Full title: Chronic oxytocin administration inhibits food intake, increases energy expenditure, and produces weight loss in fructose-fed obese rhesus monkeys

Abbreviated title: Oxytocin Signaling Reduces Weight in DIO Nonhuman Primates

James E. Blevins\textsuperscript{1,2}, James L. Graham\textsuperscript{4}, Gregory J. Morton\textsuperscript{2,3}, Karen L. Bales\textsuperscript{5}, Michael W. Schwartz\textsuperscript{2,3}, Denis G. Baskin\textsuperscript{1,2}, and Peter J. Havel\textsuperscript{4}

\textsuperscript{1}VA Puget Sound Health Care System, Office of Research and Development Medical Research Service, Department of Veterans Affairs Medical Center, Seattle, WA 98108, USA

\textsuperscript{2}Division of Metabolism, Endocrinology and Nutrition, Department of Medicine, University of Washington School of Medicine, Seattle, WA, USA

\textsuperscript{3}Diabetes and Obesity Center of Excellence, University of Washington School of Medicine, Seattle, WA, USA

\textsuperscript{4}Department of Nutrition and Department of Molecular Biosciences, School of Veterinary Medicine, University of California, Davis, CA, USA

\textsuperscript{5}Department of Psychology, University of California, Davis, CA, USA
Corresponding author’s address:

James E. Blevins, Ph.D.
VA Puget Sound Health Care System
Research-151
1660 South Columbian Way
Seattle, WA 98108

Key words: Obesity, food intake, energy expenditure, oxytocin

GRANTS
This material is based upon work supported by the Office of Research and Development, Medical Research Service, Department of Veterans Affairs (VA). The research in our laboratory has been supported by the California National Primate Research Center (CNPRC) Pilot Award (core grant #0D011107) and the Department of VA Merit Review Research Program. PJH’s research program also receives research support from NIH grants DK-095980, HL-091333, HL-107256, HL-107256 and a Multi-campus grant from the University of California Office of the President. DGB is the recipient of a VA Senior Research Career Scientist award.

ACKNOWLEDGMENTS
The authors thank the technical support of Sarah Davis and Vanessa Bakula at the UC Davis CNPRC. The authors also thank Marinelle Nunez and Guoxia Chen for their technical support.
Abstract:

Despite compelling evidence that oxytocin (OT) is effective in reducing body weight (BW) in diet-induced obese (DIO) rodents, studies of the effects of OT in humans and rhesus monkeys have primarily focused on non-ingestive behaviors. The goal of this study was to translate findings in DIO rodents to a pre-clinical translational model of DIO. We tested the hypothesis that increased OT signaling would reduce BW in DIO rhesus monkeys by inhibiting food intake and increasing energy expenditure (EE). Male DIO rhesus monkeys from the California National Primate Research Center were adapted to a 12-h fast and maintained on chow and a daily 15% fructose-sweetened beverage. Monkeys received 2x daily subcutaneous vehicle injections over 1 week. We subsequently identified doses of OT (0.2 and 0.4 mg/kg) that reduced food intake and BW in the absence of nausea or diarrhea. Chronic administration of OT for 4 weeks (0.2 mg/kg for 2 weeks; 0.4 mg/kg for 2 weeks) reduced BW relative to vehicle by 3.3±0.4% (≈0.6 kg; P<0.05). Moreover, the low dose of OT suppressed 12-h chow intake by 26±7% (P<0.05). The higher dose of OT reduced 12-h chow intake by 27±5% (P<0.05) and 8-h fructose-sweetened beverage intake by 18±8 % (P<0.05). OT increased EE during the dark cycle by 14±3% (P<0.05) and was associated with elevations of free fatty acids and glycerol and reductions in triglycerides suggesting increased lipolysis. Together, these data suggest that OT reduces BW in DIO rhesus monkeys through decreased food intake as well as increased EE and lipolysis.
Introduction:

Obesity and its associated metabolic disorders (18, 23, 28) are growing health concerns (57). The obesity epidemic has escalated in recent years and currently impacts over 78 million adults and 12.5 million children and adolescents in the U.S (49). This recent surge is attributed, in part, to increased intake of sucrose and high fructose corn syrup (10, 29, 42) which is implicated in promoting metabolic abnormalities associated with the metabolic syndrome (e.g. weight gain, visceral adiposity, insulin and leptin resistance, dyslipidemia) in humans (10, 29, 42, 43, 58, 59) and diet-induced obese (DIO) rhesus monkeys maintained on a high fructose diet (12). The ensuing impairments in the response (19-21) and/or secretion (54) of peripheral satiety signals in response to chronic exposure to high fat or high fructose diets are implicated as contributing factors in the progressive rise of obesity. However, existing weight loss strategies are ineffective and there is an urgent need for improved treatments for these diseases.

