

INGESTION OF PALATABLE SUBSTANCES IN OXYTOCIN KNOCKOUT MICE

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Mice deficient in oxytocin (OT) (OT KO), those that do not synthesize or release OT at any point in the life cycle, have shown significantly enhanced ingestion of sweet (sucrose and saccharin) and non-sweet carbohydrate-containing solutions (Polyose and cornstarch) as compared to wildtype (WT) mice of the same C57BL/6 background. The purpose of this work is two-fold: to determine if the effects observed with sweet and non-sweet carbohydrate-containing solutions extend to a palatable non-carbohydrate containing emulsified fat solution (Intralipid), and to further elucidate the drinking behavior of WT and OT KO mice exposed to sucrose solutions. Mice were exposed to a variety of two-bottle access tests to compare ingestion of Intralipid, a fat-containing emulsion, as well as sucrose and aversive (sodium chloride (NaCl) and citric acid) solutions mixed with sucrose.

On the first day of exposure to Intralipid OT KO animals consumed significantly greater volumes of Intralipid as compared to WT mice. Animals showed preference for the Intralipid emulsion over water at a variety of concentrations, however, no genotypic differences were observed in Intralipid ingestion beyond the first exposure day, suggesting that the increased ingestion of palatable liquids observed in OT KO mice does not extend to Intralipid solutions.

OT KO mice continually consumed more 10% sucrose than WT mice during repeated four-day trials and during a two-week trial. Sucrose bottle placement did not have a significant effect on sucrose consumption. OT KO mice also consumed significantly more sucrose at

concentrations of 0.625%, 1.25%, 2.5%, and 5% during four-day trials. Similar drinking patterns were observed in male and female animals and across generations.

When exposed to two-bottle access to 0.5M NaCl or 30mM citric acid mixed with 10% sucrose and water, OT KO animals consumed more of the aversive solution mixed with 10% sucrose than WT counterparts.

The results of this thesis suggest that OT plays a role in the ingestion of sweet carbohydrate-containing solutions. However, it appears that these effects do not extend to the ingestion of Intralipid, a palatable fat-containing emulsion. The exact function of OT in these ingestion behaviors has yet to be determined.

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1.0 OVERVIEW

1.1 INTRODUCTION

In order to maintain weight and nutrient balance, animals must regulate their intake of water and solute. A variety of interacting systems are involved in this regulation including sensory and metabolic signals. Research on the neurotransmitters that influence feeding may help to further elucidate the physiological mechanisms that drive food intake and reward.

Oxytocin (OT), a peptide often associated with pregnancy and lactation has also been shown to influence feeding behavior. Recently, our research group has reported that male and female mice lacking the oxytocin gene (OT knockout mice (OTKO)) ingest significantly more sucrose solution at concentrations of 10%, 5%, and 2.5% as well as 0.2% saccharin solution, cornstarch, and Polycose as compared to wildtype (WT) counterparts (Amico et al, 2005; Billings et al, 2006; Sclafani et al, 2007).

1.2 OXYTOCIN

Oxytocin (OT) is a nine amino acid neuropeptide that consists of a six amino acid ring with a three amino acid side chain. The ring is created by a disulfide bridge connecting the Cys residues in the 1 and 6 positions. OT is encoded in a single gene containing three exons and two

introns. The gene itself is similar in structure across all mammalian species in which it has been studied (Land et al, 1983).

OT in the brain is synthesized mainly in the paired paraventricular (PVN) and supraoptic nuclei (SON) of the hypothalamus (Rhodes et al, 1981; van den Pol, 1982). The PVN contains subpopulations of magnocellular and parvocellular neurons, while the SON contains only magnocellular neurons. Magnocellular OT neurons project to the posterior pituitary gland, while parvocellular neurons project to various sites in the central nervous system including the nucleus of the solitary tract, locus coeruleus, and dorsal motor nucleus of the vagus nerve (Swanson and Kuypers, 1980; van den Pol, 1982).

OT released from the posterior pituitary and into the peripheral circulation targets the uterus, mammary glands, and kidneys as well as other organs. It is important in female reproductive function as it facilitates uterine contraction during labor and milk ejection during lactation (Higuchi et al, 1985). Some peripheral tissues such as the heart (Jankowski et al, 1998), ovary (Ivell and Richter, 1984), uterus (Lefebvre et al, 1992), and testis (Ivell et al, 1997) can also synthesize OT.

1.3 OXYTOCIN INVOLVEMENT IN FEEDING

In addition to the peripheral effects seen in lactation and uterine contraction, OT acts in the central nervous system as a neurotransmitter to inhibit the ingestion of solute (both food and salt) in rodents (Arletti et al, 1989; Olson et al, 1991; Rinaman et al, 2005; Amico et al, 2001). Studies in rats have shown that centrally administered OT inhibits intake of sodium induced by angiotensin and hypovolemia (Blackburn et al, 1992; Blackburn et al, 1992b). In addition,

administration of a specific OT agonist causes dose related inhibition of food intake (Olson et al, 1991). Very large doses of OT administered peripherally have also been shown to decrease food intake in rats (Arletti et al, 1990). However, systemic OT does not inhibit salt intake in rats (Stricker and Verbalis, 1987). Additional research also suggests that OT is involved in the control of meal size and satiation (Lokrantz et al, 1997).

Previous work in our laboratory has demonstrated that male and female OT KO mice in contrast to WT mice display an immediate and sustained preference for water sweetened with sucrose beginning on the first day of exposure (Amico et al, 2005). With continued exposure to either 10% sucrose (i.e. up to 8 days) or 0.2% saccharin solution (i.e. up to 4 days), OT KO mice persist in consuming significantly greater daily volumes compared to WT cohorts (Amico et al, 2005; Billings et al, 2006). Recent work has shown that OT KO mice also consume greater amounts of other non-sweetened carbohydrate-containing solutions (Polydose and cornstarch) (Sclafani et al, 2007).

1.4 OXYTOCIN DEFICIENT MOUSE

OT KO mice of C57BL/6 background have a deletion of the OT gene and do not produce OT at any point in their lifetime (Young et al, 1996; Nishimori et al, 1996). However, OT KO mice do have OT receptors. No differences in OT receptor distribution or binding have been observed in the studied brain regions as compared to WT mice (Amico et al, 2004b). Because of their complete lack of OT, OT KO mice provide an ideal model to study the effects of central and peripheral OT on various behaviors. Temporary pharmacologic blockade of OT may produce short term behavioral effects, but will not elucidate the chronic effects of OT blockade. Also,

administering exogenous OT to an animal with endogenous OT already present is not the same as giving exogenous OT to an animal lacking the endogenous peptide and may produce different results.

Several behavioral and physiological traits distinguish OT KO mice from age- and sex-matched cohorts. Female OT KO mice exhibit normal parturition, but do not milk eject (Young et al, 1996). Female OT KO mice also show increased anxiety (Amico et al, 2004; Mantella et al, 2003b) and release more corticosterone after a psychogenic stressor (Mantella et al, 2004). Male OT KO release more corticosterone following food and water deprivation as compared to WT animals (Mantella et al, 2005). Male and female OT KO mice display inappropriate intake of sodium solutions after overnight dehydration (Amico et al, 2001; Amico et al, 2003), but not during need free conditions (Vollmer et al, 2006). Also, the anorexia normally observed during forced dehydration is attenuated in OT KO mice (Rinaman et al, 2005). However, we have shown that age- and sex-matched OT KO and WT mice have similar body weights, and display similar hourly and cumulative daily food and water intake during basal conditions (Mantella et al, 2003), similar water intake after overnight water deprivation (Amico et al, 2001), and similar food intake after an overnight fast with water available *ad libitum* (Mantella et al, 2003).

1.5 CENTRAL NERVOUS SYSTEM REGULATION OF FEEDING

Feeding is regulated by a variety of interconnected neural and somatic systems. The taste and nutrient content of a substance can influence ingestion behaviors. In humans, sweet taste is first detected by the heterodimer of G-protein coupled receptors, T1R2 + T1R3 (Nelson et al, 2001; Zhao et al, 2003). Further research is still needed to determine the function of the candidate taste

receptors for fat, salts, acids, and complex carbohydrates. In the case of sweet taste detection, transduction initiates a series of downstream events including release of neurotransmitter from the taste cell.

The nucleus of the solitary tract (NTS) acts as a first order gustatory relay. Rostral portions of the NTS receive afferent taste projections from the mouth including those from the VIIth nerve (anterior tongue and hard and soft palates), IXth nerve (taste buds in circumvallate and foliate papillae), as well as oral branches of the Vth nerve. In general, somatosensory input tends to be lateral to gustatory input (Travers and Norgren, 1995). The parabrachial nucleus of the pons (PBN) receives overlapping projections from the NTS including rostral (taste) as well as caudal (visceral) inputs. The nucleus accumbens (NAcc) also receives information relating to taste and visceral function via input from the NTS. The NTS, PBN, and amygdala are all activated following gustatory stimulation. It is not surprising that OT is involved in regulating these pathways, due to presence in these regions. OT shows high expression in the paraventricular nucleus (PVN), which has projections to the medial amygdala, NTS, and PBN (Krukoff et al, 1994).

1.6 SPECIFIC OBJECTIVES OF THE RESEARCH

The overall objective of this research was to evaluate the ingestive behavior of palatable solutions (Intralipid and sucrose) in WT and OT KO mice of C57BL/6 background.

The specific objectives of this study are:

1. To determine the effects of OT on the ingestion of Intralipid at a variety of concentrations. This was achieved by exposing male and female WT and OT KO

mice to a standard two-bottle access test containing water and an Intralipid emulsion and recording ingestion volume, fluid preference, total volume consumed, and total calories (from food and fluid) consumed.

2. To further determine the effects of OT on the ingestion of sucrose through modification of the two-bottle access paradigm. These modifications included: prolonged need-free exposure, initial ingestions and re-exposure to ascending sucrose concentrations, overnight fluid deprivation, and introduction of aversive tastants to the 10% sucrose solution. Ingestion volume, fluid preference, total volume consumed, and calories (from food and fluid) consumed were recorded in experimental groups of male and female WT and OT KO mice.

2.0 MATERIALS AND METHODS

All animal work was conducted in the Central Animal Facility, south wing of the Biomedical Science Tower, viral free quarters under a 12 h light, 12 h dark cycle (lights on at 0700 h) at the University of Pittsburgh and in accordance with the Institutional Animal Care and Use Committee (IACUC). All animals were obtained from the colony bred and maintained at the University of Pittsburgh. Mice were housed in standard suspended cages with no more than four animals per cage and had free access to food and water (Prolab RMH 3000/Purina) unless otherwise stated. The animals used in these studies were adult mice (5-20 months of age) and were acclimated to single housing three days prior to any experimental manipulation.

2.1 ANIMAL BREEDING

Male and female WT and OT KO mice of C57BL/6 background were used for these studies. The OT KO mice maintained in this colony were developed by Scott Young (National Institute of Mental Health, Bethesda, MD) (Young et al, 1996). Breeding pairs were purchased from Jackson Laboratories (Bar Harbor, ME). To maintain a colony of OT KO (-/-) mice, heterozygote (OT +/-) female and male OT KO (-/-) were used for breeding. OT KO (-/-) female mice are unable to nurse their young as they lack the OT required for milk ejection. WT females and males were used to breed WT animals. Breeding of OT KO and WT mice was done

simultaneously to insure age and generation matching. Females were placed in cages with males at a one-to-three ratio and checked daily for pregnancy after 16 days. Females were separated into individual cages for delivery. Pups were nursed for 24-26 days, weaned and placed into cages with a maximum of four per cage.

2.2 GENOTYPIC DETERMINATION BY POLYMERASE CHAIN REACTION (PCR)

Genotyping was done at the time of weaning. A small tail sample (about 2 mm) was removed with a sterile scalpel and used for DNA extraction. The sample was then prepared for polymerase chain reaction (PCR) using methods adapted from Young et al 1996. Samples were dissolved in 100 μ L of 10mM Tris-HCl and 1mM EDTA. The PCR reaction mix (50 μ L) containing 2 μ L (100 ng) of DNA, 5.0 μ L 10x PCR buffer minus Mg (Gibco BRL, Gaithersburg, MD), 2.5 μ L 10mM dNTPs (Invitrogen, Carlsbad, CA), 2.5 μ L 50mM MgCl₂ (Gibco BRL, Gaithersburg, MD), and 0.5 μ L Taq DNA polymerase (Gibco BRL, Gaithersburg, MD) was heated for 5 min at 95°C and then cycled 30 (35 for OT) times at 94°C for 40 sec (45 sec for OT) and 63°C (55°C for OT) for 1 min.

