sucrose or fat.

Recent studies indicate that in addition to suppressing food intake, oxytocin may also reduce body weight in rodents and nonhuman primates by increasing energy expenditure (10, 63, 90, 99, 102–104). Conversely, animals with either partial or complete loss in oxytocin signaling show decreased energy expenditure (18, 45, 93, 99, 104) and brown adipose tissue thermogenesis (18, 45, 93). Whether changes of energy expenditure contribute to the antiobesity effect of oxytocin in rats with established diet-induced obesity, however, has not been fully established. Thus we also investigated whether changes of energy expenditure contribute to oxytocin-mediated weight reduction in DIO rats.

Our findings demonstrate that chronic third ventricular (3V) oxytocin infusion (≈21–26 days) both reduces weight gain associated with the progression of diet-induced obesity and elicits sustained weight loss in rats with established diet-induced obesity, that this effect arises from reduced fat mass with no loss of lean mass, and that these chronic oxytocin effects are due, in part, to the fact that energy expenditure is maintained at preintervention levels despite ongoing weight loss. Chronic oxytocin treatment was also associated with evidence of increased fat oxidation as well as an enhanced satiety response to cholecystokinin (CCK) and an associated reduction of meal size. In addition, we show that the effectiveness of oxytocin to reduce weight gain occurs independently of whether sucrose is added to the HFD. After cessation of treatment, weight gain in oxytocin-treated animals remains below vehicle-treated controls for ≈10 days. Finally, we demonstrate that, like chronic CNS administration, chronic subcutaneous administration of oxytocin is sufficient to reduce body weight gain in animals maintained on a HFD at a dose that does not appear to elicit aversive behavioral responses (e.g., nausea or malaise).

**METHODS**

**Animals**

Adult male Sprague-Dawley (SD-SAS and CD<sup>®</sup> IGS) (≈2.5–6 mo/323–887 g) were obtained from Charles River Laboratories International, (Wilmington, MA). CD<sup>®</sup> IGS rats were used for all central infusion studies as well as for the 3-day peripheral infusion study and 2-h saccharin preference ratio study, whereas SD-SAS rats were used for all other peripheral infusion studies. By design, we chose the CD<sup>®</sup> IGS rat model because they grow more rapidly compared with SD-SAS rats and thus are a more suitable model to determine whether chronic increases in CNS oxytocin signaling (>15 days) are sufficient to prevent or delay the progression of diet-induced obesity during the 26-day minipump infusion. SD-SAS rats were used to extend previous studies (59) to determine whether chronic systemic infusions of oxytocin can recapitulate the effects of CNS administration to reduce food intake and weight gain at doses that do not elicit nausea or malaise. All animals were housed individually in Plexiglas cages in a temperature-controlled room under a 12:12-h light-dark cycle (lights off at 1 p.m.). Animals had ad libitum access to water and either a low-fat chow diet containing 13% kcal from fat (Harlan Teklad, Madison, WI) or a HFD containing 60% kcal from fat (Research Diets, D12492, New Brunswick, NJ), unless otherwise stated. A HFD containing 60% kcal from fat lacking sucrose was used in study 7 and study 8 (Research Diets, D08060104; corn starch replaced sucrose in D12492). Kaolin pellets were also purchased from Research Diets. The current research protocols were approved by both the Institutional Animal Care and Use Committee of the Veterans Affairs Puget Sound Health Care System (VAPSHCS) and the University of Washington in accordance with National Institutes of Health Guidelines for the Care and Use of Animals.

**Drug Preparation**

Fresh solutions of oxytocin acetate salt (Bachem Americas, Torrance, CA) were prepared the day of each experiment. Oxytocin was solubilized in sterile water and diluted with sterile saline. CCK-8 (Bachem) was dissolved in saline with 0.1% bovine serum albumin (Bachem Americas).

**3V Cannulations**

Animals were implanted with a cannula within the 3V with a side port that was connected to an osmotic minipump (model 2004, DURECT, Cupertino, CA). Briefly, animals under isoflurane anesthesia were placed in a stereotaxic apparatus with the incisor bar positioned 3.3 mm below the interaural line. A 26-gauge cannula (Plastics One, Roanoke, VA) was stereotaxically positioned into the 3V [8.1 mm anterior to the interaural line, 0 mm lateral to the midline, and 8.6 mm ventral to the skull surface] and secured to the surface of the skull with dental cement and stainless steel screws. A 2.4" piece of plastic tubing (Tygon Microbore Tubing, 0.020" × 0.060"OD, 100 ft/roll; Cole-Parmer, Vernon Hills, IL) was tunneled subcutaneously along the midline of the back and connected to the 21-gauge sidearm osmotic minipump-cannula assembly. A stainless steel 22-gauge pin plug (Instech Laboratories, Plymouth Meeting, PA) was temporarily inserted at the end of the tubing during a 2-wk postoperative recovery period, after which it was replaced by an osmotic minipump (DURECT) containing saline or oxytocin. Animals were treated with the analgesic ketoprofen (5 mg/kg; Fort Dodge Animal Health, Fort Dodge, IA) and the antibiotic baytril (5 mg/kg; Patterson Veterinary, Devens, MA) at the completion of the 3V cannulations and were allowed to recover at least 10 days before implantation of osmotic minipumps.

**Subcutaneous Osmotic Minipump Implantations For Systemic Delivery**

Osmotic minipumps (model 1003D or 2002, DURECT) were implanted subcutaneously along the midline of the back into animals that were placed under isoflurane anesthesia. The interscapular incision was closed with standard metal wound clips.

**CCK-8 Injections**

Intraperitoneal injections were administered to rats via a 1.0-ml syringe with a 25-gauge needle (1 ml/kg injection volume) immediately before the start of the dark cycle, i.e., at a time when the animals normally begin eating and when CCK-8 has a potent effect on reducing food intake.

**Body Composition**

Determinations of lean body mass and fat mass were made on chow and HFD-fed animals, including HFD-fed DIO animals, by quantitative magnetic resonance using an EchoMRI 4-in-1-700 instrument (Echo Medical Systems, Houston, TX) at the VAPSHCS Rodent Metabolic and Behavioral Phenotyping Core. Body composition measurements were also made in DIO rats using an EchoMRI-700 instrument (Echo Medical Systems) at the University of Washington (UW) Nutrition Obesity Research Center Energy Balance and Glucose Metabolism Core.
Indirect Calorimetry, Locomotor Activity, and Meal Pattern Measurements

Energy expenditure, locomotor activity, and meal pattern measures were obtained using a computer-controlled indirect calorimetry system (Prometheon; Sable Systems International, Las Vegas, NV) located in the Energy Balance and Glucose Metabolism Core of the Nutrition Obesity Research Center at the University of Washington as previously described (59). Calorimetry cages (similar to home cages with bedding) were each equipped with water bottles and food hoppers connected to load cells for continuous food and water intake monitoring and housed in a temperature- and humidity-controlled Caron environmental chambers (Caron Products and Services, Marietta, OH). Respiratory quotient (RQ) was calculated as the ratio of CO₂ production over O₂ consumption. Energy expenditure was calculated using the Weir equation: kcal/h = 60 × (0.033941 × V₀₂ + 0.001106 × VCO₂) (95) and expressed in units of kilocalories per hour. Ambulatory activity and meal patterns were determined simultaneously with the collection of the calorimetry data (59). Consecutive adjacent infrared beam breaks in the y-axes, i.e., the length of the cage, were scored as an activity count and a tally was recorded every 10 min. Meal patterns were defined using the following criteria: An individual meal was defined based on our previously published criteria (58) of at least 0.2 g of HFD that was separated from the end of the previous meal by at least 15 min. Daily food intake data were analyzed using a computer program (ExpeData version 1.7.22, Sable Systems International). Meal duration was defined as the time from the beginning to the end of a single meal and the intermeal interval as the time from the end of one meal to the beginning of the next. Average meal size was calculated by dividing the number of feeding bouts (individual meals) by the total amount of food consumed (in grams) over the 72-h test period.