While the nonapeptide oxytocin (OT) is well known for its peripheral effects on uterine contraction during parturition and milk ejection during lactation (26), growing evidence suggests that OT plays an important role in the regulation of energy homeostasis (22, 40, 45, 71). For example, mice deficient in either OT (15) or OT receptors (OTRs) (62) exhibit a late-onset obesity phenotype and variations in copy number associated with the OTR gene (OXTR) are linked with severe early-onset obesity in humans (66). In addition, impairments in OT release within the hypothalamic paraventricular nucleus (PVN) occur in DIO mice (71) which may contribute, in part, to the corresponding reductions in circulating levels of OT observed in DIO mice (71) as
well as in obese Zucker rats (25). Additionally, the pathogenesis of Prader-Willi
syndrome, a human genetic disorder characterized by hyperphagia and obesity, is
linked to a reduced number and size of OT neurons in the PVN (60). Reductions in PVN
OT expression and obesity are also associated with mutations of the single-minded 1
gene, SIM1 (39), which contribute to obesity in Sim1 haploinsufficient mice and humans
(33, 61). The increased body weight (BW) gain observed in Sim1 haploinsufficient mice
can be ameliorated with OT treatment (39). Furthermore, we and others have
demonstrated that acute or chronic central or systemic administration of OT elicits BW
loss and/or reductions in BW gain in DIO (22, 40, 45, 71, 72) and genetically obese
rodent models (1, 39, 41, 45) which is sustained over time (1, 22, 40, 44, 71, 72).
Importantly, OT elicits these effects in rodents fed a low fat/high carbohydrate chow diet
(22, 40, 45, 71) as well as in insulin and leptin resistant DIO rats maintained on a high
fat diet (HFD) (45, 71, 72). Together, these data suggest that OT plays an important role
in energy homeostasis, although the mechanisms underlying these effects remain to be
firmly established.

OT-elicited reductions in food intake appear to contribute to its ability to elicit BW
loss in rodent models. The ability of OT to dose-dependently reduce food intake,
whether given systemically or directly into the brain, is well documented (22, 40, 45, 71).
We and others have shown that chronic systemic administration of OT recapitulates the
effects of central administration of OT to reduce BW and reduce BW gain in DIO
rodents (1, 22, 40, 44, 71, 72). Similar to its effects on BW, OT also inhibits food intake
in DIO (22, 40, 45, 71, 72) and genetically obese rodent models (1, 39, 41, 45). In
addition to reducing intake of a low fat/high carbohydrate diets, including standard
rodent chow (22, 40, 45, 71), OT also reduces consumption of sucrose (47) as well as high fat diets (45, 71, 72). Conversely, impairments of OT signaling are associated with increased consumption of carbohydrates, including sucrose (2, 30, 47, 52), fructose (30), and glucose (30), as well as fat (71, 72), implicating a potential physiological role for OT to limit consumption of both simple sugars and fat.

Recent studies indicate that in addition to suppressing food intake, OT may also reduce BW in rodents by increasing energy expenditure (EE). Reductions of OT signaling are linked to obesity as well as decreases of EE (15, 35, 62, 67, 72), including impairments in sympathetic nervous system activity, thermogenesis by brown adipose tissue (BAT) (15, 35, 62) and oxygen consumption (67, 72), in the absence of increases of food intake (2, 15, 62, 67, 68) in mice. On the other hand, acute administration of OT into the CNS produces short-term increases of EE (48, 71, 72) in addition to heart rate (32, 70) and body temperature (70) in rodents. When administered by chronic subcutaneous infusion to DIO rats, OT also reduces BW at doses that are ineffective at reducing food intake (22). In cases where chronic subcutaneous OT treatment inhibits both food intake and BW, its ability to reduce BW is maintained for 9 days after treatment has ended (40) or food intake has returned to baseline pre-treatment levels (40). These findings are consistent with data which show that BW loss attributed to systemic treatment with OT exceeds that of pair-fed control animals (22, 45). OT also activates sympathetic preganglionic neurons (6), including the stellate ganglia (5). With well characterized polysynaptic projections to BAT (50), stellate ganglia (34), and the spinal cord (55), these findings suggest that OT may have an important role in regulating sympathetic nervous system activity.
However, despite compelling evidence that OT has important effects on energy balance and BW in rodents, studies in humans and nonhuman primates (NHPs) have primarily focused on the role of OT in mood, trust, and pair-bonding. The goal of this study was therefore to establish a proof-of-principle that systemic OT treatment is effective at inducing weight loss in a NHP model of DIO. We determined the extent to which chronic administration of OT induces long-term BW loss in DIO rhesus monkeys and assessed whether these effects on BW loss were maintained following cessation of treatment. We further examined whether this OT-induced BW loss is mediated by reductions in food intake as well as increases of EE and/or lipolysis.