Primer pairs, synthesized at the University of Pittsburgh Sequence facility, were designed for PCR to detect either the wild type allele (OT, 332 bp) or the mutant allele (neomycin resistance cassette, 430 bp). Primer pairs for the wild type allele were (forward) TCG CTC TGC CAC AGT CCG GAT TC and (reverse) TCA GTG TTC TGA GCT GCA AAC C, and for the mutant allele, they were (forward) AGA GGC TAT TCG GCT ATG ACT G and (reverse) TTC GTC CAG ATC ATC CTG ATC.

2.3 TWO BOTTLE ACCESS TEST

During the experiment two bottles of fluid were placed in the cage. Each animal was given two-bottle access to water and an experimental solution (sucrose, Intralipid, sodium chloride, quinine, citric acid, or a second bottle of water) and had *ad libitum* access to each bottle. Bottles were monitored and refilled daily with fresh solutions. Daily (24 hour) consumption was recorded for the length of the experiment. Unless otherwise stated, each experiment concluded after four days of monitoring. Except for experiments involving a food deprivation paradigm, animals had free access to standard laboratory chow (Prolab RMH 3000/Purina 1% sucrose, 0.1% glucose, 0.2% fructose, 0% lactose, 5% crude fat by weight; 4.1 kcal/g) throughout the study period. Powdered food (Prolab RMH 3000/Purina, finely ground) was used in some experiments to allow close monitoring of the animal's total caloric intake. In such studies powdered food was available *ad libitum* and was refilled daily. For studies involving powdered food, 24 hour intake was recorded on all days that intake of fluids was monitored.

2.4 METABOLIC PARAMETER DETERMINATION

To obtain blood for metabolic parameter analysis, animals were gas sacrificed. Blood was collected from the heart via venipuncture and placed in heparinized tubes. Tubes were kept on ice and quickly centrifuged at 12000 RPM. Blood was then analyzed for plasma sodium, plasma osmolality, and total protein. Blood for determining hematocrit level was collected using microhematocrit heparinized capillary tubes at time of sacrifice and was centrifuged using an IEC MB Centrifuge (International Equipment Company). Hematocrit level was determined

using a visual scale. Blood glucose was also detected at time of sacrifice using an Ascensia Elite Glucometer (Bayer Corporation, Mishawaka, IN).

2.5 STATISTICAL ANALYSIS

Data are grouped by genotype (and treatment) and are presented as group means \pm SEM. Statistical analysis was performed using SPSS software (SPSS Inc., Chicago, IL). Differences between treatments and groups were analyzed through a repeated measures two-way analysis of variance (ANOVA) with corrections for repeated measures. If the ANOVA showed a statistically significant difference in the overall F ratio, the Bonferroni *post hoc* test was used to determine pair-wise differences. Differences of body weight and other simple comparisons were made using a t-test. Statistical significance was set at $P < 0.05$.

3.0 INTRALIPID INGESTION

Results from this chapter have been submitted in manuscript form and are under review at the *American Journal of Physiology Regulatory Integrative and Comparative Physiology*.

Previous work has shown that oxytocin (OT) signaling pathways modulate intake of sweet and non-sweet carbohydrate-containing solutions in mice. Compared to wild type (WT) mice of the same C57BL/6 background strain, male and female OT gene knockout (OT KO) mice consume larger volumes of sucrose or saccharin solutions during initial and sustained exposure (Amico et al, 2005; Billings et al, 2006). OT KO mice also consume greater amounts of non-sweet carbohydrate containing solutions (Polycose and cornstarch) than WT cohorts (Sclafani et al, 2007). Although WT mice eventually develop a strong preference for sucrose or saccharin solution over water, initial intake is attenuated compared to OT KO mice (Amico et al, 2005). Further, WT mice reach a plateau in daily sucrose or saccharin intake that is significantly less than the daily intake by OT KO mice (Amico et al, 2005; Billings et al, 2006). Thus, the genetic absence of OT in male and female mice enhances their initial and sustained daily consumption of sweet and non-sweet carbohydrate-containing solutions.

Given the exaggerated intake of palatable carbohydrate-containing solutions by OT KO compared to WT mice, the study presented in this section was designed to determine whether OT KO mice also display enhanced intake of a palatable fat-containing solution. Mice and rats

readily ingest Intralipid, a commercial non-sweetened stable soybean oil emulsion that is useful for studies requiring an easily quantifiable and palatable liquid source of fat (Hartfield et al, 2003; Lewis et al, 2006; Sclafani, 2007; Sclafani et al, 2007). If OT signaling pathways are generally involved in limiting intake of all palatable liquids, then OT KO mice should consume significantly more Intralipid than WT mice. Conversely, if OT signaling pathways play a special role in limiting intake of palatable carbohydrate-containing solutions, then intake of fat (Intralipid emulsions) should be similar between genotypes. A recent study in which mice were exposed to a 4% Intralipid emulsion did not reveal differences in intake between OT KO and WT mice (Sclafani et al, 2007). To expand these initial observations, daily fluid intake was monitored in male or female OT KO and WT mice during novel (first-time) exposure to 10% or 4.1% Intralipid emulsion as well as re-exposure to a range of Intralipid concentrations presented in descending or ascending order. Intralipid emulsion was provided *ad libitum* along with water and laboratory chow in consecutive tests. Caloric intake of both food and fluids was calculated daily. Hourly intake of Intralipid and water was also compared to the hourly intake of sucrose and water (in separate tests) during a 6 hr evening period corresponding to the transition from day to night (i.e., 2 hrs before lights out until 4 hrs after lights out).

Intralipid (20%, Fresenius Kabi, Uppsala, Sweden) was stored at 4°C and was freshly diluted each day with tap water. Each 100 ml of a 20% stock solution contains 20 g of purified soybean oil, 1.2 g of purified egg phospholipids, 2.25 g of glycerol, and water. The caloric content of the stock Intralipid emulsion is 2 kcal/ml. Water and test solution were presented simultaneously to mice (available *ad libitum*) via two stainless steel drinking spouts affixed to graduated cylinders calibrated in 0.1 ml increments. The position of water and test solution remained constant during each experiment.

3.1 EXPERIMENTAL DESIGN

1. Daily intake of 10% Intralipid in male mice during first-time exposure

The purpose of this experiment was to determine whether OT KO and WT mice differ in their initial or sustained daily intake of a lipid-rich emulsion. Male WT (n=8) and OT KO (n=8) mice (F10 generation, age 5-6 months at the start of the experiment) were given two-bottle access to water and 10% Intralipid available *ad libitum* for three days. Volumes of water and Intralipid consumed were recorded daily at 1000 h. Intralipid preference scores were calculated each day by dividing Intralipid intake by total fluid intake. On the third day of exposure, hourly intakes of each fluid were recorded from 1700 h to 2300 h (i.e., from 2 hrs before lights out until 4 hrs after lights out). This time period was selected based upon previous experience that this time corresponds to the maximal rate of palatable fluid intake. For comparative purposes, this protocol was repeated in a separate group of age-matched male OT KO (n=8) and WT (n=8) mice given two-bottle access to water and 10% sucrose solution (instead of Intralipid) available *ad libitum* along with pelleted chow for three days including hourly recordings of 10% sucrose and water intake from 1700 h to 2300 h on the third day. 10% sucrose was chosen because at this concentration OT KO mice consume more sucrose solution than WT mice (Amico et al, 2005; Billings et al, 2006).

2. Daily intake over a range of descending lipid concentrations in non-naive animals

The purpose of this experiment was to determine if animals differ in their initial and sustained daily intake upon re-exposure to descending concentrations of Intralipid emulsions. The same male mice exposed to 10% Intralipid emulsion in Experiment 1 were switched back to water as the only available fluid for one week and were then re-exposed to two-bottle access to tap water and 10% Intralipid presented *ad libitum* for three days. Intake of each fluid was recorded daily

as described for Experiment 1. Mice were then switched back to water as the only available fluid for 48 hours, then were exposed to two-bottle access to tap water and 5% Intralipid presented *ad libitum* for three days. This process was repeated for exposure to 2.5% Intralipid. As in Experiment 1, hourly fluid intake of 5% and 2.5% Intralipid and water was measured for 6 hours during the transition from day to night (1700 h to 2300 h) on exposure day 3.

3. Daily intake of 4.1% Intralipid in female mice during first-time exposure

The purpose of this experiment was two-fold: to extend these observations to female mice, and to examine intake of Intralipid at a concentration that is calorically matched to 10% sucrose. Naive female WT (n=8) and OT KO (n=8) mice (F11 generation, age 5 months) were given two-bottle access to water and 4.1% Intralipid available *ad libitum* for three days along with pelleted chow using the same protocol as Experiment 1.

4. Daily caloric intake over a range of ascending Intralipid concentrations

In Experiment 2, no differences were seen between genotypes in intake of descending Intralipid concentrations. To further study the effect of concentration on Intralipid ingestion and total caloric intake, we examined a broader range of concentrations presented in ascending order. Intralipid concentrations were isocalorically matched to previously tested sucrose concentrations (Amico et al, 2005). Female WT (n=8) and OT KO (n=8) mice (F11 generation, age 13-16 months) were presented with a series of two-bottle access tests using ascending Intralipid concentrations on consecutive weeks. Food and fluid intake were recorded daily. Following one week of acclimation to powdered food, animals were given two-bottle access to 0.5% Intralipid and water along with powdered chow for three days, then switched to water as the only fluid source and pelleted chow for 48 hrs. This pattern was repeated with ascending concentrations of

Intralipid: 1.0%, 2.1%, 4.1%, and 8.2%. Daily intakes at each Intralipid concentration were compared using data from the third day of exposure.

3.2 RESULTS

1. Daily intake of 10% Intralipid emulsion in male mice during first-time exposure

Male OT KO mice showed an immediate and sustained preference for Intralipid emulsion (near 100%) on the first day of exposure. In contrast, male WT mice showed an incremental increase in preference (56% day 1, 70% day 2, 86% day 3). Preference for Intralipid was significantly different between genotypes [ANOVA, $F(1,14) = 4.922$, $P = 0.044$] only on exposure day 1 (Bonferroni t-test $P < 0.05$) (Figure 1A).

There was a significant interaction between genotype and exposure day on Intralipid intake across the three days of exposure [ANOVA, $F(2,28) = 4.568$, $P = 0.019$]. On the first day, OT KO mice consumed almost twice as much Intralipid emulsion as WT mice (post hoc Bonferroni t-test, $P < 0.05$) (Figure 1A), but this difference was not sustained on subsequent days of exposure. In WT mice, Intralipid intake on day 1 was significantly lower than on days 2 and 3. In contrast, daily intake of Intralipid by OT KO mice did not differ among days. Mice normally consume 4-5 ml of water a day during baseline conditions (Vollmer et al, 2006). In both genotypes the volume of Intralipid consumed was greater than normal daily water consumption. Water intake on each of the three days was less than 3 ml for WT mice and less than 0.5 ml for OT KO mice. There was a significant difference in water consumption between genotypes [ANOVA, $F(1,14) = 5.69$, $P = 0.032$, post hoc Bonferroni t-test, $P < 0.05$] on day 1

(Figure 1B). Total daily fluid intake was not different between genotypes on any of the three days.