Study Protocols

Study 1: effects of chronic 3V oxytocin infusions on food intake, body weight gain, and body composition in HFD-fed and chow-fed rats. 3V cannulated rats received implantations of 28-day minipumps to infuse vehicle (saline) or oxytocin (16 nmol/day) directly into the brain and were maintained on either chow or HFD for 26 days. Food intake and body weight were recorded daily in ad libitum fed rats over 26 days. Dosing was based on recently published data in a DIO rat model (23).

Study 2: effects of chronic 3V oxytocin infusions on kaolin intake in chow-fed rats. Ad libitum fed rats were maintained on chow before receiving implantations of 3V cannulas and 28-day minipumps to infuse vehicle (saline) or oxytocin (16 nmol/day). The amount of kaolin intake (g) was assessed across 15 days. The placement of kaolin and chow were reversed every other day within each treatment condition.

Study 3: effects of chronic 3V oxytocin infusions on food intake, body weight gain, and body composition in HFD-fed and chow-fed rats. Ad libitum fed rats were maintained on chow or HFD for 2 and 2.5 mo before receiving implantations of 3V cannulas and 28-day minipumps to infuse vehicle (saline) or oxytocin (16 nmol/day), respectively (2-wk interval between 3V cannula and minipump implantations). Food intake and body weight were recorded daily over 26 days.

Study 4: effects of chronic 3V oxytocin infusions on food intake, body weight, and body composition in rats with established diet-induced obesity. Ad libitum fed rats were maintained on chow or HFD for 4 and 4.5 mo before receiving implantations of 3V cannulas and 28-day minipumps to infuse vehicle (saline) or oxytocin (16 nmol/day) over 21 days, respectively. Daily body weight was recorded on days 1–5, 9, 12, 14, 16, and 20–21. Daily food intake was recorded on days 1–5 and 21, whereas 72-h measurements were completed between days 9 and 12.

Study 5: effects of chronic 3V oxytocin infusions on energy expenditure, locomotor activity, RQ, and meal patterns in DIO rats. Ad libitum fed rats were maintained on a HFD for 4 and 4.5 mo before receiving implantations of 3V cannulas and 28-day minipumps to infuse vehicle (saline) or oxytocin (16 nmol/day), respectively. DIO rats (from study 3A) were acclimated to the Sable Systems indirect calorimetry cages for approximately 1 wk before continuous measurement of energy intake for meal pattern analysis (58, 59), energy expenditure (59), locomotor activity (59), and RQ (59) between infusion days 12 and 14.

Study 6: effects of chronic 3V oxytocin infusions on CCK-8-induced satiety in HFD-fed rats. Rats received implantations of 3V cannulas and 28-day minipumps before receiving vehicle (saline) or oxytocin (16 nmol/day) and were maintained on a 6-h fast. After minipump implantations were completed, rats were immediately placed on the HFD. Beginning on infusion day 2, animals received an intraperitoneal injection of either vehicle or CCK-8 (0.25, 0.5, 1, 2 nmol/kg) immediately before the start of the dark cycle at 48-h intervals. Food intake was measured at 0.5 h following access to food and the start of the dark cycle.

Study 7: effects of chronic 3V oxytocin infusions on food intake, body weight gain, and body composition in DIO rats maintained on a HFD lacking sucrose. Ad libitum fed rats were maintained on HFD lacking sucrose for 4 and 4.5 mo before receiving implantations of 3V cannulas and 28-day minipumps to infuse vehicle (saline) or oxytocin (16 nmol/day) over 28 days, respectively. Daily food intake and body weight were recorded in 3-h fasted animals over 28 days.

Study 8: effects of treatment cessation on food intake, body weight gain, and body composition in DIO rats maintained on a HFD lacking sucrose. After completion of study 7 on day 28, a subset of animals was euthanized and the remaining DIO animals had minipumps removed on day 38. Daily food intake and body weight were recorded in 3-h fasted animals for an additional 28 days.

Study 9: effects of chronic subcutaneous oxytocin infusions on food intake, body weight gain, 2-h saccharin preference ratio, and kaolin intake in chow-fed rats. Ad libitum fed rats were implanted with 3- (0, 50, 100, 200 nmol oxytocin/day) or 14-day osmotic minipumps (0, 50 nmol oxytocin/day). Daily food intake and body weight were measured across the 3- and 12-day infusion period. Dosing was based on recently published data in a DIO rat model (23).

Study 10: effects of chronic subcutaneous oxytocin infusions on body weight gain in HFD-fed and chow-fed rats. Ad libitum fed rats that had been maintained on chow were implanted subcutaneously with 14-day osmotic minipumps to infuse vehicle (saline) or oxytocin (50 nmol/day) systemically. Animals were then placed on either chow or HFD for the remainder of the 13-day infusion period. Daily food intake and body weight were measured across the 13-day infusion period.

Blood Collection

Blood was collected in either 3- (study 4) or 6-h (study 10) fasted rats at the end of the light cycle within a 2-h window (11:00 a.m.–1:00 p.m.). Treatment groups were counterbalanced at time of
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euthanasia to avoid bias. Blood samples (3 mL) were collected immedi-
ately before transcardiac perfusion by cardiac puncture in chilled
serum separator tubes (SST-amber; Becton-Dickinson, Franklin
Lakes, NJ). Whole blood was centrifuged at 6,000 rpm for 1.5 min at
4°C; serum was removed, aliquoted, and stored at −80°C for subse-
quent analysis.

Serum Hormone Measurements

Serum adiponectin and leptin were measured using electrochemi-
luminescence detection [Meso Scale Discovery (MSD), Rockville,
MD] using established procedures (17). Intra-assay coefficient of
variation (CV) for adiponectin and leptin were 0.8 and 3.6%, respec-
tively. The limits of detectability for these assays are as follows:
adiponectin (0.11–200 ng/ml) and leptin (0.055–100 ng/ml). Serum
fibroblast growth factor-21 (FGF-21) (R&D Systems, Minneapolis,
MN), irisin (AdipoGen, San Diego, CA), and serum oxytocin (ENZO
Life Sciences, Farmingdale, NY) levels were determined by ELISA.
The intra-assay CV for FGF-21, irisin, and oxytocin were 3.3, 8.0, and
6.0%, respectively; the limits of detectability were 13.4–2,000 pg/ml
(FGF-21), 0.1–5 pg/ml (irisin) and 15–1,000 pg/ml (oxytocin). ENZO
Life Sciences recently switched to a different rabbit polyclonal de-
tection antibody and, based on the information available from the and
comparisons with other assays across several species, results in at
least 1.78-fold higher baseline levels.

Glucose and Lipid Measurements

Blood was collected for glucose measurements by tail vein nick
and measured by the glucometer using the AlphaTRAK 2 blood
glucose monitoring system (Abbott Laboratories, Abbott Park, IL)
(11). Total cholesterol, triglycerides (TGs), and free fatty acids
(FFAs) were measured using an enzymatic-based kit (Wako Chemi-
cals, Richmond, VA). Intra-assay CVs for total cholesterol, FFAs, and
TGs were 2.9, 2.2, and 3.5%, respectively. These assay procedures
have been validated for rodents (22).

Statistical Analyses

All results are expressed as means ± SE. Comparisons between
multiple groups involving between-subjects designs were made using one-
or two-way ANOVA as appropriate, followed by a post hoc
Fisher’s least significant difference test. Comparisons involving with-
in-subjects designs were made using a one-way repeated-measures
ANOVA followed by a post hoc Fisher’s least significant difference
test. Analyses were performed using the statistical program SYSTAT
(Systat Software, Point Richmond, CA). Differences were considered
significant at P < 0.05, two-tailed.