Methods.

Animals. Adult male rhesus monkeys (N=5) (age: 10-18 years, BW: 17.5±1.1 kg) (37.9±1.9% fat) from the California National Primate Research Center (CNPRC) Primate Resource were maintained at the CNPRC at the University of California, Davis. All animals were housed individually in a temperature controlled room under a 12:12-h light-dark cycle (lights off at 6 PM; lights on at 6 AM). Animals had ad libitum access to water throughout the course of the study and were adapted to a daily 12-h fast (during dark phase from 6 PM to 6 AM) with the exception of when the animals were placed into indirect calorimetry cages for their EE measurements at baseline and at the conclusion of week 4 of the treatment period. The research protocols were approved both by the Institutional Animal Care and Use Committees of the University of California, Davis and conducted in accordance with the Department of Agriculture Animal Welfare Act and NIH Guidelines for the Care and Use of Animals.
Injections and Drug Preparation

Fresh solutions of OT acetate salt (American Peptides, Sunnyvale, CA) were prepared on each day of the experiment within 30-45 minutes of administration. OT was solubilized in sterile water and diluted with sterile saline. Both vehicle and OT were filtered (0.22 μm filter, EMD Millipore, Billerica, MA) prior to administration. Vehicle injections (0.1 ml/kg injection volume) were administered subcutaneously 2x daily between 6:45-7:00 AM and 2:45-3:00 PM during week 1 (FIGURE 1). OT injections were administered in identical fashion during weeks 2-3 (0.2 mg/kg) and 4-5 (0.4 mg/kg). A pilot study was undertaken prior to the start of the experiment to confirm effective dosing based on an acute decrease of food intake. These animals were the same as those used in the chronic administration study. Each animal served as its own control and received 1x daily injections of vehicle or OT (0.04, 0.2 mg/kg; 0.1 ml/kg injection volume) in randomized fashion between 6:45-7:00 AM at 48 h intervals.

Diet and energy intake measurements

Animals were maintained on a standard monkey chow diet (High Protein Monkey Diet Jumbo 5047, Advance Protocol Old World Primate; LabDiet, St. Louis, MO) which consists of 11% kcal from fat, 30% kcal from protein, and 59% kcal from carbohydrate. Fresh chow was given to the animals at 7 AM and was replaced daily at 3 PM. In addition, all animals were provided 1x daily with a 15% fructose-sweetened beverage flavored with unsweetened Kool-Aid® (Kraft Foods Group, Inc., Northfield, IL) at 7 AM. Cumulative energy intake (chow diet + sweetened beverage) was measured 2x daily at 3 and 7 PM using previously established methods in the CNPRC (12). Cumulative 8-
and 12-h food intake data were averaged across the 1-week vehicle treatment period, 2-week OT treatment periods and the first two weeks of the washout period. During the pilot study cumulative food intake was measured at 0.5, 1, 8, and 12 h following access to food at 7:00 AM. Cages were inspected daily for signs of nausea or diarrhea.

**Indirect calorimetry**

EE was assessed 2x (1x at baseline and 1x at conclusion of week 4 treatment) over 24-h periods using indirect calorimetry ($O_2$, $CO_2$) in metabolic chambers at the CNPRC (12) Exposure Facility. The indirect calorimetry measurements were completed in two airtight 32"h x 27"d x 24"w chambers at 30 min intervals (12). EE was calculated using the Weir equation: kcal/min = (3.941 × $\dot{V}_{O_2}$ + 1.106 × $\dot{V}_{CO_2}$) (65). To control for the influence of body size variation on total EE (13), group comparisons involving this outcome were analyzed following normalization to body weight and lean body mass. Monkeys were placed into the chambers between 7-9 AM and were given ad libitum access to the fructose-sweetened beverage, standard monkey chow, and water and remained in the chambers until 6 AM. The area under the curve (AUC) was calculated using the trapezoidal method.