Figure 1. **Daily intake of 10% Intralipid and water in male mice during first-time exposure**

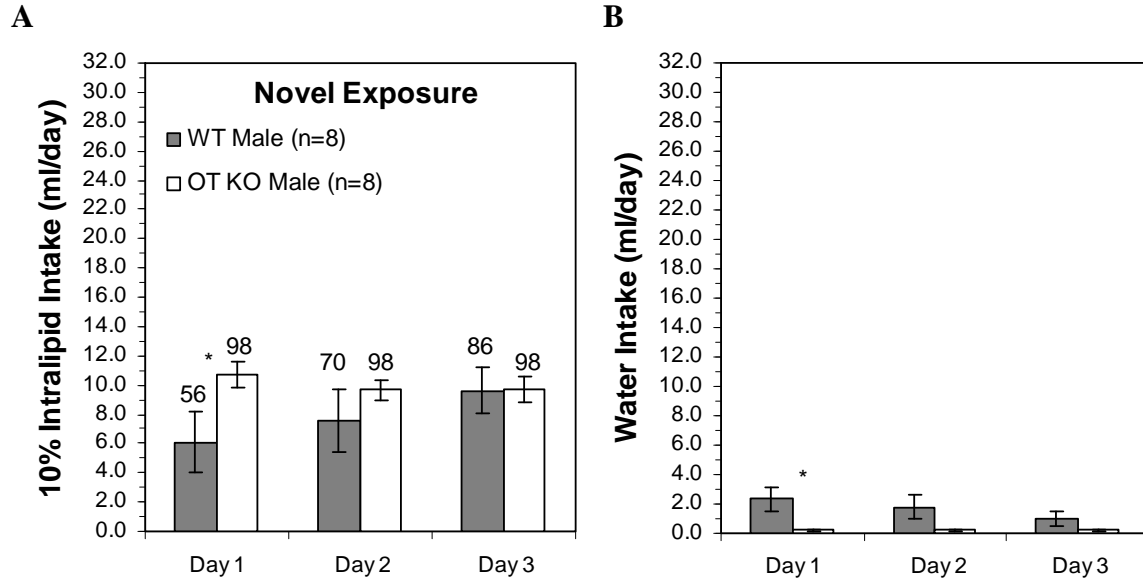


Figure 1. Daily intake of 10% Intralipid (A) and water (B) upon first time exposure in oxytocin knockout (OT KO) and wild type (WT) male mice presented with two-bottle access over a three-day exposure period. Numbers above the bars represent preference scores for Intralipid intake. Preference for Intralipid on day 1 was significantly different between genotypes (ANOVA, $P = 0.044$). There was also an interaction between genotype and time such that OT KO mice consumed significantly greater volumes of Intralipid as compared to WT mice (ANOVA, $P = 0.019$) on day 1. Consumption of Intralipid by WT mice on day 1 was significantly lower than on days 2 and 3. In contrast Intralipid intake by OT KO mice was the same each day. Water intake also differed between the genotypes on day 1 (ANOVA, $P = 0.032$). Values represent means \pm SEM. * indicates a significant difference between genotypes ($P < 0.05$)

At the end of the third exposure day, hourly monitoring of 10% Intralipid and water intake for 6 hrs during the evening (1700 h to 2300 h) confirmed that intake was not different between the genotypes (Figure 2A). In a parallel experiment, hourly intakes of 10% sucrose solution and water were monitored during the same evening period in a separate group of OT KO and WT male mice at the end of three days of exposure to 10% sucrose available *ad libitum* along with water and pelleted chow. OT KO mice consumed significantly more 10% sucrose compared to intake by WT mice at each time point after lights out [ANOVA, $F(1,14) = 8.35$, $P = 0.012$; Bonferroni t-test, $P < 0.05$] (Figure 2B).

There was no genotypic difference in body weight at the beginning or end of the three-day Intralipid experiment. Pre- and post-exposure body weights for OT KO mice were 28.8 ± 1.0 g and 28.7 ± 1.1 g and for WT mice were 30.3 ± 1.2 g and 31.1 ± 1.4 g. There was also no genotypic difference in body weight at the beginning or end of the three-day sucrose experiment. Pre- and post-exposure body weights for OT KO mice were 26.5 ± 0.4 g and 27.5 ± 0.6 g and for WT mice were 26.4 ± 0.9 g and 26.6 ± 1.2 g.

Figure 2. **Nighttime intake over a range of Intralipid concentrations in non-naive animals.**

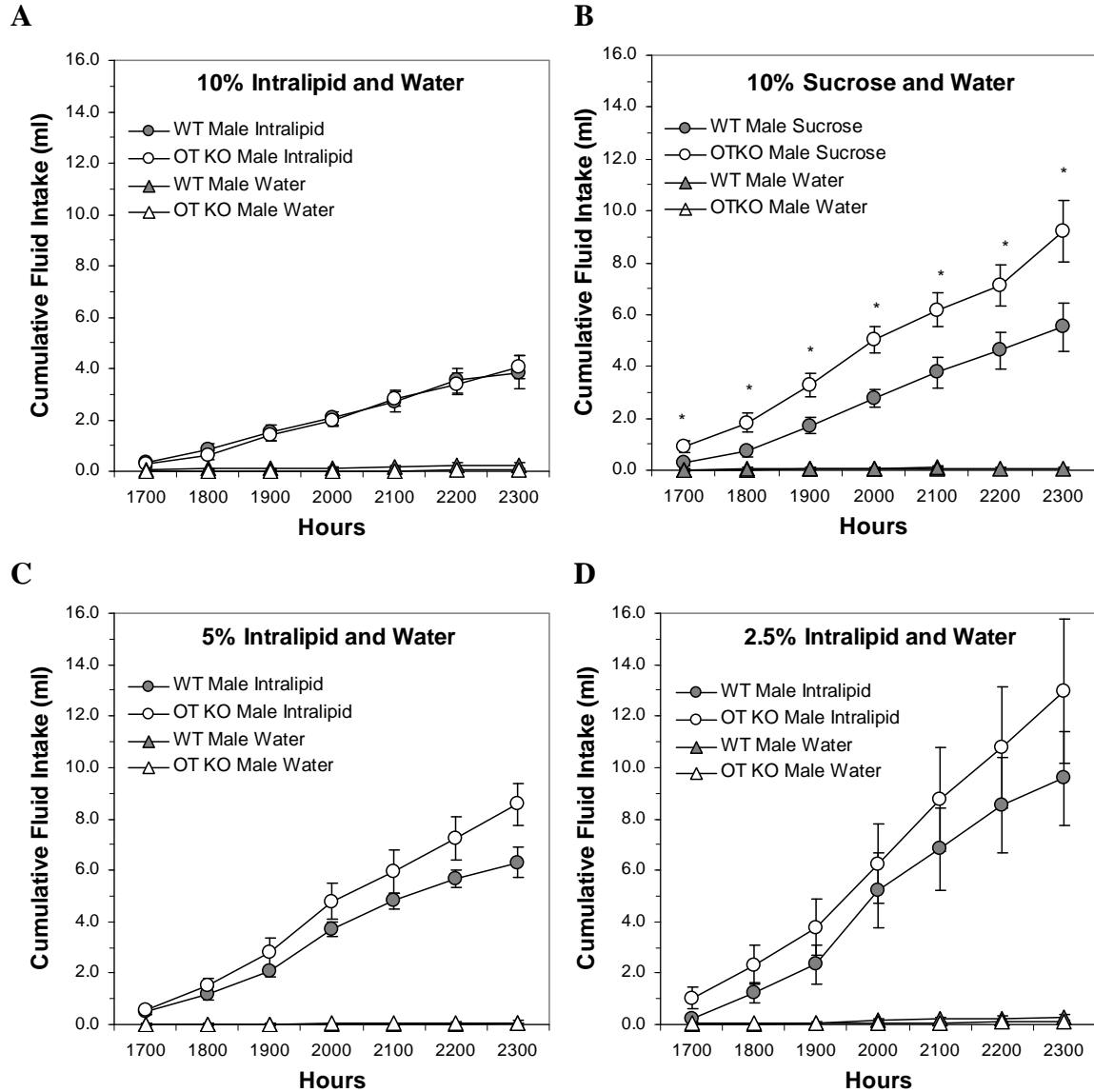


Figure 2. Hourly intake of 10% Intralipid (A), 10% sucrose (B), 5% Intralipid (C), and 2.5% Intralipid (D) in groups of oxytocin knockout (OT KO) and wild type (WT) male mice given two-bottle access to each solution paired with water. Hourly cumulative intake of 10% sucrose was greater in OT KO than WT mice (ANOVA, $P = 0.012$), whereas intakes of 10%, 5%, and 2.5% Intralipid during the same time period did not differ between genotypes. Water intake was negligible and was not different between genotypes. Values are means \pm SEM. * indicates a significant difference between genotypes ($P < 0.05$)

2. Daily intake over a range of descending lipid concentrations in non-naive animals

Male OT KO and WT mice demonstrated a marked preference for Intralipid over water when re-exposed to a 10% Intralipid emulsion during a three-day period that began one week after the conclusion of Experiment 1. Mice drank approximately 12-15 ml of 10% Intralipid daily with no significant differences between genotypes or across exposure days (Figure 3A). Intralipid preference was greater than 98% on all three days in both genotypes. Water intake was minimal, averaging less than 1.0 ml per day and did not differ between genotypes (Figure 3B).

As with the 10% Intralipid concentration, there were no significant differences between genotypes in their intake of 5% (Figure 3B) or 2.5% (Figure 3C) Intralipid over three days of exposure. OT KO and WT mice previously exposed to three days of access to 10% Intralipid consumed significantly larger daily volumes of 5% [ANOVA, $F(1,30) = 4.22$, $P = 0.049$; Bonferroni t-test, $P < 0.05$] on day 2, and 2.5% [ANOVA, $F(1,30) = 6.31$, $P = 0.018$; Bonferroni t-test, $P < 0.05$] on days 1 and 2 as compared to intake of 10% Intralipid emulsion (Figure 3). However, there were no significant differences between genotypes in intake of either 5% or 2.5% Intralipid emulsion, and no differences in volume consumed at the two lower Intralipid concentrations.

Figure 3. Daily intake over a range of Intralipid concentrations in non-naive animals.

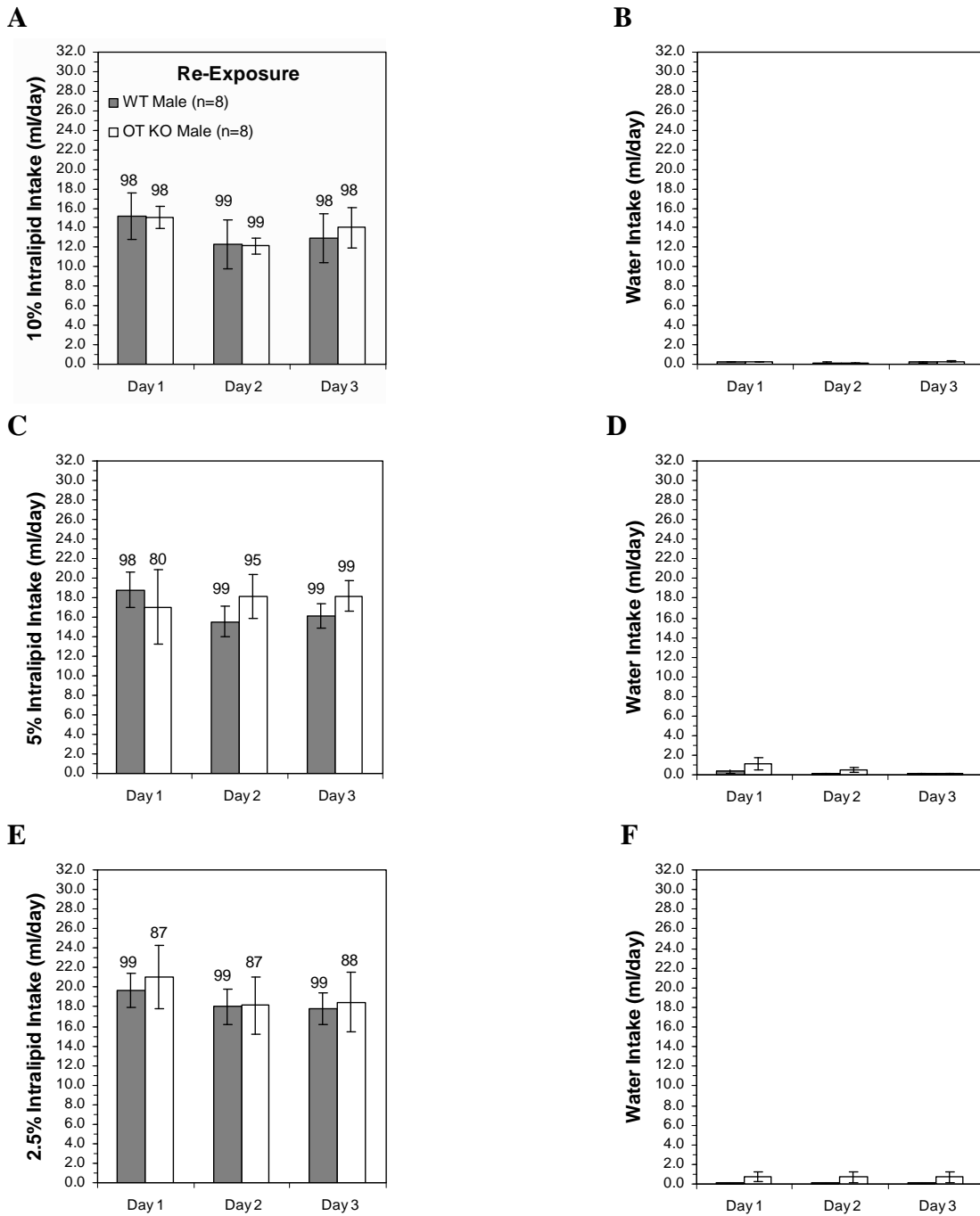


Figure 3. Daily intake over a range of descending Intralipid concentrations. No genotypic differences in daily intake of 10% Intralipid (A), water (B), or preference for Intralipid were observed following re-exposure to two-bottle access in OT KO and WT male mice. There were also no genotypic differences in daily intake of 5% (C) or 2.5% (E) Intralipid or the corresponding water intakes (D, F). Numbers above the bars represent preference scores for Intralipid intake. Values represent means \pm SEM.