RESULTS

Studies 1–2: Effects of Chronic 3V Oxytocin Infusions on
Food Intake, Body Weight Gain, and Body Composition in
HFD-Fed and Chow-Fed Rats

We first determined whether the effects of long-term (~26 days)
infusion of oxytocin into the CNS was sufficient to prevent body
weight gain associated with the progression of diet-induced obesity.
At study onset, HFD-fed animals had lower body weights relative to
chow-fed animals (HFD: 362 ± 7 g; chow: 408 ± 6 g; P < 0.05),
but there was no significant difference in body adiposity between
groups (HFD: 29.7 ± 1.7 g; chow: 28.2 ± 1.6 g).

High-fat diet. Relative to HFD-fed animals that received vehicle,
there was a significant effect of 3V oxytocin to attenuate weight gain
over a period of 26 days (Fig. 1A; P < 0.05), and 3V oxytocin also
tended to reduce body weight throughout the study (vehicle: 487 ±
16 g; oxytocin: 415 ± 16 g) (P = 0.058). There was a significant
main effect of oxytocin to selectively reduce body fat (data not
shown; P < 0.05) as well as body adiposity gain (Fig. 1B; P < 0.05)
and serum leptin levels (data not shown; P < 0.05), but it had no
significant effect on lean mass gain. The effect of oxytocin to reduce
body weight gain and body adiposity was mediated, at least in part,
by a sustained reduction of energy intake (Fig. 1, C and D; P < 0.05).
To determine whether this suppression of food intake was secondary
to an aversive effect of central oxytocin, we measured its effect on
kaolin intake over the 15-day testing period in a separate cohort of
chow-fed rats that received the same dose of oxytocin. Chronic 3V
administration of oxytocin failed to increase kaolin consumption
(data not shown). These findings extend a previous report showing
that acute 3V administration of oxytocin has no effect on kaolin
consumption in chow-fed mice (103).

Chow. We then examined if these effects of oxytocin to reduce
weight gain and food intake in HFD-fed rats were maintained in
chow-fed control rats. By comparison, while there was only a tran-
sient effect of oxytocin to reduce body weight gain (Fig. 1E; P <
0.05), there was no significant main effect of oxytocin to reduce body
weight in chow-fed animals (490 ± 12 g) relative to vehicle controls
(475 ± 15 g). Similarly, there was no significant main effect of
chronic 3V oxytocin to reduce body adiposity gain (Fig. 1F) or
serum leptin (data not shown) in chow-fed animals and in fact, oxytocin
caused an increase in lean mass gain relative to vehicle (P < 0.05) in
the absence of any effect on food intake (Fig. 1, G and H).

Two-way ANOVA revealed a significant diet×drug interaction
of oxytocin to reduce weight gain across days 4–26 and
to reduce energy intake in animals on HFD relative to chow-
fed controls on days 1–4, 6–10, 15, 18, 20–23, and 25–26
(P < 0.05). This interactive effect was maintained when
weekly energy intake data were compiled across weeks 1–4
(P < 0.05). There was a near-significant diet×drug interactive
effect of oxytocin to reduce weight gain on days 1 (P = 0.091)
and 3 (P = 0.054) as well as on energy intake on days 5 (P =
0.075), 14 (P = 0.051), 16 (P = 0.052), and 17 (P = 0.076).
In addition, there was a significant main effect of oxytocin to
reduce total fat [F(1,24) = 7.933; P < 0.05] and fat mass gain
[F(1,24) = 12.204; P < 0.05] as well as an interactive effect
of oxytocin to reduce both total fat [F(1,24) = 5.636; P < 0.05]
and fat mass gain in HFD-fed animals relative to chow-
fed controls [F(1,24) = 10.101; P < 0.05]. Overall, the data
indicate that augmented CNS oxytocin signaling elicits sus-
tained reductions in both body weight gain and body adiposity
while preserving lean mass in HFD-fed rats, effects that were
mediated, in part, through a reduction in energy intake. In
contrast, increased 3V oxytocin administration was largely
ineffective on these outcomes in chow-fed rats.

Study 3: Effects of Chronic 3V Oxytocin Infusions on Food
Intake, Body Weight Gain, and Body Composition in
HFD-Fed and Chow-Fed Rats

Following the previous experiment, we then asked if 1) the
effects of oxytocin to reduce food intake and body weight gain
involved a potential macronutrient preference toward fat, and 2)
the enhanced responsiveness to oxytocin among HFD-fed animals
was maintained throughout the progression of diet-induced obe-
sity. At study onset, following exposure to chow or HFD for 2.5
mo, there was no significant difference in body weight between
HFD-fed animals and chow-fed animals (HFD: 531 ± 16 g;
chow: 544 ± 13 g), but HFD-fed animals had greater body
adiposity (HFD: 131 ± 12 g; chow: 67 ± 4 g; P < 0.05).

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Fig. 1. Effects of chronic third ventricular (3V) oxytocin infusions on food intake, body weight gain, and body composition in high-fat diet (HFD)-fed and chow-fed rats. Ad libitum fed rats were either placed on HFD (60% kcal from fat; N = 5–6/group) or maintained on chow (N = 8–9/group) at onset of continuous infusions of vehicle or oxytocin (16 nmol/day). A and E: change in body weight gain in animals maintained on HFD or chow; B and F: change in fat mass and lean mass in animals maintained on HFD or chow; C and G: change in daily energy intake (kcal/day) in animals maintained on HFD or chow; D and H: change in weekly energy intake (kcal/wk) in animals maintained on HFD or chow. Note week 4 data represent data across only 5 days. Data are expressed as means ± SE. *P < 0.05 oxytocin vs. vehicle.
High-fat diet. Relative to HFD-fed animals that received vehicle, central administration of oxytocin reduced weight gain throughout the infusion period (Fig. 2A; \( P < 0.05 \)), but there was no significant effect on body weight (vehicle: 586 ± 29 g; oxytocin: 553 ± 20 g). These effects of oxytocin were associated with a reduction in body adiposity gain (Fig. 2B; \( P < 0.05 \)), serum leptin (\( P < 0.05 \); data not shown), and a slight increase, and therefore protective effect, on lean mass (\( P < 0.05 \)). This effect was accompanied by a reduction in daily energy intake (kcal/day); \( D \) and \( H \): change in weekly energy intake (kcal/wk) in animals maintained on HFD or chow. Note week 4 data represent data across only 5 days. Data are expressed as means ± SE. *\( P < 0.05 \) oxytocin vs. vehicle.
energy intake that was maintained until the last week of the study (Fig. 2, C and D). A lower dose of oxytocin (1.6 nmol/day) was also examined in HFD-fed rats, but it was ineffective in reducing weight gain, fat mass, or energy intake (data not shown).

Chow. We next asked if the effects of oxytocin to reduce weight gain and food intake were attenuated in weight-matched chow-fed control rats. In contrast to its effect in HFD-fed rats and consistent with our earlier observations, 3V oxytocin (16 nmol/day) reduced weight gain only on days 2 and 3 (Fig. 2E; $P < 0.05$) but had no significant effect on body weight in chow-fed rats relative to vehicle controls (vehicle: 583 ± 22 g; oxytocin: 593 ± 21 g). In addition, there was no significant effect of oxytocin to reduce fat ($P = 0.053$) or lean mass gain (Fig. 2F), serum leptin levels (data not shown), or energy intake (Fig. 2, G and H).