**BW**: BW was determined 1x weekly at the end of baseline, vehicle treatment, OT treatment and weeks 1-3 and 7 of the washout period.

**Plasma measurements**: Fasting blood samples (15 ml) were drawn into EDTA Vacutainer tubes 1x weekly between 6:15-6:30 AM immediately prior to administration
of vehicle or OT. Samples were collected at the conclusion of baseline, vehicle or OT
treatment from a cephalic vein in conscious monkeys using the arm-pull technique
following an overnight fast.

Plasma Assays

Whole blood was centrifuged at 6,000 rpm for 15-min at 4°C, plasma was removed, and
it was aliquoted and stored at −80°C for subsequent analysis.

Adiponectin, insulin, leptin and OT

Plasma adiponectin, insulin and leptin were measured by RIA (EMD Millipore, Billerica,
MA). These assay procedures have been validated for rhesus monkeys using already
established procedures (12). Intra-assay CVs for adiponectin, insulin, and leptin were
5.4, 6.5, and 3.5%, respectively. The limits of detectability for the RIAs are as follows:
adiponectin (0.8–100 ng/mL), insulin (2.7–200 µU/mL) and leptin (1–100 ng/mL).
Plasma OT levels were determined by ELISA (ENZO Life Sciences, Farmingdale, NY).
The intra-assay CV for OT was 4.5% and the limit of detectability was 15-1000 pg/mL.

Glucose, lipid and lipoproteins

Glucose, TGs, total cholesterol, HDL, direct LDL, apolipoprotein A1 (ApoA1) and
apolipoprotein C3 (ApoC3) concentrations were measured using an enzymatic based
Polychem Chemistry Analyzer (MedTest DX, Canton, MI). Free fatty acids (FFAs) were
measured using an enzymatic based kit (Wako Chemicals USA, Inc., Richmond, VA).
These assay procedures have been validated for rhesus monkeys (12). Intra-assay CVs
for glucose, total cholesterol, HDL, LDL, ApoA1, ApoC3, FFAs and TGs were 0.6, 2.9, 2.0, 0.5, 2.4, 0.7, 2.2, and 3.5%, respectively.

Statistics: All results are expressed as means ± SE. Comparisons between treatments were made using a one-way repeated measures ANOVA with a Fisher's least significant difference post hoc test. Analyses were performed using the statistical program SYSTAT (Systat Software, Point Richmond, CA). To control for the influence of body size variation on total EE (13), group comparisons involving this outcome were adjusted for total body mass and lean mass using the statistical program GraphPad Prism (GraphPad Software, Inc., La Jolla, CA). Differences were considered significant at \( P<0.05 \).

Results

**BW Gain Following Exposure to Fructose-Sweetened Beverage:**

Animals were maintained on 500 ml/day of a 15% fructose-sweetened beverage and *ad libitum* access to their usual standard chow diet for 10.8±0.1 months prior to study onset where animals weighed 15.0±1.2 kg at baseline and 17.5±1.1 kg (ΔBW=+2.5±0.7 kg) after exposure to the intervention diet (\( P<0.05 \)). We have previously reported that supplementation of an *ad libitum* chow diet with 15% fructose-sweetened beverages over 6- and 12-month periods result in similar increases in BW at 6 and 12 months (11, 12). These differences were associated with 29% and 35% increases of fat mass at 6 and 12 months, respectively (12).
Effects of OT on Body Weight:
The initial goal of this study was to determine if chronic OT treatment would elicit BW loss in a more translational NHP model of DIO. Overall, there was a significant main effect of OT to reduce BW ($F(4,16)=15.760, P<0.05$). OT elicited BW loss compared with vehicle treatment after 2 (-0.41±0.09 kg), 3 (-0.41±0.08 kg), and 4 weeks (-0.58±0.04 kg) of treatment (FIGURE 2A; $P<0.05$). At the end of vehicle and OT treatment, animals weighed 17.7±1.1 kg and 17.1±1.10 kg, respectively ($P<0.05$). Chronic OT treatment elicited BW loss in all 5 animals relative to vehicle treatment. These data are the first to demonstrate that chronic OT induces BW loss in a NHP model of DIO.