In both genotypes, most fluid intake occurred after 1600 h. At the end of the third day of exposure to 5% and 2.5% Intralipid, hourly monitoring of fluid intake (Intralipid and water) for 6 hrs during the evening (1700 h to 2300 h) confirmed that intake was not different between genotypes at the 5% (Figure 2C) or 2.5% (Figure 2D) Intralipid concentrations.

There was no effect of genotype on body weight measured at the beginning and end of each three day exposure to Intralipid. There was also no effect of genotype on body weight measured before the re-exposure to 10% Intralipid and the end of 2.5% Intralipid exposure. Pre- and post-exposure body weights for OT KO mice were 28.8 ± 1.1 g and 29.7 ± 1.2 g and for WT mice were 30.3 ± 1.2 g and 31.0 ± 1.4 g.

3. Daily intake of 4.1% Intralipid in female mice during first-time exposure

The preference of female OT KO mice for Intralipid was similar on all three days with 96% preference on day 1, 97% on day 2, and 87% on day 3. In contrast, the preference of female WT mice for Intralipid was only 58% on day 1, and then reached levels equal to those of OT KO mice, 97% and 98% on days 2 and 3 respectively. There was a significant interaction between genotype and exposure day on preference for 4.1% Intralipid [ANOVA, $F(2,28) = 6.850$, $P = 0.004$] on day 1 (Bonferroni t-test $P < 0.05$). Female OT KO mice consumed approximately twice as much 4.1% Intralipid emulsion as WT mice on the first day of exposure (Figure 4A). The effect of genotype on Intralipid intake on experimental day 1 in naive female OT KO mice was similar to that seen on the first day of exposure to 10% Intralipid in male OT KO mice (Experiment 1). There was a significant interaction between genotype and exposure day in daily intake of 4.1% Intralipid in naive female mice [ANOVA, $F(2,28) = 4.622$, $P = 0.018$] on day 1 (Bonferroni t-test $P < 0.05$). There was also an overall significant interaction between genotype and exposure day in daily water intake [ANOVA, $F(2,28) = 6.161$, $P = 0.006$] (Figure 4B).

Water intake of WT mice on day 1 was significantly higher than water intake by WT mice on days 2 and 3 (Bonferroni t-test $P < 0.05$). However, total daily fluid intake did not differ between genotypes.

There was no effect of genotype on body weight measured at the beginning or end of the three-day exposure to 4.1% Intralipid. Pre- and post-exposure body weights for OT KO mice were 28.8 ± 1.0 g and 28.7 ± 1.1 g and for WT mice were 30.3 ± 1.2 g and 31.1 ± 1.4 g.

Figure 4. **Daily intake of 4.1% lipid and water in female mice during first-time exposure**

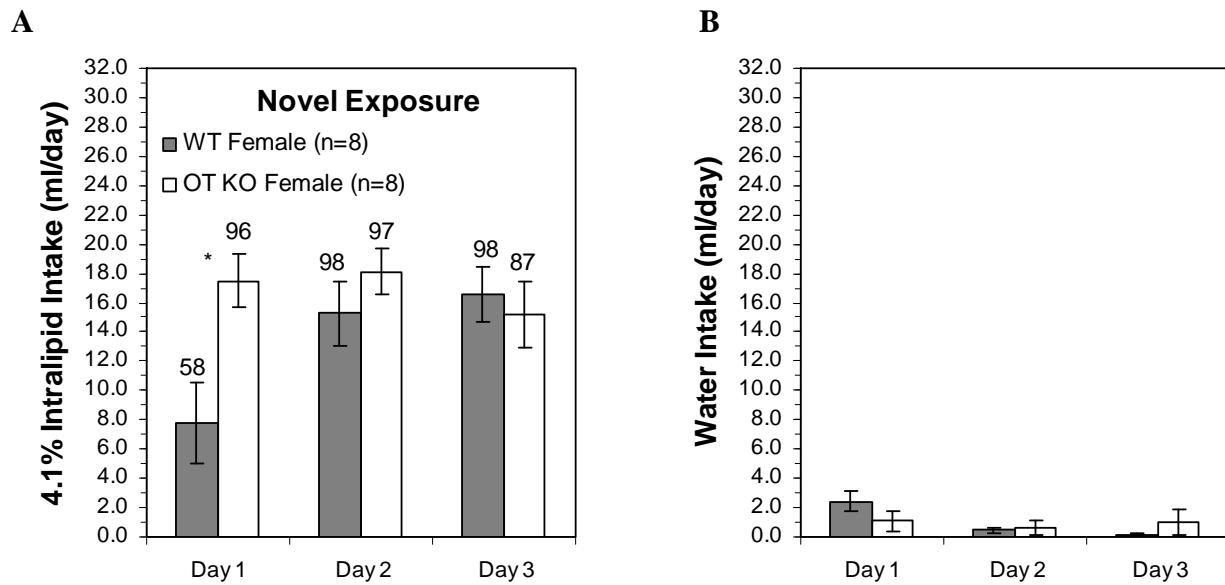


Figure 4. Daily intake of 4.1% Intralipid (A) and water (B) upon first time exposure in oxytocin knockout (OT KO) and wild type (WT) female mice presented with two-bottle access over a three-day exposure period. Numbers above the bars represent preference scores for Intralipid intake. There was a significant interaction between genotype and time such that OT KO mice consumed significantly greater volumes of Intralipid on day 1 (ANOVA, $P = 0.018$) and significantly less water (ANOVA, $P = 0.006$) compared to WT mice. There was also a significant interaction between genotype and time in Intralipid preference on day 1 (ANOVA, $P = 0.004$). Values represent means \pm SEM. * indicates a significant difference between genotypes ($P < 0.05$)

4. Daily caloric intake over a range of ascending Intralipid concentrations

A separate group of female OT KO and WT mice naive to Intralipid were exposed to ascending concentrations of Intralipid (0.5%, 1.0%, 2.1%, 4.1%, and 8.2%) for three days each during consecutive weeks. When mice were presented with a broader range of Intralipid concentrations in ascending order, there were no differences between genotypes in the volume of Intralipid consumed at each of the tested concentrations (Figure 5A). However, there was a significant concentration related effect on the volume of Intralipid consumed [ANOVA, $F(1,4) = 8.25$, $P < 0.001$]. Intralipid consumption increased as concentration increased, up to 4.1%, but declined during exposure to the highest concentration tested (8.2%). As Intralipid concentration increased, calories consumed from Intralipid also increased [ANOVA, $F(1,4) = 54.51$, $P < 0.001$] (Figure 5C). However, total calories consumed (chow plus Intralipid) did not differ between genotype or between concentrations (Figure 5B). To compensate for the increased calories consumed from Intralipid, WT and OT KO animals decreased food intake (Figure 5D). There was no difference between genotypes in calories consumed from food, but there was a significant effect of Intralipid concentration on calories consumed from food [ANOVA, $F(1,4) = 15.99$, $P < 0.001$]. Significantly fewer calories were obtained from food during exposure to 8.2% Intralipid compared to calories consumed from food during exposure to any of the other Intralipid concentrations (Bonferroni t-test $P < 0.05$). As Intralipid concentration and consumption increased, food consumption decreased similarly in both genotypes.

There was no effect of genotype on body weights measured at the beginning or end of the experiment or between any of the three day exposures. Pre- and post-exposure body weights for OT KO were 27.4 ± 1.0 g and 26.9 ± 0.8 g and for WT were 27.3 ± 0.9 g and 27.1 ± 0.9 g.

Figure 5. Daily intake over a range of ascending Intralipid concentrations

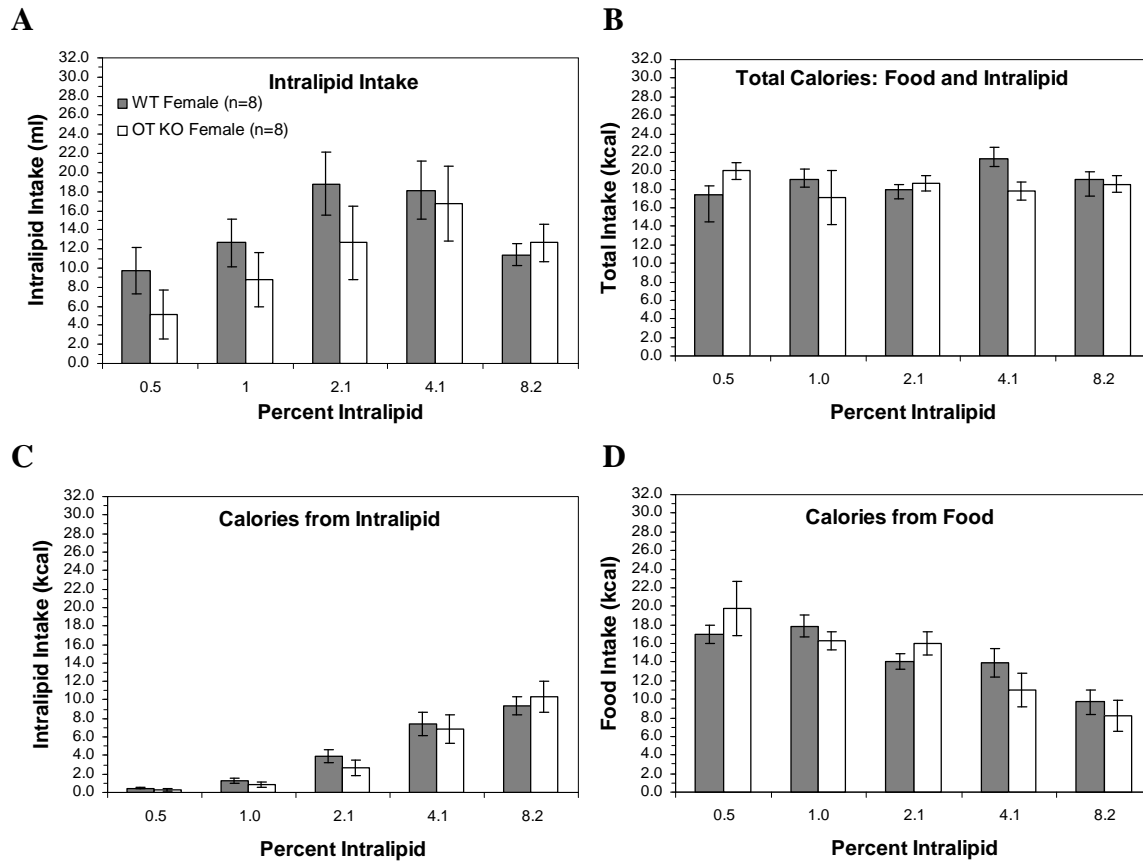


Figure 5. Volume of liquid and calories consumed from an ascending range of Intralipid concentrations in oxytocin knockout (OT KO) and wild type (WT) mice given two-bottle access to water paired with Intralipid. Data shown represent intake on day 3. There was no effect of genotype on volume consumed (A) or on calories derived (C) from any of the Intralipid concentrations tested. There was an effect of concentration on the total volume of Intralipid consumed (ANOVA, $P < 0.001$). There were also main effects of concentration on the calories consumed from Intralipid (C) (ANOVA, $P < 0.001$) and on the calories consumed from food (D) (ANOVA, $P < 0.001$). However, total calories (food plus Intralipid) did not differ between genotypes or by Intralipid concentration (B). Values represent means \pm SEM.