Two-way ANOVA revealed there was a significant diet×drug interactive effect for oxytocin to preferentially reduce weight gain in HFD-fed rats relative to chow-fed controls across days 5–6, 11, 13–16, and 22–26 ($P < 0.05$). There was also a near-significant diet×drug interaction of oxytocin to reduce weight gain on days 4 ($P = 0.055$), 7 ($P = 0.078$), 8 ($P = 0.073$), 9 ($P = 0.051$), 10 ($P = 0.096$), 12 ($P = 0.084$), 18 ($P = 0.091$), 19 ($P = 0.055$), 20 ($P = 0.050$), and 21 ($P = 0.106$). In addition, there was a near-significant diet×drug interactive effect for oxytocin to reduce energy intake in HFD-fed animals relative to chow-fed controls on day 22 of the infusion period ($P = 0.060$). Collectively, the data showed that there was enhanced sensitivity to oxytocin to elicit sustained reductions in weight gain and energy intake in weight-matched preobese rats through a specific reduction in body fat in HFD-fed rats at a dose that was largely ineffective in chow-fed rats.

Study 4: Effects of Chronic 3V Oxytocin Infusions on Food Intake, Body Weight Gain, Body Weight, and Body Composition in Rats With Established Diet-Induced Obesity

Following the previous experiment we next asked if long-term infusions of oxytocin into the 3V reduce food intake and body weight in an established DIO rat model. By design, DIO rats weighed more (706 ± 20 g) and had increased adiposity (205 ± 13 g) relative to chow-fed rats (583 ± 15 g; 94 ± 7 g) ($P < 0.05$) at study onset (after exposure to chow or HFD for 4.5 mo). We have found that 3–4 mo of exposure to our HFD is required to elicit diet-induced obesity in male CD\textsuperscript{®} IGS rats (both an increase in body weight and body adiposity relative to age-matched chow-fed control rats).

High-fat diet. DIO rats that received 3V oxytocin treatment over 21 days experienced much greater weight loss than 3V vehicle-treated controls (Fig. 3, A and B; $P < 0.05$). Consistent with our previous studies, 3V oxytocin also reduced body weight gain relative to vehicle treatment throughout the infusion period (Fig. 3B; $P < 0.05$). Oxytocin-elicited weight loss was attributed to a sustained reduction of body fat stores and was accompanied by a corresponding decrease of serum leptin concentrations ($P < 0.05$; Table 1) and a slight increase, and therefore protective effect, on lean mass (Fig. 3C; $P < 0.05$). These effects were also accompanied by a transient reduction of energy intake (Fig. 3D) during days 1–5 that persisted through days 9–12 (vehicle: 222 ± 6 kcal; oxytocin: 195 ± 10 kcal) ($P < 0.05$). However, these effects were no longer significant by day 21.

Chow. In contrast, oxytocin failed to reduce body weight or body weight gain in age-matched chow-fed control rats (Fig. 3, E and F), although body adiposity gain following 3V oxytocin was reduced relative to vehicle treatment (Fig. 3G; $P < 0.05$). Oxytocin also failed to significantly reduce energy intake throughout the infusion period in chow-fed animals (Fig. 3H).

Two-way ANOVA revealed a significant diet×drug interactive effect of 3V oxytocin to produce a more pronounced reduction of body weight in addition to body weight gain in HFD-fed rats compared with chow-fed rats across days 2–5, 9, 12, 14, 16, and 20–21 as well as energy intake in HFD-fed rats relative to chow-fed rats across days 4 and 5 ($P < 0.05$). There was a near-significant diet×drug interactive effect of 3V oxytocin to suppress energy intake on days 2 ($P = 0.132$) and 3 ($P = 0.063$). Overall, these findings implicate a preferential effect of oxytocin to reduce energy intake and produce sustained weight loss by decreasing fat mass while sparing lean mass in DIO rats at a dose that was largely ineffective in chow-fed rats.

Study 5: Effects of Chronic 3V Oxytocin Infusions on Energy Expenditure, Locomotor Activity, RQ, and Meal Patterns in DIO Rats

Energy expenditure. To gain insight into the mechanisms whereby oxytocin induces weight loss in rats with diet-induced obesity induced by a HFD, we measured energy expenditure using respirometric indirect calorimetry across 72 h during oxytocin or vehicle infusions spanning days 12–14 of treatment. We found that, despite a marked effect of oxytocin to induce weight loss at this time point, total energy expenditure was similar to that of vehicle-treated controls ($P = NS$; Fig. 4A/Fig. 4D) and there was no significant main effect of oxytocin to increase energy expenditure relative to vehicle treatment during the light cycle ($F_{(1,13)} = 0.447$), dark cycle ($F_{(1,13)} = 0.224$), or total daily energy expenditure ($F_{(1,13)} = 0.337$) (Fig. 4D). Energy expenditure data were examined for possible confounding by differences in measures of body size (43, 44). There were no significant correlations or suggestive trends when any measure of energy expenditure was regressed on any measure of body size either within groups or for both groups combined. Accordingly, we did not adjust energy expenditure for body mass, fat mass, or lean mass.

Locomotor activity. We next examined the effectiveness of long-term oxytocin infusions to alter locomotor activity in DIO rats. Although oxytocin treatment appeared to reduce activity during the early part of the dark cycle ($P < 0.05$; Fig. 4B/4E), consistent with earlier reports (23, 53), there was no significant main effect of oxytocin to increase activity during the light cycle ($F_{(1,13)} = 0.822$), dark cycle ($F_{(1,13)} = 0.638$) or when measured as total daily activity ($F_{(1,13)} = 0.779$) (Fig. 4E).

Respiratory quotient. Our next goal was to measure RQ to assess if long-term administration of oxytocin into the 3V may impact fat oxidation in DIO rats. In rats with diet-induced obesity, 3V oxytocin treatment lowered RQ during both the light and dark period ($P < 0.05$; Fig. 4C/4F), suggesting an effect of oxytocin to increase fatty acid utilization. Consistent with this, we found a significant main effect of oxytocin to reduce RQ during the light cycle ($F_{(1,13)} = 7.710, P < 0.05$), dark cycle ($F_{(1,13)} = 5.610, P < 0.05$), as well as total daily

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Fig. 3. Effects of chronic 3V oxytocin infusions on food intake, body weight, and body composition in rats with established diet-induced obesity. Ad libitum fed rats were either maintained on HFD (60% kcal from fat; \( N = 7–8/\text{group} \)) or chow (\( N = 7–8/\text{group} \)) for 4.5 mo before receiving continuous infusions of vehicle or oxytocin (16 nmol/day). A and E: change in body weight in HFD-fed diet-induce obese (DIO) or chow-fed control animals; B and F: change in body weight gain in HFD-fed DIO or chow-fed control animals; C and G: change in fat mass and lean mass in HFD-fed DIO or chow-fed control animals; D and H: daily energy intake (kcal/day) in HFD-fed DIO or chow-fed control animals. Data are expressed as means ± SE. *\( P < 0.05 \) oxytocin vs. vehicle.
Serum measurements following 3V infusions of oxytocin or vehicle in chow-fed or HFD-fed DIO rats

Table 1. Serum measurements following 3V infusions of oxytocin or vehicle in chow-fed or HFD-fed DIO rats