In order to determine if the BW loss resulting from OT administration extended beyond cessation of treatment, BW was determined during a washout period out to 7 weeks post-treatment. There was a significant main effect of OT to maintain BW loss relative to vehicle treatment throughout the washout period ($F(4,16)=14.112, P<0.05$). Weight loss remained below that following vehicle treatment during the week 1 ($P<0.05$), 2 ($P<0.05$), and 7 ($P=0.056$) of the washout period. Animals did not begin to gain significant BW relative to the end of the OT treatment until week 3 of the washout period (FIGURE 2B; $P<0.05$).

Effects of OT on Consumption of Chow and Fructose-Sweetened Beverage:
To determine if the effects of OT to elicit weight loss is due, in part, to reductions of energy intake from chow and fructose, cumulative 8- and 12-h food intake was
measured throughout the course of vehicle and OT treatment. While there was no
significant main effect of OT to reduce chow consumption at 8 hours (F(2,8)=3.602,
P=0.077), there was a significant main effect of OT to reduce chow consumption at 12
hours (F(2,8)=11.816, P<0.05). Specifically, we found that the low dose of OT (0.2
mg/kg) suppressed 12-h chow intake by 26±7% (FIGURE 3A; P<0.05). The higher dose
of OT (0.4 mg/kg) suppressed 8- and 12-h chow intake by 13±9 and 27±5% (P<0.05),
respectively.

There was no significant main effect of OT to reduce 8-h fructose sweetened beverage
intake (Kool-Aid®) (F(2,8)=2.808, P=0.119). While the lower dose of OT (0.2 mg/kg)
failed to significantly reduce fructose-sweetened beverage intake at 8 hours, the higher
dose of OT (0.4 mg/kg) reduced 8-h fructose-sweetened beverage intake by 18±8%
(FIGURE 3B; P<0.05). Importantly, there were no signs of nausea or diarrhea following
administration of OT at either dose upon daily inspection.

Moreover, we found that there were no differences in 8- or 12-h chow (FIGURE 3A) or
Kool-Aid® (FIGURE 3B) consumption in animals treated with OT treatment (0.4 mg/kg)
relative to the same animals 2-weeks after the washout period (P=NS). These findings
suggest that the effects of OT to reduce food intake are prolonged, and secondly,
prevent the rebound hyperphagia that is characteristic following weight loss.
Effects of OT on Energy Expenditure:

To determine if increased EE may also contribute to the effects of OT to produce weight loss, we measured EE using indirect calorimetry (normalized to BW) at both baseline and during the last week of treatment. Our findings show that there was a main effect of OT to increase total EE ($F(1,4)=18.060, P<0.05$). This effect of systemic OT to increase EE was primarily due to an increase in EE during the dark cycle ($F(1,4)=35.068, P<0.05$) (FIGURE 4A) with a tendency to increase EE during the light cycle ($F(1,4)=4.992, P=0.089$). Specifically, systemic OT increased EE by 14.4±3.0 and 9.2±1.8% during the 12-h dark cycle and entire 24-h period, respectively (FIGURE 4B). OT did not significantly stimulate EE during the course of the 9-h light cycle measurements (3.8±1.7%; $P=0.09$). Similar results were also observed when the data were normalized to lean body mass.

Plasma Measurements following OT treatment

OT treatment resulted in increased plasma OT, FFA and glycerol ($P=0.05$) concentrations, in addition to reductions of plasma glucose and TG concentrations, and modest decreases of total and LDL cholesterol, and ApoC3 ($P<0.05$) (Table 1).

Discussion

The goal of this study was to translate the previous findings in DIO rodent models to a pre-clinical translational NHP model of DIO. Here we show for the first time that chronic administration of OT is sufficient to elicit long-term weight loss in fructose-fed DIO rhesus monkeys. Furthermore, we observed that this weight loss remained below
that of vehicle treatment for 7 weeks into the washout period and significant weight
regain did not begin until week 3 of the washout period. In addition, we determined that
the ability of OT to elicit weight loss appear to be attributed, in part, to reductions in
consumption of both chow and fructose-sweetened beverage in the absence of nausea
or diarrhea. We also identified that chronic systemic administration of OT increases EE
in addition to increasing FFAs and reducing TGs as well as total cholesterol. Together,
these findings provide evidence that chronic systemic OT effectively produces long-term
reductions in BW in DIO rhesus monkeys through mechanisms that may include
reductions of food intake, in addition to increases of EE and possibly increased lipolysis.