3.3 DISCUSSION

Recent work has shown that OT KO mice consume greater daily volumes of sweetened (sucrose and saccharin) as well as non-sweetened carbohydrate-containing solutions (i.e., Polyose and cornstarch) (Amico et al, 2005; Billings et al 2006; Sclafani et al, 2007). The present work sought to determine whether the enhanced intake of palatable sweetened and non-sweetened carbohydrate-containing solutions manifested by OT KO mice extends to enhanced intake of palatable but non-sweetened Intralipid.

Results shown here demonstrate that WT and OT KO mice prefer Intralipid in concentrations between 0.5% and 10% to water, consistent with previous reports that lipid emulsions in this concentration range are highly palatable to rats (Hartfield et al, 2003) and mice (Lewis et al, 2006; Sclafani, 2007). Interestingly, the only time that OT KO mice showed greater preference for Intralipid compared to WT mice was on the first day of exposure to the novel emulsions at concentrations of 10% and 4.1%. On subsequent Intralipid exposure days there were no differences between genotypes in daily intake or preference for Intralipid at any of the eight concentrations tested, including no differences in hourly evening intakes. These new observations contrast with previous findings that OT KO mice persistently consume significantly more sucrose, saccharin, and non-sweetened carbohydrate-containing solutions than WT cohorts.

These cumulative findings support the view that the congenital absence of OT in OT KO mice is not associated with enhanced intake of a palatable fat-enriched emulsion (Intralipid), but is associated with enhanced intake of saccharin and carbohydrate-containing liquids. When male (Experiment 1) and female (Experiment 3) OT KO and WT mice were naive to Intralipid, OT KO mice consumed almost twice as much lipid emulsion and displayed nearly twice as much preference for Intralipid over WT mice on the first day of exposure. During initial exposure, OT

KO mice demonstrate no delay in consuming novel palatable liquids, including both Intralipid (present work) and sucrose solutions (Amico et al, 2005). After mice gain experience with the palatable solutions, both genotypes consume equivalent amounts of Intralipid (present work), but OT KO mice continue to consume greater amounts of palatable sweetened (sucrose and saccharin) and non-sweetened carbohydrate-containing (Polydose and cornstarch) solutions (Amico et al, 2005; Billings et al, 2006; Sclafani et al, 2007).

These experiments included a range of Intralipid concentrations from 0.5% to 10%, (0.05 kcal/ml and 1 kcal/ml, respectively) including 4.1% Intralipid which is isocalorically matched to 10% sucrose (0.41 kcal/ml). Therefore, it is unlikely that daily Intralipid intake by mice of either genotype is limited by a ceiling effect of caloric density. Although very high or very low concentrations of Intralipid could mask a potential genotypic effect on intake of these palatable emulsions, the results between genotypes are similar through all concentrations. As shown in Experiment 4, the absence of OT does not interfere with the ability of mice to regulate their total daily caloric intake. Mice of both genotypes maintained a relatively constant daily caloric intake by reducing chow intake as calories derived from Intralipid increased. The compensatory reduction in solid food intake was also previously observed in OT KO and WT mice drinking sucrose solution (Amico et al, 2005).

Because OT gene deletion has not been shown to disrupt food or water intake under basal conditions or after overnight deprivation, the immediate and sustained excessive intake of carbohydrate-containing solutions by male and female OT KO mice is striking (Amico et al, 2005; Billings et al, 2006). Such observations support the hypothesis that OT signaling pathways in mice play a special role in limiting excessive intake of highly palatable ingesta. Integration of past findings with the new data reported here suggest that this hypothesis should

be refined, as OT signaling pathways do not appear to be involved in limiting intake of Intralipid.

4.0 SUCROSE INGESTION

To further expand the initial observations with 10% sucrose, a variety of conditions were used to modify the standard four-day sucrose-water two-bottle access procedure. These conditions included: prolonged need-free exposure, initial ingestion and re-exposure to ascending sucrose concentrations, overnight fluid deprivation, and introduction of aversive tastants to 10% sucrose solution. Sucrose, water, and food ingestion were monitored in OT KO and WT mice for two consecutive weeks to determine ingestion throughout a longer monitoring period. In a separate set of studies, naive animals were exposed to a range of sucrose concentrations in ascending order to see if an effect remained throughout the concentration curve. The two-bottle access paradigm was further adjusted to include overnight fluid deprivation and exposure to aversive solutions combined with 10% sucrose. WT and OT KO mice do not readily consume a 0.5M sodium chloride (NaCl) solution at baseline conditions (Vollmer et al, 2006). However, previous findings have shown that OT KO mice will inappropriately ingest NaCl solution following overnight fluid deprivation (Amico et al, 2001). Aversive substances (NaCl and citric acid solutions) were sweetened to determine if animals would inappropriately ingest a normally aversive solution if combined with sucrose. Solutions were provided *ad libitum* along with water and laboratory chow. Intake was calculated daily.

Sucrose (Sigma-Aldrich, St. Louis, MO) solutions were mixed each day with tap water. A 10% sucrose solution contains 0.41 kcal/ml. NaCl (Sigma-Aldrich, St. Louis, MO) and citric

acid (Sigma-Aldrich, St. Louis, MO) solutions were also prepared with tap water on the morning of exposure. Water and test solution were presented simultaneously to mice (available *ad libitum*) via two stainless steel drinking spouts affixed to graduated cylinders calibrated in 0.1 ml increments. The position of water and test solution remained constant during each experiment unless otherwise stated.

4.1 EXPERIMENTAL DESIGN

1. Daily intake of 10% sucrose solution

The purpose of this experiment was to follow food and fluid intake in WT and OT KO mice over a two-week period. Non-naive animals (male and female 12-20 months of age, F8 and F9 generation) were given two-bottle access, either 10% sucrose/water or water/water. Twenty-four animals (WT n=12; OT KO n=12) were given *ad libitum* access to 10% sucrose and water, while fifteen animals (WT n=8; OT KO n=7) were given *ad libitum* access to two bottles of water for the length of the study. Bottle placement (left, right) was switched to remove any place preference. All animals had free access to laboratory chow throughout the study. On days when data are not shown, fluid intake was not recorded daily, but animals were given free access to two 300 CC bottles of fluid (10% sucrose and water, or two bottles of water, depending on treatment). Following the same paradigm, but after eighteen days total exposure to sucrose and/or water, blood was collected from 19 animals (WT n=9; OT KO n=10) to determine hematocrit, glucose, plasma sodium, plasma osmolality, and total protein.

2. Daily caloric intake over a range of sucrose concentrations

The purpose of this experiment was to determine if animals differ in their initial and sustained daily intake upon exposure to ascending concentrations of sucrose solutions. Naive female WT (n=8) and OT KO (n=8) mice (F10 generation, age 8-12 months at the start of the experiment) were given two-bottle access to water and sucrose solution presented *ad libitum* for four days. Animals then received water as the only available source of fluid for three days and were then given two-bottle access to water and sucrose (for four days) in a concentration that was higher than solution given previously. This pattern continued over four weeks (four sucrose concentrations: 0.625%, 1.25%, 2.5%, and 5%). Intake of each fluid was recorded daily and compared across concentrations using data from the second day of exposure. Laboratory chow was available *ad libitum* through the course of all studies.

3. Fluid intake following overnight fluid deprivation

The purpose of this study was to determine if animals would drink more fluid in their response to overnight water deprivation when given a 10% sucrose solution following the eighteen-hour deprivation period. Male WT (n=8) and OT KO (n=8) (F9 generation, age 6-7 months) were given two bottles of water for two full days, on the third day at 1600 h both bottles of water were removed from the cage. The following morning at 1000 h fluid was replaced. Each animal received two-bottle access to water and 10% sucrose solution. Fluid intake was recorded at 5, 10, 20, 30, 40, 50, and 60 minutes post-fluid introduction as well as at 6 and 24 hours. Food was available *ad libitum* throughout the study.

4. Intake of aversive solutions combined with 10% sucrose

The purpose of this study was to determine if animals would consume substances normally considered aversive if combined with 10% sucrose. Through previous studies 0.5M sodium

chloride (NaCl) has been shown to be equally aversive to WT and OT KO mice (Amico et al, 2001; Vollmer et al, 2006). Therefore, following a two-bottle access procedure male WT (n=8) and OT KO (n=8) mice (F10 generation, age 5-6 months) were given a 0.5M NaCl/10% sucrose mixture and water for four consecutive days. A separate group of male WT (n=8) and OT KO (n=8) mice (F10 generation, age 5-10 months) were given one bottle of water and one bottle of 30mM citric acid (sour) for four consecutive days. The aversive citric acid concentration was chosen based on published C57BL/6 ingestion curves (Finger et al, 2005). The same animals previously exposed to 30mM citric acid were then exposed a 30mM citric acid/10% sucrose mixture paired with water for four consecutive days. 24-hour fluid intake was recorded and food was available *ad libitum* during each study. Between experiments food and water (as the only fluid source) were available *ad libitum*.

4.2 RESULTS

1. Daily intake of 10% sucrose solution

In agreement with previously published results over four and eight days of exposure, OT KO mice continued to show increased consumption of 10% sucrose as compared to WT mice [ANOVA, $F(1,22) = 23.871$, $P < 0.001$, post hoc Bonferroni t-test, $P < 0.05$] over the eleven days of exposure (Figure 6A). Total fluid intake by OT KO mice was also increased [ANOVA, $F(1,22) = 26.971$, $P < 0.001$, post hoc Bonferroni t-test, $P < 0.05$] on each day as compared to WT cohorts (Figure 6D). Water intake was negligible and was not different between genotypes (Figure 6B). Food intake was increased in WT mice as compared to OT KO cohorts [ANOVA,

F (1,22) = 12.124, P = 0.02, post hoc Bonferroni t-test, P < 0.05] on days 1, 2, 8, 9, and 10 (Figure 6C).