<table>
<thead>
<tr>
<th>3V Treatment</th>
<th>Vehicle</th>
<th>Oxytocin</th>
<th>HFD</th>
<th>Oxytocin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leptin, ng/ml</td>
<td>8.5 ± 2.0a</td>
<td>4.7 ± 0.5a</td>
<td>20.5 ± 2.6b</td>
<td>11.6 ± 2.1c</td>
</tr>
<tr>
<td>Adiponectin, µg/ml</td>
<td>5.4 ± 0.5ab</td>
<td>5.0 ± 0.3c</td>
<td>6.4 ± 0.4b</td>
<td>6.0 ± 0.4b</td>
</tr>
<tr>
<td>FGF-21, pg/ml</td>
<td>67.9 ± 12.8a</td>
<td>80.5 ± 16b</td>
<td>119.9 ± 13.3b</td>
<td>128 ± 14.1a</td>
</tr>
<tr>
<td>Irisin µg/ml</td>
<td>4.9 ± 0.2</td>
<td>4.9 ± 0.4</td>
<td>4.9 ± 0.5</td>
<td>5.3 ± 0.3</td>
</tr>
<tr>
<td>Blood glucose, mg/dl</td>
<td>97 ± 4.3a</td>
<td>96 ± 3.0b</td>
<td>108 ± 2.4a</td>
<td>107.4 ± 3.1b</td>
</tr>
<tr>
<td>FFA, mcg/l</td>
<td>0.3 ± 0</td>
<td>0.2 ± 0</td>
<td>0.3 ± 0</td>
<td>0.4 ± 0.1</td>
</tr>
<tr>
<td>TG, mg/dl</td>
<td>79.6 ± 10.9a</td>
<td>50 ± 7.0b</td>
<td>44.9 ± 5.5b</td>
<td>34.5 ± 3.6b</td>
</tr>
<tr>
<td>Total cholesterol, mg/dl</td>
<td>66.5 ± 3.3a</td>
<td>64 ± 2.7a</td>
<td>98.9 ± 4.7b</td>
<td>84.3 ± 5.5a</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 8–10/group. 3V, third ventricular; HFD, high-fat diet; FGF-21, fibroblast growth factor-21; FFA, free fatty acids; TG, triglyceride. Different letters denote significant differences between treatments. Shared letters are not significantly different from one another.

RQ $F(1,13) = 7.087, P < 0.05$ (Fig. 4F), consistent with other reports (23, 49, 53).

Serum hormones in chow-fed and HFD-fed DIO rats. There was an increase of serum leptin, FGF-21, blood glucose, and total cholesterol in vehicle-treated DIO animals relative to chow-fed vehicle-treated animals ($P < 0.05$; Table 1). Consistent with earlier reports and its selective action to reduce fat mass, oxytocin treatment was associated with a reduction in serum leptin in DIO animals (23) but not in chow-fed control animals. Oxytocin treatment also reduced serum total cholesterol levels ($P < 0.05$) in DIO animals. In addition, oxytocin treatment was not associated with a significant change in blood glucose, FFA, TG, adiponectin, irisin, or FGF-21.

Meal patterns. Given previous evidence that endogenous oxytocin signaling enhances meal-related satiety in chow-fed animals (6, 9, 57, 67), we sought to extend these findings by determining the effect of exogenous oxytocin on meal patterning in HFD-fed DIO animals that were housed in indirect calorimetry chambers equipped to continuously measure energy intake. Chronic 3V infusion of oxytocin into HFD-fed DIO rats reduced energy intake during both the light and dark cycle (Fig. 5A). This effect was due to a reduction in meal size (Fig. 5B) with no change in meal frequency (Fig. 5C) such that the total number of meals consumed was not different between groups with diet-induced obesity. Further analysis revealed a significant main effect of oxytocin to reduce meal size during the light cycle [$F(1,12) = 8.831, P < 0.05$], dark cycle [$F(1,12) = 5.173, P < 0.05$], and throughout the entire day [$F(1,12) = 9.778, P < 0.05$]. This effect of oxytocin treatment was associated with a shorter meal duration (Fig. 5D) and longer intermeal interval (Fig. 5E). Overall, these findings indicate that the effect of 3V oxytocin to reduce food intake in DIO rats occurs, in part, via reduced meal size without changing meal frequency ($P < 0.05$).

Study 6. Effects of Chronic 3V Oxytocin Infusions on CCK-8-Induced Satiety in HFD-Fed Rats

From these observations, in a separate cohort of rats, we next determined whether increased oxytocin signaling enhanced the satiety response to CCK-8 in animals maintained on a HFD. As others have reported (21, 81) we found that low doses of CCK-8 (0.25, 0.5 nmol/kg) failed to reduce food intake in animals maintained on a HFD. In the presence of increased oxytocin signaling, however, the ability of these lower doses of CCK-8 (0.25, 0.5 nmol/kg) to reduce food intake was markedly enhanced. At higher doses of CCK-8, however, the addition of oxytocin failed to enhance the effectiveness of CCK-8 to reduce food intake (Fig. 5F). Our findings show that there was an overall significant main effect of CCK-8 to $I$ reduce 0.5 h food intake in the presence of oxytocin treatment [$F(4,56) = 3.483, P < 0.05$], and 2) reduce 0.5 h food intake in the presence of oxytocin [$F(4,52) = 3.141, P < 0.05$].

Two-way ANOVA revealed a near-significant main effect of CCK-8 (0.5 nmol/kg) [$F(1,54) = 3.822, P = 0.056$], a significant main effect of oxytocin [$F(1,54) = 10.147, P < 0.05$], and a near-significant interactive effect of oxytocin and CCK-8 to suppress 0.5 h food intake [$F(1,54) = 2.433, P = 0.125$]. These findings support the hypothesis that increased oxytocin signaling enhances sensitivity to the meal-related satiety response to lower doses of CCK-8 in HFD-fed DIO rats.

Study 7: Effects of Chronic 3V Oxytocin Infusions on Food Intake, Body Weight Gain, and Body Composition in DIO Rats Maintained on a HFD Lacking Sucrose

Our next goal was to examine if our earlier findings showing oxytocin to preferentially reduce weight gain in DIO rats relative to chow-fed controls (study 4) was due, in part, to sucrose having been a component in the HFD (2, 68). Therefore, we examined the effectiveness of central oxytocin to reduce food intake and weight gain in DIO rats maintained on a HFD lacking sucrose. DIO rats maintained on the HFD lacking sucrose had similar body weights [HFD (−sucrose): 170 ± 21 g vs. HFD (+sucrose): 706 ± 20 g] and, as expected, tended to have slightly reduced adiposity [HFD (−sucrose): 170 ± 17 g vs. HFD (+sucrose): 205 ± 13 g] ($P = 0.123$) relative to DIO rats maintained on a HFD containing sucrose (study 4) after exposure to each respective HFD for 4.5 mos. Central administration of oxytocin reduced weight gain throughout the infusion period (Fig. 6A; $P < 0.05$), an effect associated with a reduction in fat mass and a slight increase, and therefore protective effect, on lean mass (Fig. 6B; $P < 0.05$). This effect was also accompanied by a reduction in energy intake that was maintained until the third week of the study (Fig. 6, C and D).

Study 8: Effects of Treatment Cessation on Food Intake, Body Weight Gain, and Body Composition in DIO Rats Maintained on a HFD Lacking Sucrose

To determine the extent to which oxytocin-elicited reductions in weight gain, body adiposity, and food intake persist following the cessation of treatment, we tracked daily food intake and body weight in animals following minipump removal in a subset of animals used in study 7. Body composition was measured at the end of the washout...
study. Oxytocin continued to reduce weight gain in this subset of animals between infusion days 29–39 (Fig. 6E). After cessation of treatment (minipump removal on day 38), weight gain in oxytocin-treated animals remained below that of vehicle-treated controls for ≈10 days (Fig. 6E; P < 0.05). This effect was associated with an increase in body adiposity (Fig. 6F; P < 0.05) and energy intake that was maintained through the second week following minipump removal (Fig. 6, G and H).