These findings are consistent with data from studies in rodents reporting that
administration of OT results in BW loss or decreased BW gain. Chronic administration
of OT over a period between 7-42 days is sufficient to reduce either BW or BW gain in
DIO mice and rats (22, 40, 44, 71, 72) as well as genetically obese mice (1). Of
particular interest in our study is the observation that BW loss persisted below that of
vehicle control treatment out to 7 weeks during the washout period and animals did not
regain significant BW until week 3 of the washout period. These prolonged effects are
consistent with other reports in obese nonhuman primates treated with adipotide (7), a
melanocortin 4 agonist, BIM-22493 (38), or fibroblast growth factor-21 (37, 64), and
appear to be mediated, in part, by reductions of food intake that persisted for the initial 2
weeks of the washout period. Similarly, Maejima and colleagues showed that BW gain
continue to remain below that of vehicle controls for 9 days following cessation of OT
treatment in DIO mice (40). In addition, the effects of OT on exploratory and anti-
aggressive behavior persist for at least 7 days following cessation of treatment in male
rats (14). Recent studies show that acute systemic administration of OT is capable of activating PVN OT neurons (16, 31) and stimulating the release of OT in the PVN in a rodent model (72). These self-stimulatory properties (22) in addition to its prolonged bioavailability of OT in the CNS are thought to contribute, in part, to its positive effects on prosocial behavior (36) and may also contribute to the prolonged effects of OT on BW loss following cessation of treatment (40).

Our findings indicate that OT reduced the intake of both standard monkey chow and a fructose-sweetened beverage and may point to a potential role of OT to limit consumption of carbohydrates or sweets in primates. These findings are consistent with those showing that exogenous administration of OT reduces sucrose intake in rodents (47) as well as consumption of chocolate cookies in humans (53). Based on recent data it appears that an important physiological role of OT is to limit intake of sucrose (2, 30, 47, 52), in addition to other simple sugars [fructose (30) and glucose (30)]. Reductions of endogenous OT signaling also stimulate intake of rodent chow, which contains up to 58% energy (kcal) from carbohydrates (3, 4, 8, 51, 71, 72). While additional studies to determine if OT impacts macronutrient preference in NHPs will be informative, together, these findings build on existing rodent data and suggest that the effects of OT to limit consumption of carbohydrates may extend to primates.

The effects of OT to increase EE also appear to contribute towards its effects to reduce BW although the site(s) of OT action in the CNS underlying these effects in rhesus monkeys remain to be identified. Recent findings show that direct administration of OT into the ventromedial hypothalamus (VMH) increases short-term EE in rats (48). Moreover, adeno-associated viral recovery of OTRs into the VMH/dorsomedial
hypothalamus (DMH) of OTR deficient mice restored impairments in cold-induced thermogenesis (35), providing further evidence that OTRs in the DMH/VMH are linked to the regulation of EE. While systemic OT increases the induction of Fos (a marker of neuronal activation) in the VMH in mice (72) and OTRs are expressed in the VMH of rhesus monkeys (9, 24) it remains to be determined whether systemic OT is capable of reaching OTRs in the DMH or VMH in sufficient concentrations to stimulate EE in rhesus monkeys. In addition to potential actions in either the VMH or DMH, OT could also increase sympathetic nervous system activity through polysynaptic projections from premotor neurons in the NTS (that potentially express OTRs) to BAT (34, 50, 55).

Future studies will be required to address the extent to which these effects are mediated through direct actions in the VMH, NTS or elsewhere in the CNS.

Based on our findings that OT increased FFA and tended to increases plasma glycerol concentrations suggest that OT may also reduce BW, in part, by increasing lipolysis. This could occur through a direct effect on adipocytes (27, 46, 56, 63). *In vitro* data from show that incubation of cultured 3T3-L1 adipocytes with OT increases enzymes associated with lipolysis (22) and results in increased glycerol release (22). In addition, chronic OT treatment *in vivo* results in reductions in fat mass (22, 40, 72), particularly adipocyte area from both the mesenteric and epididymal fat depots (40). Consistent with these findings, *in vivo* data from rodents with global loss in OT signaling report increases of abdominal fat deposition (15, 62) and increases of perirenal, mesenteric, and epididymal fat depot weights relative to wild-type littermate controls (62). Similarly, selective ablation of OT neurons in the PVN and SON (67) is associated with increases of body fat. Together, these recent studies unveil potential mechanisms
whereby the OT reduces body fat through separate or combined effects to reduce food intake, promote lipolysis and increase EE.