Figure 6. **Daily intake during two-bottle access over eleven consecutive days**

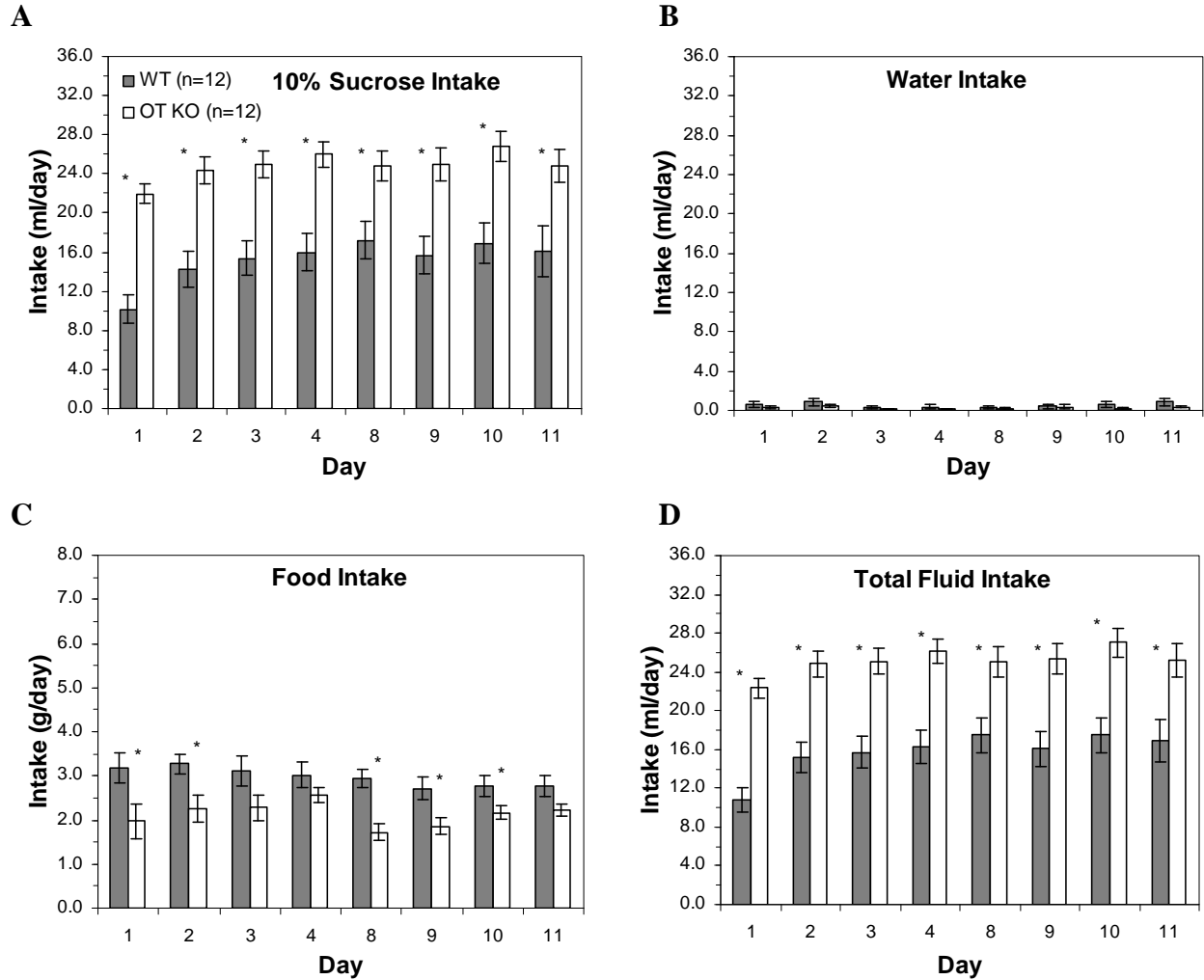


Figure 6. Daily intake of 10% sucrose (A), water (B), food (C), and total fluid (D) in oxytocin knockout (OT KO) and wild type (WT) mice presented with two-bottle access to 10% sucrose and water over an eleven day exposure period. OT KO mice consumed significantly more 10% sucrose and total fluid as compared to WT mice (ANOVA, P < 0.001). WT animals consumed significantly more food when compared to OT KO cohorts (ANOVA, P = 0.02). Water intake was negligible and did not differ between genotypes. Values represent means \pm SEM. * indicates a significant difference between genotypes (P < 0.05).

There was no influence of place preference on sucrose consumption as shown in Figure 7. Animals of each genotype consumed similar amounts of sucrose whether the bottle was placed on the left or right side of the cage.

Figure 7. Effects of bottle position on intake of 10% sucrose on consecutive exposure days

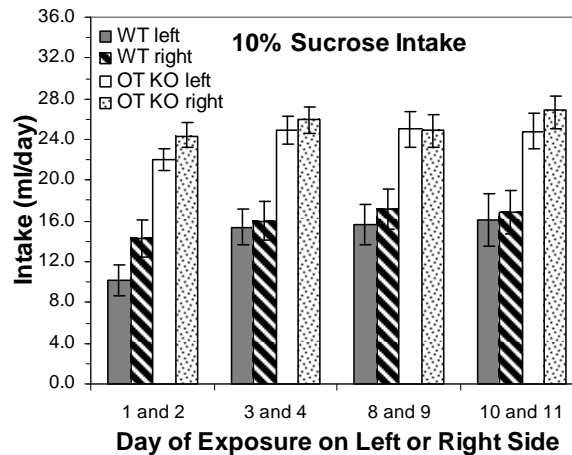


Figure 7. Daily intake of 10% sucrose in oxytocin knockout (OT KO n=12) and wild type (WT n=12) mice presented with two-bottle access to 10% sucrose and water over an eleven day exposure period. Bottle placement (left or right side) did not influence sucrose ingestion. Within each genotype animals consumed similar amounts without regard for bottle placement. Values represent means \pm SEM.

The caloric preference for sucrose (over food) was significantly greater in OT KO animals [ANOVA, $F(1,22) = 21.813$, post hoc Bonferroni t-test, $P < 0.05$] as compared to WT animals on all days. On each day over half of the calories consumed by OT KO mice were derived from the sucrose solution. However, total caloric intake did not differ between genotypes (Figure 8).

Figure 8. Daily caloric intake of food and sucrose over eleven consecutive days

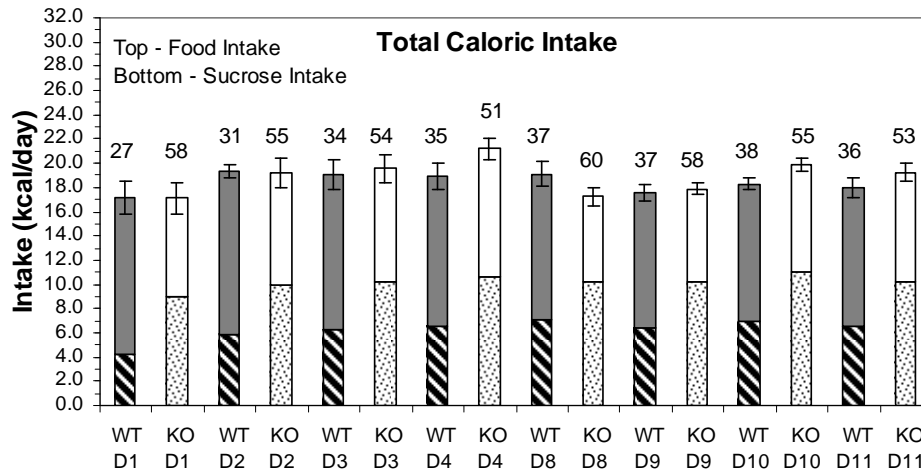


Figure 8. Daily total caloric intake in wild type (WT n=12) and oxytocin knockout (KO n=12) mice presented as two-bottle access to 10% sucrose and water and given *ad libitum* access to food over an eleven day exposure period. The numbers above the bars represent the percentage of total calories derived from sucrose. The caloric preference for sucrose was significantly higher in OT KO animals as compared to WT animals (ANOVA, $P < 0.001$). Over half of the total calories consumed on each day by OT KO animals were derived from the 10% sucrose solution. However, there were no differences in total caloric intake between genotypes. Values represent means \pm SEM.

Animals that received two bottles of water over the eleven day period showed no genotypic differences in water consumption, food consumption, or calories consumed (Figure 9). Calories consumed from food under this baseline condition did not differ from the caloric level consumed by animals that had received sucrose and water throughout the course of the study.

Figure 9. Daily intake during access to two bottles of water over eleven consecutive days

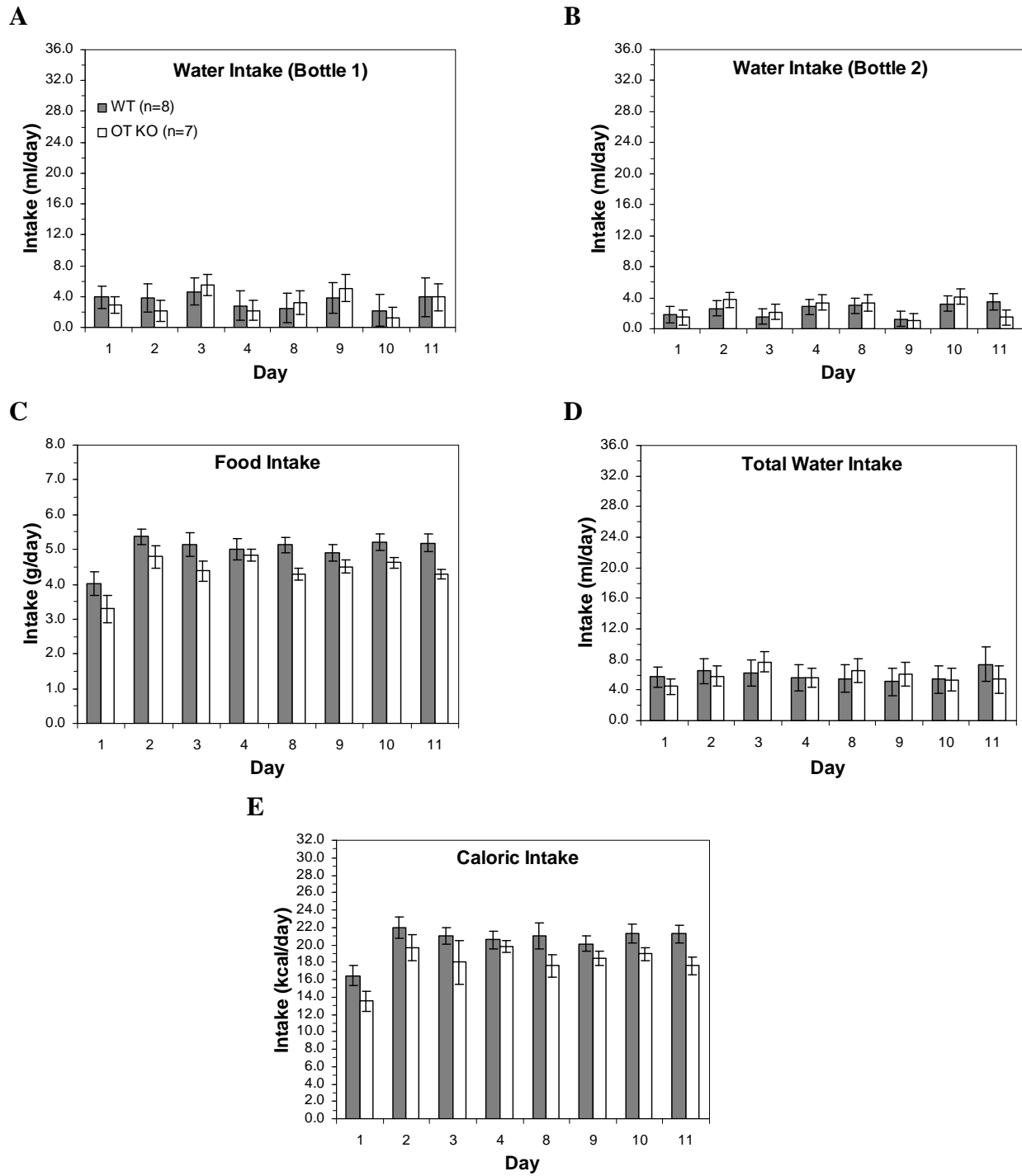


Figure 9. Daily intake of two bottles of water (A, B, and D), food (C), and calories (E) in oxytocin knockout (OT KO) and wild type (WT) mice given two-bottle access over an eleven-day exposure period. There were no genotypic differences in intake of water, food, or calories over the eleven days measured. Values represent means \pm SEM.

A variety of metabolic parameters including hematocrit, glucose, plasma sodium, plasma protein, and plasma osmolality were measured in a separate group of WT and OT KO animals following eighteen days of exposure to 10% sucrose and water or two bottles of water. However, there were no genotypic or treatment differences found in any of the metabolic parameters measured (Table 1).

There were no genotypic differences in body weight at the beginning or end of the experiment. Pre- and post-sucrose exposure body weights for OT KO mice were 31.9 ± 1.3 g and 33.5 ± 1.5 g and for WT mice were 30.2 ± 0.8 g and 30.9 ± 0.6 g.

Table 1. **Metabolic data following sucrose/water (S/W) or water/water (W/W) two-bottle access**

Genotype	Treatment		Hematocrit	Glucose (mg/dL)	p[Na] (mEq/L)	PP (g/dL)	pOsm (mOsm/L)
WT n=5	S/W	Mean	48.1%	107.0	153.4	6.0	324.8
		SE	<i>0.9%</i>	<i>12.4</i>	<i>0.7</i>	<i>0.1</i>	<i>1.9</i>
WT n=4	W/W	Mean	44.5%	101.5	154.8	6.4	331.7
		SE	<i>0.3%</i>	<i>25.2</i>	<i>1.5</i>	<i>0.6</i>	<i>0.7</i>
KO n=5	S/W	Mean	46.8%	123.2	154.2	6.3	330.5
		SE	<i>1.5%</i>	<i>10.9</i>	<i>0.7</i>	<i>0.2</i>	<i>1.5</i>
KO n=5	W/W	Mean	42.9%	113.2	156.8	6.0	328.4
		SE	<i>0.8%</i>	<i>9.0</i>	<i>0.5</i>	<i>0.4</i>	<i>1.5</i>

Table 1. Metabolic measurements (hematocrit, glucose, plasma sodium (p[Na]), plasma protein (PP), and plasma osmolality (pOsm)) of wild type (WT) and oxytocin knockout (KO) mice following eighteen days of exposure to two-bottle access to 10% sucrose and water (S/W) or two bottles of water (W/W). There were no genotypic or treatment differences found in any of the metabolic parameters measured.