Study 9: Effects of Chronic Subcutaneous Oxytocin Infusions on Food Intake, Body Weight Gain, 2-h Saccharin Preference Ratio, and Kaolin Intake in Chow-Fed Rats

As a first step to assessing its translational potential to prevent or treat obesity, we determined whether chronic systemic administration of oxytocin was effective at reducing weight gain without inducing nausea or food aversion. To address this, we sought to identify the lowest dose of oxytocin that, following 3-day subcutaneous infusion, reduced body weight gain without producing nausea. Oxytocin reduced weight gain beginning on day 1, and this effect persisted throughout the 3-day measurement period (Fig. 7A). The effect of subcutaneous oxytocin on food intake was less clear at these low doses. Oxytocin tended to reduce energy intake on day 2 at 50 (P = 0.055), 100 (P = 0.1), and 200 nmol/day (P = 0.092; Fig. 7B). Oxytocin (50, 100, 200 nmol/day) also failed to significantly alter 2-h saccharin preference ratios relative to vehicle (Fig. 7C), and oxytocin (50 nmol/day) also failed to significantly increase kaolin consumption throughout the 12-day analysis period (Fig. 7D). Overall, the data indicate that chronic subcutaneous administration of oxytocin, at doses that reduce weight gain and food intake in chow-fed rats, is unlikely to elicit nausea. These findings extend other reports

Fig. 4. Effects of chronic 3V oxytocin infusions on energy expenditure, locomotor activity, and respiratory quotient (RQ) in DIO rats. Ad libitum fed DIO rats received continuous infusions of vehicle or oxytocin (16 nmol/day) over 21 days and were maintained on HFD (60% kcal from fat; N = 7–8/group). A: 24-h profile of energy expenditure during vehicle or oxytocin infusions; B: energy expenditure measurements during the light, dark, and throughout the 24-h period; C: 24-h profile of locomotor activity; D: locomotor activity measurement during the light, dark, and throughout the 24-h period; E: 24-h profile of RQ; F: RQ measurement during the light, dark, and throughout the 24-h period. Data are expressed as means ± SE. *P < 0.05 oxytocin vs. vehicle.
showing that acute bolus injections of oxytocin into the CNS or periphery fail to increase kaolin intake (103) or elicit a conditioned taste aversion (42, 63, 103).

Study 10: Effects of Chronic Subcutaneous Oxytocin Infusions on Body Weight Gain in HFD-Fed and Chow-Fed Rats

After completion of the previous experiments, we next asked if chronic 13-day subcutaneous administration of oxytocin (50 nmol/day), at a dose that failed to induce nausea (study 9), was sufficient to mimic the effects of 3V administration to prevent diet-induced obesity.

High-fat diet. In rats fed a HFD, subcutaneous administration of oxytocin significantly reduced body weight gain throughout the 13-day infusion period (Fig. 8A; P < 0.05). These effects were associated with a transient reduction in food intake on days 2 and 4 (Fig. 8B).

Chow. By comparison, oxytocin produced only a transient reduction of weight gain of chow-fed rats on days 1–3 (P < 0.05; Fig. 8C), an effect that was not associated with a significant reduction in energy intake (Fig. 8D).

There was a significant diet×drug interactive effect of oxytocin to preferentially reduce body weight gain on day 4 (P < 0.05) and a near-significant interactive effect on days 3 and 7 (P = 0.108). Similarly, there was also an interactive effect of oxytocin to preferentially reduce energy intake in HFD-fed rats relative to chow-fed controls across days 2 and 4 (P < 0.05) and a near-significant interactive effect on day 7 (P = 0.091). We also compared the effects of chronic 14-day infusions of vehicle and oxytocin in a separate group of chow-fed rats (N = 10/group). Oxytocin reduced body weight gain over days 9–10 and 12–14 (P < 0.05). Consistent with our earlier findings oxytocin had a transient effect to reduce food intake over days 2–3 (P < 0.05). When comparing chow-fed rats to the HFD-fed group there tended to be a significant diet×drug interactive effect of oxytocin to preferentially reduce body weight gain (P = 0.070) as well as reduce energy intake on day 4 (P < 0.05). Overall, the data showed that there was an enhanced effectiveness of oxytocin, following chronic subcutaneous administration, to reduce weight gain and energy consumption in HFD-fed rats at a dose that was largely ineffective in chow-fed rats.

Fig. 5. A–F: effects of chronic 3V oxytocin infusions on meal patterns in DIO rats or on cholecystokinin (CCK-8)-elicited satiety in HFD-fed rats. Ad libitum fed DIO rats received continuous infusions of vehicle or oxytocin (16 nmol/day) and were maintained on HFD (60% kcal from fat; N = 7–8/group). A: change in energy intake (kcal/day); B: meal size; C: meal frequency; D: meal duration; E: intermeal interval; F: rats (6-h fasted) received continuous infusions of oxytocin vehicle or oxytocin (16 nmol/day) in combination with intraperitoneal injections of CCK-8 (immediately prior to start of dark cycle). Data are expressed as means ± SE. A–E: *P < 0.05 oxytocin vs. vehicle, F: *P < 0.05 oxytocin vehicle + CCK-8 vs. oxytocin + CCK-8.
Serum Hormones in Chow-Fed and HFD-Fed Rats

There was an increase of serum leptin in vehicle-treated HFD-fed animals relative to chow-fed vehicle-treated animals ($P < 0.05$; Table 2). In contrast to our central infusion data from DIO rats, peripheral infusions of OT failed to reduce serum leptin in preobese rats maintained on a HFD. As expected (23, 25, 73, 92), serum oxytocin levels were lower among rats receiving subcutaneous vehicle relative to chronic infusions of OT.

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**Fig. 6.** A–D: effects of chronic 3V oxytocin infusions on food intake, body weight gain, and body composition in DIO rats maintained on a HFD lacking sucrose. DIO rats were maintained on HFD (60% kcal from fat; $N = 8–10$/group) for 4.5 mo before receiving continuous infusions of vehicle or oxytocin (16 nmol/day). A subset of these rats was euthanized on day 28 while minipumps were left in place in the remaining rats until day 38 as indicated below. E–H: effects of treatment cessation on food intake, body weight gain, and body composition in DIO rats maintained on a HFD lacking sucrose. DIO rats were maintained on HFD (60% kcal from fat; $N = 3–5$/group) for the duration of the washout study. Minipumps were removed on day 38 (indicated by arrow) from DIO rats that had previously received continuous infusions of vehicle or oxytocin (16 nmol/day). A and E: change in body weight gain; B and F: change in fat mass and lean mass; C and G: daily energy intake (kcal/day); D and H: change in weekly energy intake (kcal/wk). Data are expressed as means ± SE. *$P < 0.05$ oxytocin vs. vehicle. For Washout, *$P < 0.05$ postoxytocin vs. postvehicle.
to oxytocin treatment on infusion day 13, irrespective of whether animals were maintained on HFD or chow (P < 0.05 oxytocin vs. vehicle) (Table 2). There were no differences in oxytocin levels between vehicle-treated animals maintained on chow or HFD, consistent with our previous data in rats (59). Peripheral oxytocin treatment was also not associated with a significant change in blood glucose, FFA, total cholesterol, adiponectin, irisin, or FGF-21.

**DISCUSSION**

Our findings demonstrate that a chronic increase (~21–26 days) of CNS oxytocin signaling both reduced weight gain associated with the progression of diet-induced obesity and evoked sustained weight loss in rats with established diet-induced obesity by selectively reducing fat mass while preserving lean mass, even when administered at a dose that did not appreciably affect energy balance in chow-fed controls. We also report oxytocin-induced weight loss involves 1) maintenance of energy expenditure at preintervention levels despite ongoing weight loss, 2) a robust reduction of respiratory quotient, consistent with increased fat oxidation, and 3) an enhanced satiety response to CCK-8 and associated reduction of meal size. Moreover, oxytocin’s weight-reducing effects occur independently of whether sucrose is added to the HFD and persist for ~10 days following cessation of treatment. While the CNS mechanisms underlying this antiobesity effect remain unresolved, these data indicate that long-term CNS oxytocin administration can both prevent and treat diet-induced obesity and that these effects are selective for loss of body fat. Combined with our evidence that chronic subcutaneous infusions of oxytocin are sufficient to reduce body weight gain in animals maintained on a HFD at a dose that does not appear to elicit aversive behavioral responses (e.g., nausea or malaise), these findings identify oxytocin as a viable agent for obesity prevention and treatment.