**Perspectives.** Chronic consumption of a high fructose diet produces weight gain and metabolic perturbations (insulin resistance and dyslipidemia) associated with the metabolic syndrome and T2DM in rhesus monkeys (12). With the disturbing rise in obesity in recent years attributed, in part, to increased sugar intake, there is urgent need to translate the promising results reported in DIO rodent models to examine the extent to which OT pharmacotherapy reduces BW in DIO primates. Given the need for new therapeutic strategies for the treatment of obesity it is surprising that, up to now, there has yet to be a single clinical trial performed to systematically investigate the effects of chronic OT administration on food intake and BW in DIO NHPs or in obese humans. While one preliminary study by Zhang and colleagues demonstrated that chronic intranasal OT reduced BW over an 8-week period in prediabetic obese humans (73) they did not examine the extent to which these effects may be attributed to reductions in food intake as well as increases in EE and/or lipolysis. Here we provide the first key evidence that chronic administration of OT is a potential therapy that can produce long-term reductions in BW in a nonhuman primate model of diet-induced obesity through mechanisms that may involve reductions of energy intake as well as increases of EE and lipolysis. “While this % weight loss is less than that achieved in long-term (≥1 year) studies in humans treated with FDA-approved drugs such as Qsymia (phentermine + topiramate) (≈10.9% of initial BW), it is similar in magnitude to BW loss following either orlistat (≈3.1% of initial BW) or lorcaserin (Belviq; ≈3.2% initial BW) (7, 28). It should be
stressed also that in most human obesity studies, subjects are placed on a “healthy”
diet intended to enhance weight loss (often in conjunction with increased physical
activity and other “lifestyle” interventions), whereas in our study, subjects were
maintained on the same obesogenic diet throughout the study. Last, the amount of BW
loss we observed is similar to what Kievet and colleagues reported following chronic
administration of a selective melanocortin receptor 4 agonist BIM-22493 over 4 weeks
(≈1 kg) in DIO rhesus monkeys maintained on a high fat diet (13).” Together, these
findings provide key translational data to support future larger scale and longitudinal
studies that examine the effects of chronic administration of OT on weight loss,
macronutrient preference and feeding reward in both male and female obese nonhuman
primates, as well as in clinical studies in humans.
Figure 1. Experimental Paradigm for Chronic Administration of OT or vehicle into DIO NHPs.

Figure 2. Effect of Chronic OT Administration on Weight Loss in DIO NHPs.
12-h fasted rhesus monkeys received 2x daily injections of vehicle or escalating doses of OT (0.2 for 2 weeks and 0.4 mg/kg for 2 weeks) and were maintained on standard monkey chow and fructose-sweetened beverage (Kool-Aid). A, Cumulative BW loss following OT treatment. B, Cumulative BW loss during 7-week washout period. Data are expressed as mean±SEM. A: *P<0.05 OT vs. vehicle; B *P<0.05 washout vs. OT, †0.05<P<0.1 washout vs. OT.

Figure 3. Effect of OT Administration on Food Intake in DIO NHPs.
12-h fasted rhesus monkeys received 2x daily injections of vehicle or OT (0.2, 0.4 mg/kg). A, Cumulative 8- and 12-h intakes of standard monkey chow. B, Cumulative 8- and 12-h intakes of fructose-sweetened beverage (Kool-Aid). Data are expressed as mean±SEM. †0.05<P<0.1 OT vs. vehicle, *P<0.05 OT vs. vehicle.

Figure 4. Effect of OT Administration on Food Intake in DIO NHPs. Indirect calorimetry was used to measure EE at baseline or following OT treatment (0.4 mg/kg) in ad libitum fed rhesus monkeys. A, 21-h profile of EE at baseline or following OT treatment. B, EE measurement depicted as area under the curve at both baseline and following OT treatment. Data are expressed as mean±SEM. †0.05<P<0.1 OT vs. baseline, *P<0.05 OT vs. baseline.