2. Daily intake over a range of sucrose concentrations

When naive female mice were presented with a range of sucrose concentrations in ascending order there were significant differences between genotypes in volume consumed. OT KO mice consumed significantly more sucrose [ANOVA, $F(1,14) = 13.419$, $P = 0.003$, post hoc Bonferroni t-test, $P < 0.05$] at concentrations of 0.625%, 1.25%, 2.5%, and 5% (Figure 10A). In contrast, WT mice consumed significantly more water [ANOVA, $F(1,14) = 4.739$, $P = 0.047$, post hoc Bonferroni t-test, $P < 0.05$] while also given access to the 0.625% and 2.5% sucrose concentrations (Figure 10B). While measurable water intake was recorded from WT animals, OT KO animals showed a preference of over 80% for the sucrose solutions at all concentrations. OT KO animals also consumed more calories from sucrose at all concentrations as compared to WT animals [ANOVA, $F(1,14) = 14.065$, $P = 0.002$, post hoc Bonferroni t-test, $P < 0.05$] (Figure 10C). As sucrose concentration increased, the calories consumed from sucrose also increased. There was also a significant effect of concentration on total fluid intake. Total fluid intake increased as sucrose concentration increased. OT KO mice consumed more total fluid when presented with 2.5% and 5% sucrose as compared to the intake of WT animals at those same concentrations [ANOVA, $F(1,14) = 14.065$, $P = 0.002$, post hoc Bonferroni t-test, $P < 0.05$] (Figure 10D).

There were no genotypic differences in body weight at the beginning or end of the four experimental weeks. Pre- and post-sucrose exposure body weights for OT KO mice were 27.1 ± 1.3 g and 27.0 ± 1.1 g and for WT mice were 26.4 ± 0.9 g and 26.6 ± 0.7 g.

Figure 10. Daily intake over a range of ascending sucrose concentrations

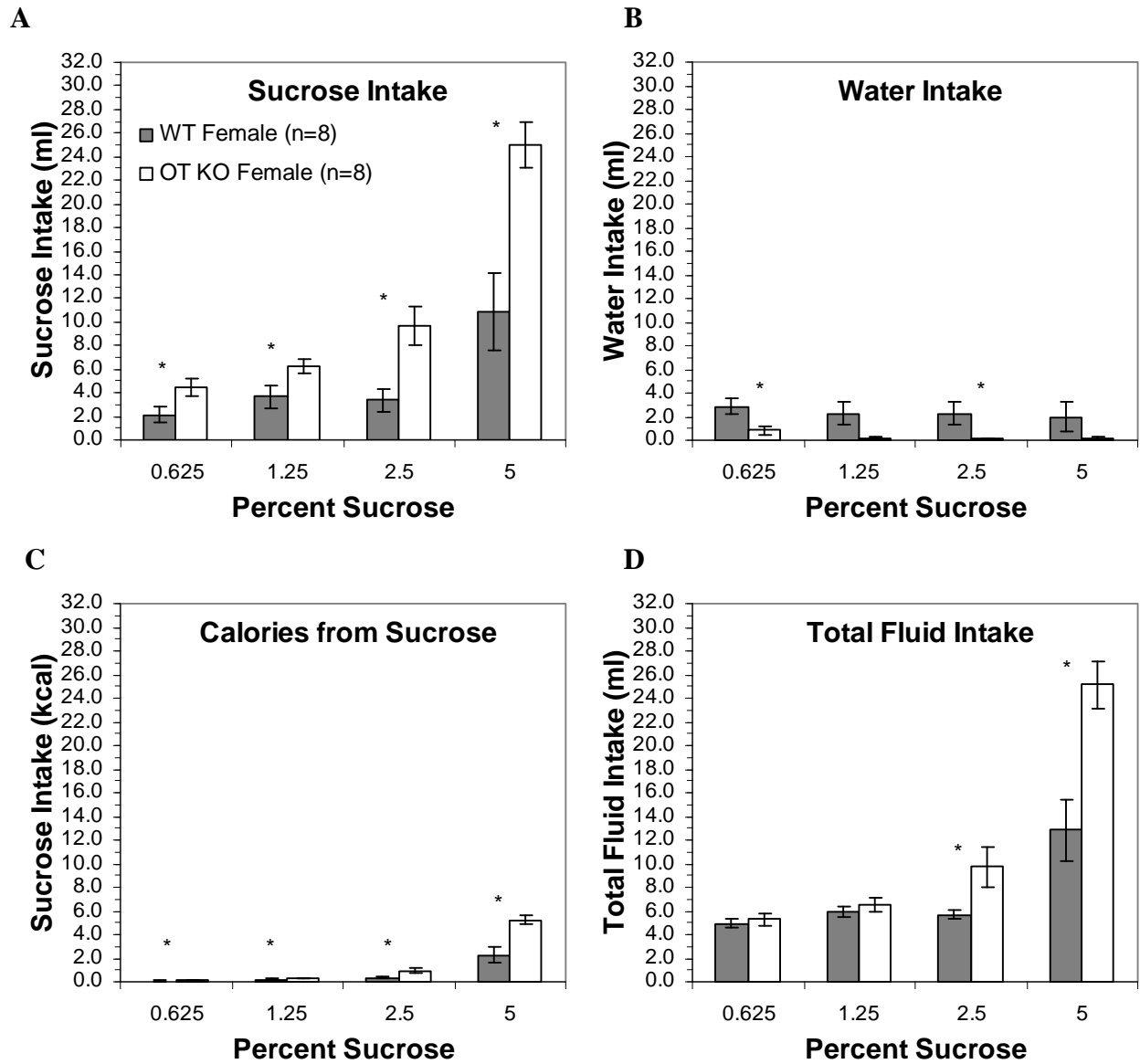


Figure 10. Volume of liquid and calories consumed from an ascending range of sucrose concentrations in oxytocin knockout (OT KO) and wild type (WT) mice given two-bottle access to water paired with sucrose. Data shown represent intake on day 2. There were significant effects of genotype on the volume of sucrose consumed (ANOVA, $P = 0.003$) (A), volume of water consumed (ANOVA, $P = 0.047$) (B), calories derived from sucrose (ANOVA, $P = 0.002$) (C), and volume of total fluid consumed (ANOVA, $P = 0.005$) (D). Values represent means \pm SEM. * indicates a significant difference between genotypes ($P < 0.05$)

3. Fluid intake following overnight fluid deprivation

Male WT and OT KO animals previously exposed to water were given an eighteen hour fluid deprivation. Following the deprivation period, two bottles of fluid (10% sucrose and water), were inserted into each cage and consumption was monitored. There was a significant difference in sucrose consumption immediately following the re-introduction of fluids. OT KO mice consumed more sucrose at all intervals measured within the first hour [ANOVA, $F(1,14) = 13.44$, $P = 0.003$, post hoc Bonferroni t-test, $P < 0.05$] as compared to WT mice (Figure 11A). OT KO mice also consumed significantly more sucrose at the 1 and 24 hour intervals [ANOVA, $F(1,14) = 9.78$, $P = 0.007$, post hoc Bonferroni t-test, $P < 0.05$] as compared to WT mice (Figure 11B). Before fluid deprivation and before being introduced to sucrose both genotypes recorded similar consumption of total fluid (water) on days 1, 2, and 3. After fluid deprivation and the introduction of sucrose, OT KO animals consumed significantly more total fluid (sucrose and water) [ANOVA, $F(1,14) = 5.696$, $P = 0.032$, post hoc Bonferroni t-test, $P < 0.05$] as compared to WT animals (Figure 11C).

Body weight was measured at the beginning and end of eighteen-hour fluid deprivation. Animals lost approximately 20% of their body weight over the eighteen-hour fluid deprivation, demonstrating a dehydrated state.

Figure 11. Fluid intake before and after overnight fluid deprivation

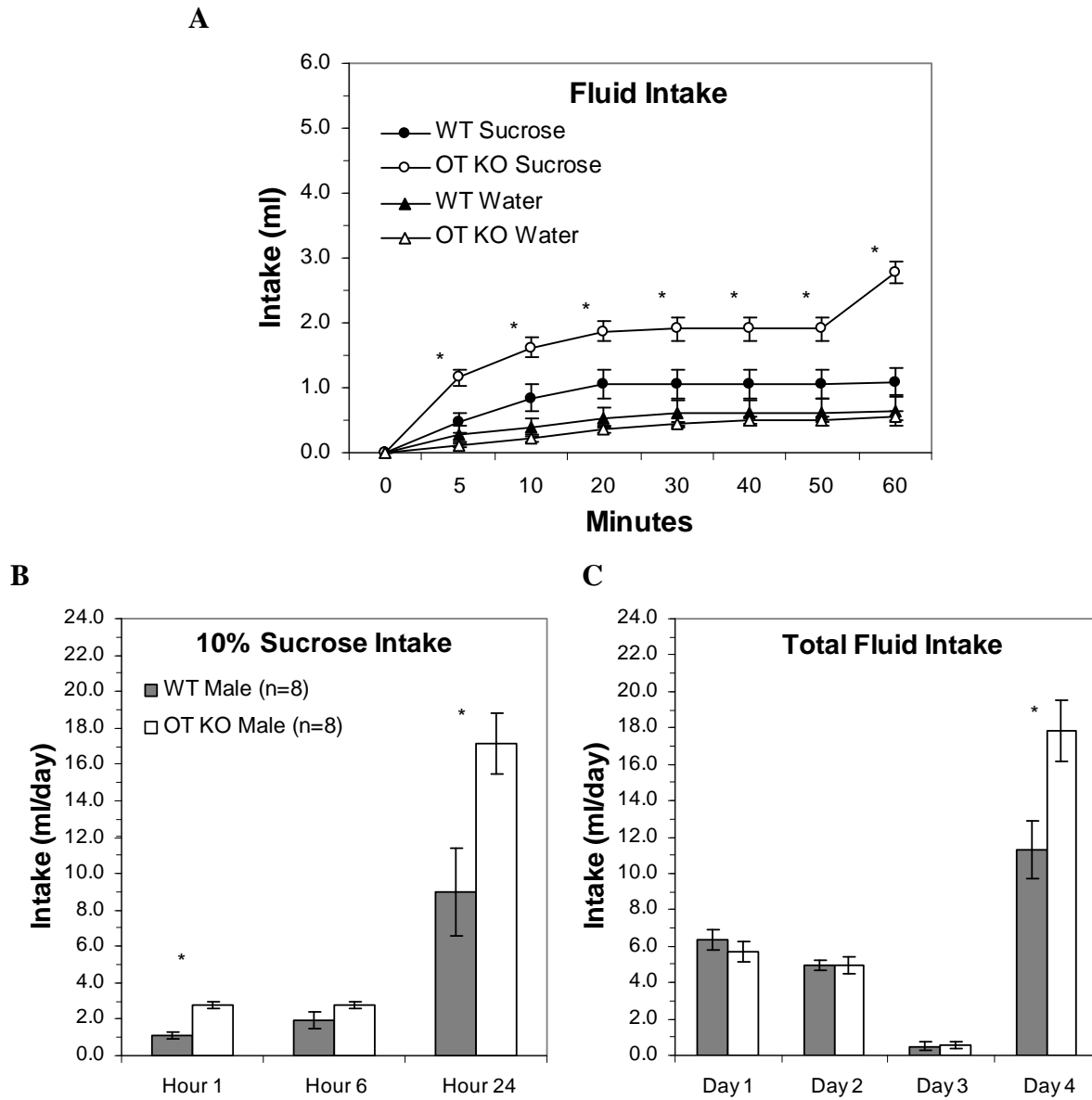


Figure 11. Following an eighteen-hour fluid deprivation on day 3, male oxytocin knockout (OT KO) and wild type (WT) mice were given two-bottle access to 10% sucrose and water. OT KO animals consumed significantly more sucrose at each interval within a one-hour time period (ANOVA, $P = 0.003$) as compared to WT mice (A). There were no genotypic differences in water intake within the one-hour interval. OT KO animals also consumed more sucrose at the 1 hour and 24 hour time points (ANOVA, $P = 0.007$) as compared to WT animals (B). Total fluid intake did not differ between genotypes on days 1, 2, and 3 when water only was available. However, when sucrose was introduced on day 4, OT KO mice consumed significantly more total fluid (sucrose and water) compared to WT animals (ANOVA, $P = 0.032$) (C). Values represent means \pm SEM. * indicates a significant difference between genotypes ($P < 0.05$)