These findings are key steps in extending our understanding of the CNS mechanisms by which oxytocin may influence and potentially regulate energy homeostasis and body adiposity in particular. Our findings that oxytocin limits food intake, body weight, and body adiposity gain in animals on a HFD fed, but not on a chow diet, irrespective of whether obesity is present, suggests that the effect of oxytocin to inhibit food intake depends on the diet being consumed. Consistent with this view, acute or chronic central or systemic oxytocin administration reduces consumption of HFD (23, 53, 59, 103, 104), whereas impairment of oxytocin signaling is associated with increased consumption of fat (103, 104), implying a physiological role for oxytocin to limit fat consumption. These findings are also consistent with studies showing that ingestion of Intralipid stimulates Fos (marker of neuronal activation) in PVN oxytocin neurons (68). This observation raises the possibility that the...
mechanism of oxytocin’s effects on body weight may involve a preferential macronutrient consumption of fat. While additional studies to determine if chronic increases in oxytocin signaling impact macronutrient preference will be helpful, together, these findings build on existing rodent data to suggest that oxytocin’s effects to limit consumption of preferred (palatable) macronutrients helps to drive its effects on food intake and body weight. Collectively, these findings suggest that the effect of central oxytocin to reduce food intake, body weight, and body adiposity is specific to consumption of a HFD, irrespective of whether obesity is present.

Our findings are consistent with earlier reports in which daily acute 3V injections of oxytocin over 1 wk into DIO mice were sufficient to reduce body weight gain through a specific reduction in fat mass (104). Deblon and colleagues (23) also reported that chronic infusions of oxytocin into the lateral ventricle reduce weight gain and adiposity gain in HFD-fed rats. However, oxytocin was infused into the lateral cerebral ventricle for a shorter period of time (14 days); it was also not clear to what extent obesity was actually induced in the rats because they were on the HFD for only 5 wk before oxytocin treatment. Those reports also did not include body weight or total fat mass following diet exposure in either HFD-fed rats or age-matched chow-fed control rats (23) but instead focused on the effects of chronic subcutaneous infusions of oxytocin on changes of body weight gain and body composition relative to vehicle treatment. Consistent with other studies (23, 73), oxytocin treatment limited fat mass while eliciting either no

Table 2. Serum measurements following subcutaneous infusions of oxytocin or vehicle in chow-fed or HFD-fed DIO rats

<table>
<thead>
<tr>
<th>SC Treatment</th>
<th>Chow</th>
<th>HFD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vehicle</td>
<td>Oxytocin</td>
</tr>
<tr>
<td>Leptin, ng/ml</td>
<td>2.9 ± 0.6a</td>
<td>3.2 ± 0.1bc</td>
</tr>
<tr>
<td>Adiponectin, μg/ml</td>
<td>4.1 ± 0.3</td>
<td>5.4 ± 0.2</td>
</tr>
<tr>
<td>FGF-21, pg/ml</td>
<td>122.6 ± 26</td>
<td>76.2 ± 9.3</td>
</tr>
<tr>
<td>Irisin, μg/ml</td>
<td>4.4 ± 0.4</td>
<td>4.3 ± 0.2</td>
</tr>
<tr>
<td>Blood glucose, mg/dl</td>
<td>99.3 ± 2.9bc</td>
<td>99.2 ± 4.4bc</td>
</tr>
<tr>
<td>FFA, meq/l</td>
<td>0.18 ± 0.02</td>
<td>0.21 ± 0.01</td>
</tr>
<tr>
<td>Total cholesterol, mg/dl</td>
<td>67.8 ± 17</td>
<td>51.3 ± 6.2</td>
</tr>
<tr>
<td>Oxytocin, pg/ml</td>
<td>1513.9 ± 253.3a</td>
<td>2882.9 ± 383.1b</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 5–6/group. FGF-21, fibroblast growth factor-21; FFA, free fatty acids. Different letters denote significant differences between treatments. Shared letters are not significantly different from one another.
change or a slight increase (and therefore a protective effect) on lean mass. Insight into the mechanism(s) underlying oxytocin’s protective effect on lean mass awaits further investigation.

The findings across all studies consistently show that oxytocin reduces weight gain in preobese HFD-fed animals regardless of whether the animals were weight matched at study onset. Potential limitations in this study, however, are that, despite both groups of animals in study 1 being age-matched, these groups were not weight matched at study onset. Animals arrived at the VAPSHCS at different ages before study onset, which likely contributed to different growth rates. Nevertheless, these discrepancies are unlikely to have influenced the major overall conclusion because oxytocin also produced consistent and preferential reductions of weight gain in HFD-fed rats that were both age and weight matched to chow-fed controls in study 3. In addition, the HFD used in this study and other studies (23, 53, 59, 103, 104) also contains more sucrose (6.7% kcal from sucrose) relative to chow (3.7% kcal from sucrose), and although relatively small, it may have also contributed to the enhanced effectiveness of oxytocin in this model (2, 10, 38, 61, 68). However, we found that chronic 3V infusions of oxytocin reduce weight gain, body adiposity, and energy consumption in DIO rats irrespective of whether sucrose is added to their HFD. Collectively, these findings suggest that oxytocin preferentially reduces consumption of highly palatable foods that are high in fat and/or high in fat and sugar.

In contrast to the reduction in circulating levels of oxytocin reported in DIO mice (103, 104), we found no difference in circulating levels between vehicle-treated chow-fed and HFD-fed rats. One potential explanation for the lack of observed reduction in serum oxytocin may be due, in part, to the length of time animals were placed on the HFD and severity of obesity. Serum oxytocin was measured from rats that were on the HFD for only 13 days (study 10), whereas previous studies reported reductions in serum oxytocin in DIO mice that were maintained on the HFD for 12 (104) or 16 wk (103). Furthermore, Zhang and colleagues (104) demonstrated that circulating levels of oxytocin were lower in DIO mice particularly when blood was sampled during the mid to late light cycle. By design, blood sampling in our study occurred toward the end of the light cycle to match the time period where such differences occur between lean and DIO mice. While not the goal of our study, future studies aimed at collecting blood across multiple circadian time points in rats with already established DIO will be helpful in addressing the extent to which reductions in serum oxytocin may potentially contribute to the pathogenesis of diet-induced obesity in a rat model.

We and others have demonstrated that in HFD-fed animals oxytocin reduces body weight, in part, by reducing food intake (23, 53, 59, 103, 104), and that the underlying mechanism, based on data from Chow-fed animals, may involve increased sensitivity to meal-related satiety signals, such as CCK (6, 9, 57, 67). Leptin is similarly hypothesized to inhibit food intake by enhancing the hindbrain neuronal and satiety response to CCK (12, 34, 57, 58, 97), although this effect is impaired in diet-induced obesity (26, 59). Indeed the ability of low doses of CCK to reduce food intake and activate Fos expression (a marker of neuronal activation) in hindbrain neurons linked to the control of meal size are also impaired in animals maintained on a HFD (20, 21, 81). These observations raise the possibility that the effect of oxytocin, a downstream target of leptin action (12, 57, 70, 99), to inhibit food intake in DIO models is intact (10, 23, 53, 59, 103, 104) because oxytocin acts downstream of the site of leptin resistance. To our knowledge, there are no published studies investigating whether increased oxytocin signaling is sufficient to restore the impaired satiety response to low doses of CCK-8 in animals maintained on a HFD. Accordingly, we hypothesized that increased oxytocin signaling circumvents leptin resistance to reduce food intake in DIO animals by enhancing the satiety response to CCK. Our findings are consistent with this hypothesis, since oxytocin both reduced meal size (without affecting the number of meals consumed) and enhanced sensitivity to the effect of CCK to reduce food intake. One recent report, however, shows chronic administration of oxytocin into the lateral cerebral ventricle reduces meal frequency without impacting meal size in HFD-fed Wistar rats (23). However, it is not clear which criteria were used to define a meal in this study and further studies need to clarify whether strain, route of administration, extent of leptin resistance, or obesity in HFD-fed animals at time of oxytocin treatment impacts meal patterning. Nevertheless, combined with previous evidence that reduced oxytocin signaling reduces responsiveness to CCK (6, 9, 67) and is linked to increases in meal size (13, 100), these observations support a physiological role for oxytocin to regulate responsiveness to meal-related satiety signals and hence to participate in control of meal size.