Table 1. Plasma Measurements Following Daily Subcutaneous Administration of OT or Vehicle
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6:45 a.m./2:45 p.m.  
2x daily SQ injections

7:00 a.m./3:00 p.m.  
Access to chow/Kool-aid®

3:00 p.m.  
Chow replaced

3:00 p.m./7:00 p.m.  
Quantify food intake

7:00 p.m.  
Fast animals

1x weekly BW measurements

FIGURE 1
Table 1. Plasma Measurements Following Daily Subcutaneous Administration of OT or Vehicle

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Vehicle</th>
<th>OT (week 1)</th>
<th>OT (week 2)</th>
<th>OT (week 3)</th>
<th>OT (week 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leptin (ng/mL)</td>
<td>62.3 ± 7.5</td>
<td>65.9 ± 7.2</td>
<td>69 ± 7.3† †</td>
<td>61.5 ± 4.8</td>
<td>60.7 ± 6.0</td>
<td>58.9 ± 7.7</td>
</tr>
<tr>
<td>Insulin (mU/mL)</td>
<td>275 ± 51</td>
<td>243 ± 33</td>
<td>254 ± 56</td>
<td>259 ± 36</td>
<td>259 ± 47</td>
<td>276 ± 61</td>
</tr>
<tr>
<td>Adiponectin (µg/mL)</td>
<td>5.9 ± 1.3</td>
<td>5.9 ± 1.5</td>
<td>5.7 ± 1.3</td>
<td>4.7 ± 1.4</td>
<td>5.3 ± 2.0</td>
<td>5.0 ± 2.2</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>124 ± 30</td>
<td>130 ± 31</td>
<td>120 ± 27</td>
<td>115 ± 26</td>
<td>114 ± 20</td>
<td>100 ± 14*</td>
</tr>
<tr>
<td>FFA (mEq/L)</td>
<td>0.40 ± 0.02</td>
<td>0.38 ± 0.05</td>
<td>0.42 ± 0.06*</td>
<td>0.46 ± 0.06*</td>
<td>0.52 ± 0.06*</td>
<td>0.46 ± 0.02*</td>
</tr>
<tr>
<td>Glycerol (mg/dL)</td>
<td>0.64 ± 0.19</td>
<td>0.66 ± 0.25</td>
<td>0.64 ± 0.23</td>
<td>0.53 ± 0.17</td>
<td>0.79 ± 0.17</td>
<td>0.83 ± 0.14**</td>
</tr>
<tr>
<td>TG (mg/dL)</td>
<td>470 ± 234</td>
<td>406 ± 148</td>
<td>353 ± 180</td>
<td>463 ± 248</td>
<td>360 ± 140</td>
<td>279 ± 95*</td>
</tr>
<tr>
<td>Total Cholesterol (mg/dL)</td>
<td>174 ± 17</td>
<td>162 ± 9</td>
<td>150 ± 14*</td>
<td>167 ± 18</td>
<td>156 ± 12†</td>
<td>152 ± 10*</td>
</tr>
<tr>
<td>LDL (mg/dL)</td>
<td>60 ± 4</td>
<td>53 ± 3†</td>
<td>48.0 ± 3*</td>
<td>54 ± 3</td>
<td>55 ± 5†</td>
<td>59 ± 5</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>63 ± 11</td>
<td>56 ± 11†</td>
<td>58 ± 12</td>
<td>59 ± 11</td>
<td>55 ± 11*</td>
<td>55 ± 10*</td>
</tr>
<tr>
<td>ApoC3 (mg/dL)</td>
<td>10 ± 2</td>
<td>9 ± 2</td>
<td>9 ± 2</td>
<td>10 ± 2</td>
<td>9 ± 2</td>
<td>8 ± 1*</td>
</tr>
<tr>
<td>ApoA1 (mg/dL)</td>
<td>114 ± 15</td>
<td>108 ± 15</td>
<td>109 ± 14</td>
<td>112 ± 16</td>
<td>112 ± 15</td>
<td>115 ± 14</td>
</tr>
<tr>
<td>OT (pg/mL)</td>
<td>2942 ± 265</td>
<td>2594 ± 282</td>
<td>2701 ± 160</td>
<td>3259 ± 202</td>
<td>3704 ± 237*</td>
<td>4250 ± 570*</td>
</tr>
</tbody>
</table>

N=5/group
* P<0.05 vs. baseline
** P=0.05 vs. baseline
† 0.05<P<0.1 vs. baseline
†† P>0.1 vs. baseline