Our study also addressed the effects of oxytocin on energy expenditure. Although we did not detect differences in total energy expenditure between 3V vehicle and 3V oxytocin-treated animals, the latter group lost weight relative to the former and previous evidence suggests that persistent, sustained reductions in food intake and body weight are associated with a compensatory decrease of energy expenditure in both rodent models (29) and humans (77–79, 84). We therefore interpret these findings to suggest that oxytocin, like leptin, prevents the effect of weight loss to reduce energy expenditure (77–79) and in this way enhances weight loss induced by reduced food intake. Whether and how this observation is linked to the effect of oxytocin to selectively reduce body fat stores while protecting lean body mass (39, 77) awaits further study.

The extent to which oxytocin may elicit weight loss through factors that elicit “browning” of white adipose tissue has only recently been examined. One study reported that chronic subcutaneous administration of oxytocin, at a dose that reduced weight gain in obese db/db mice, also appeared to increase the number of uncoupling protein-1 (+) cells in subcutaneous and visceral fat of obese db/db mice (73). Furthermore, Lawson and colleagues (48) demonstrated that oxytocin in amenorrheic athletes is positively correlated with resting energy expenditure as well as FGF-21 and irisin, two factors that induce the conversion of white adipose tissue to the “brite” or “beige” adipose tissue (14, 28, 98). However, we did not measure a significant increase in either FGF-21 or irisin in response to 3V or subcutaneous infusions of oxytocin that was effective in limiting weight gain. As blood was sampled after the weight loss had already occurred (day 21) in DIO rats, it is possible that oxytocin may have increased FGF-21 or irisin had blood been collected before oxytocin elicited reductions in body
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weight. Future studies will assess circulating levels of FGF-21 or irisin at multiple time points with respect to the progression of diet-induced obesity.

We report that extended increases in CNS oxytocin signaling increased fatty acid oxidation (as evidenced by a decreased RQ) in DIO rats. These findings extend those of Deblon and colleagues (23) who reported both a reduced RQ and increased expression of lipolytic genes in adipose tissue following 14-day infusions of oxytocin into the lateral cerebral ventricle in HFD-fed rats. Oxytocin also increases free fatty acids, glycerol, and/or reduces triglycerides in cultered 3T3-L1 adipocytes (23, 101), rats (23), and DIO nonhuman primates (10). Lawson and colleagues (49) also reported a tendency for oxytocin to reduce triglycerides in humans. In addition, chronic central or systemic oxytocin increases epididymal white adipose tissue expression of hormone sensitive lipase (a key mediator of adipocyte lipolysis) (1, 23) while reducing expression of fatty acid synthase (an enzyme linked to lipogenesis) in rats (1). While it remains to be determined whether chronic CNS infusions of oxytocin increase circulating levels (23) in sufficient concentrations to trigger a peripheral effect on lipolysis, it is possible that these effects in our model may be attributed, in part, to a direct effect on adipocytes (60, 83, 94) where OTRs are expressed (1, 31, 32, 60, 83, 94, 101) or an indirect mechanism. The mechanism underlying these effects may involve outgoing polysynaptic sympathetic nervous system projections from PVN oxytocin neurons to both inguinal (86) and epididymal white adipose tissue (86, 89). With respect to the question of which receptor populations contribute to the effects of oxytocin to impact energy expenditure, acute microinjection of oxytocin into the median raphe increases both heart rate and body temperature in mice (102), and oxytocin administration into the VMH increases short-term energy expenditure in rats (63). Evidence that adeno-associated viral expression of OTRs into the VMH/DHM of OTR null mice restores impairments in cold-induced thermogenesis (45) and corrects defects in β3- and α2-adrenergic mRNA expression in interscapular brown adipose tissue, strengthens the link between OTRs in these areas and control of energy expenditure. It is well established that stimulation of the VMH increases activation of efferent nerves innervating brown adipose tissue (62) and induces brown adipose tissue thermogenesis (71), whereas VMH lesions are associated with deficits in sympathetic nervous system activity (80). In addition, some cells in the DMH (65) and VMH (5, 65) have polysynaptic projections to brown adipose tissue. Oxytocin may therefore stimulate brown adipose tissue thermogenesis through either direct (82) or indirect (65) projections from PVN oxytocin neurons to sympathetic premotor neurons (65).

Perspectives and Significance

Obesity and its associated metabolic complications (19, 24, 35) are major health concerns (87). According to the World Health Organization, obesity rates have increased at least twofold since 1980 with approximately 1.9 billion adults classified as being overweight as of 2014 (Fact sheet No. 311, World Health Organization). In the United States alone, obesity impacts approximately 78 million adults and 12.5 million children and adolescents (64). This increase is associated with increased consumption of diets high in fat (16), and sugars, particularly fructose corn syrup and sucrose (15, 37, 56, 88), all of which contribute to the metabolic irregularities observed in the metabolic syndrome (e.g., visceral adiposity, insulin and leptin resistance, dyslipidemia, weight gain). Unfortunately, existing pharmacotherapeutic strategies to treat obesity are relatively ineffective and there is widespread need for improved treatments. We and others have previously shown that chronic systemic administration of oxytocin elicits weight loss or reductions of weight gain in DIO and genetically obese rodent models, DIO nonhuman primates, and obese humans through mechanisms that include reduction of food intake (1, 10, 23, 49, 53, 59, 104, 105), increased energy expenditure (10), increases in lipolysis (1, 8, 23), and/or reductions in RQ (23, 49, 53). Our findings demonstrate that chronic 3V oxytocin infusion both reduced weight gain associated with the progression of HFD-induced obesity and elicited a sustained reduction of fat mass, with no decrease of lean mass, in rats with established diet-induced obesity. We further demonstrated that these chronic oxytocin effects result from 1) maintenance of energy expenditure at preintervention levels despite ongoing weight loss, 2) a robust reduction in respiratory quotient, consistent with increased fat oxidation, and 3) an enhanced satiety response to CCK-8 and associated reduction of meal size. In contrast to other anti-obesogenic therapies, including melanocortin receptor ligands [MTII (72), BIM-22493 (46)], tipramine (27), and the GLP-1 receptor agonist, lixisenatide (50), which reduce both fat mass and lean mass, oxytocin is one of the few hormones that we are aware of [aside from leptin (36, 41, 69)] that protects against the loss in lean mass and selectively reduces fat mass, thus highlighting its translational potential. Combined with our evidence that chronic subcutaneous infusions of oxytocin are sufficient to reduce body weight gain in animals maintained on a HFD at a dose that does not appear to elicit aversive behavioral responses (e.g., nausea or malaise), these findings identify oxytocin as a viable agent for obesity prevention and treatment. Future studies are warranted to determine the extent to which chronic intranasal administration of oxytocin evokes weight loss in obese men and women by reducing fat mass while preserving lean mass, which will be important if oxytocin is be considered as a potential antiobesity treatment in humans.

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