4. Intake of aversive solutions combined with 10% sucrose

As previously reported, animals will not readily consume a 0.5M NaCl solution under baseline conditions (Amico et al, 2001; Vollmer et al, 2006). However, when this same concentration of NaCl was combined with 10% sucrose and given in two-bottle access test along with water over four days, OT KO mice consumed significantly more of the 10% sucrose/0.5M NaCl solution [ANOVA, $F(1,14) = 14.87$, $P = 0.002$, post hoc Bonferroni t-test, $P < 0.05$] on days 1 and 3 as compared to WT mice (Figure 12A). Water intake was not different between genotypes, but intake levels for both genotypes were increased as compared to previous observations that did not involve pairing with a salt solution (Figure 12B). This increase in water intake on all days (water volume averaging 7-9 ml/day) and consequential decrease in the sucrose/salt mix consumption on days 2 and 4 was presumably due to the osmotic properties of the sodium chloride contained within the mixed solution. OT KO mice also consumed more total fluid [ANOVA, $F(1,14) = 9.72$, $P = 0.008$, post hoc Bonferroni t-test, $P < 0.05$] on days 1 and 3 as compared to WT mice (Figure 12C).

There were no genotypic differences in body weight at the beginning or end of the experiment. Pre- and post- exposure body weights for OT KO mice were 26.3 ± 0.5 g and 26.9 ± 0.4 g and for WT mice were 25.8 ± 1.2 g and 26.3 ± 0.9 g.

Figure 12. Daily intake of a 0.5M NaCl/10% sucrose mixture and water

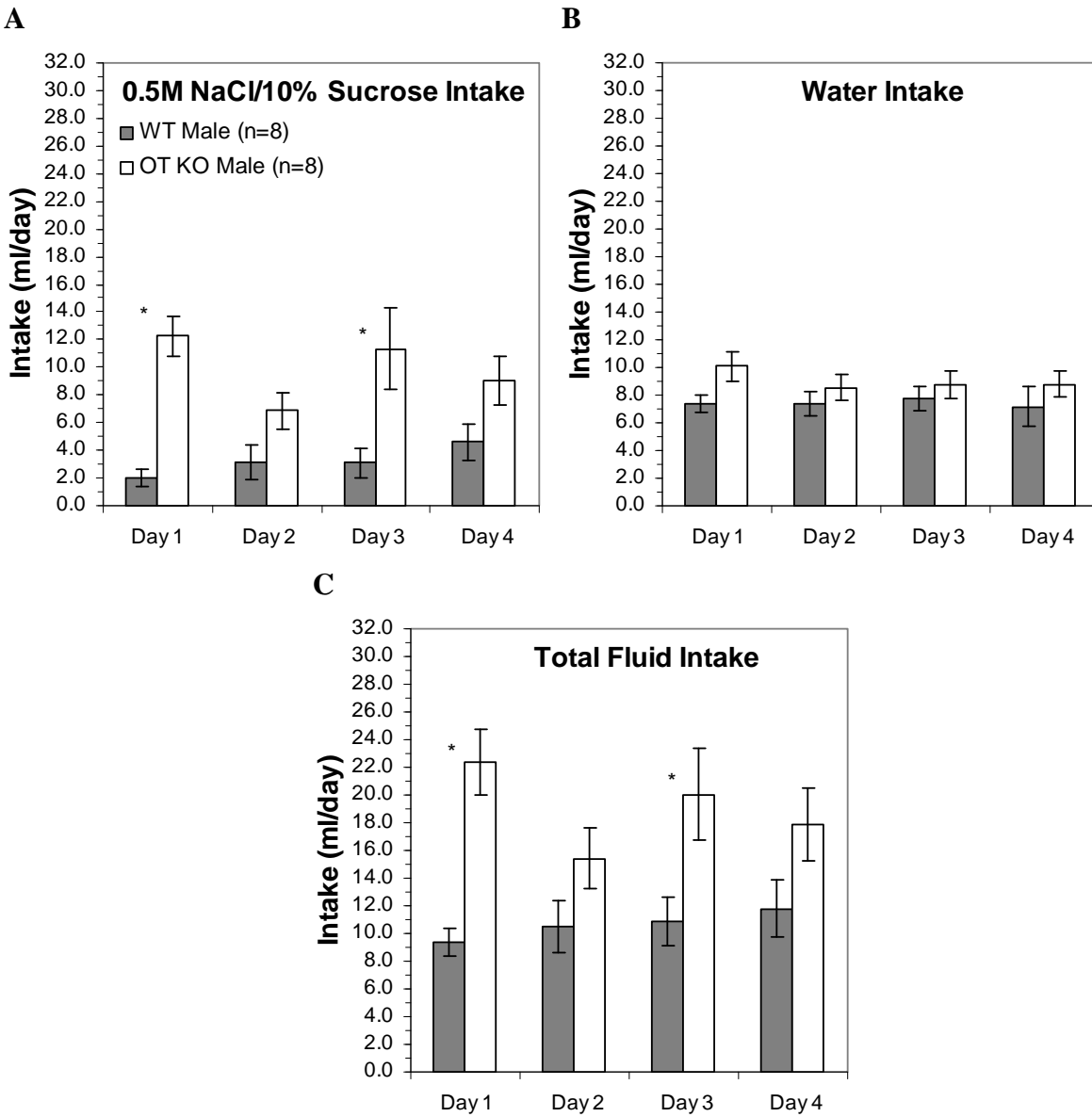


Figure 12. Daily intake of a 0.5M NaCl and 10% sucrose mixture (A), water (B), and total fluid (mixture plus water) (C) in oxytocin knockout (OT KO) and wild type (WT) male mice when presented as two-bottle access test over a four-day exposure period. OT KO mice consumed more of the 0.5M NaCl/10% sucrose mixture (ANOVA, $P = 0.002$) on days 1 and 3 as compared to WT mice. OT KO mice also consumed more total fluid (ANOVA, $P = 0.008$) on days 1 and 3 as compared to WT mice. There were no genotypic differences in water intake. Values represent means \pm SEM. * indicates a significant difference between genotypes ($P < 0.05$)

OT KO and WT mice presented with two-bottle access to a sour 30mM citric acid solution and water did not consume measurable amounts of citric acid (~ 0.1ml) (Figure 13A) and instead chose to consume only water (Figure 13B). There was also no difference in total fluid intake (citric acid plus water) between the two genotypes (Figure 13C).

Figure 13. Daily intake of 30mM citric acid and water

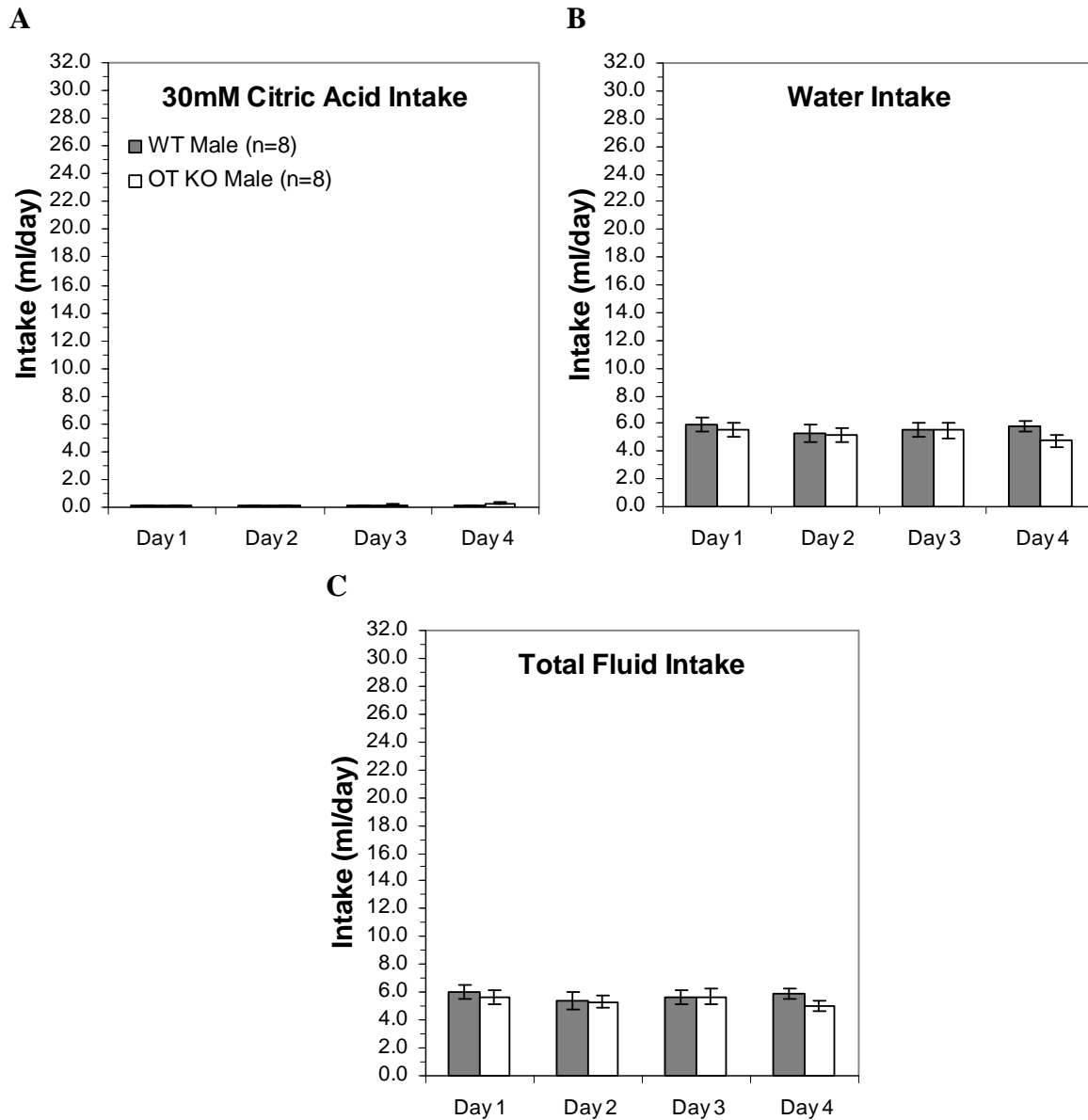


Figure 13. Daily intake of 30mM citric acid (A), water (B), and total fluid (citric acid plus water) (C) in oxytocin knockout (OT KO) and wild type (WT) male mice presented with two-bottle access over a four day exposure period. There were no genotypic differences in citric acid, water, or total fluid intake on any of the four days. Values represent means \pm SEM.

When this same concentration of citric acid (30mM) was mixed with 10% sucrose and presented as a two-bottle access test with water, OT KO animals consumed significantly more of the 30mM citric acid/10% sucrose mix [ANOVA, $F(1,14) = 25.695$, $P < 0.001$, post hoc Bonferroni t-test, $P < 0.05$] on days 1, 2, and 4 as compared to WT cohorts (Figure 14A). OT KO animals also consumed and significantly less water [ANOVA, $F(1,14) = 4.711$, $P = 0.048$, post hoc Bonferroni t-test, $P < 0.05$] on day 1 as compared to WT animals (Figure 14B). However, total fluid intake (mixture plus water) was not different between WT and OT KO animals (Figure 14C).

Figure 14. Daily intake of a 30mM citric acid/10% sucrose mixture and water)

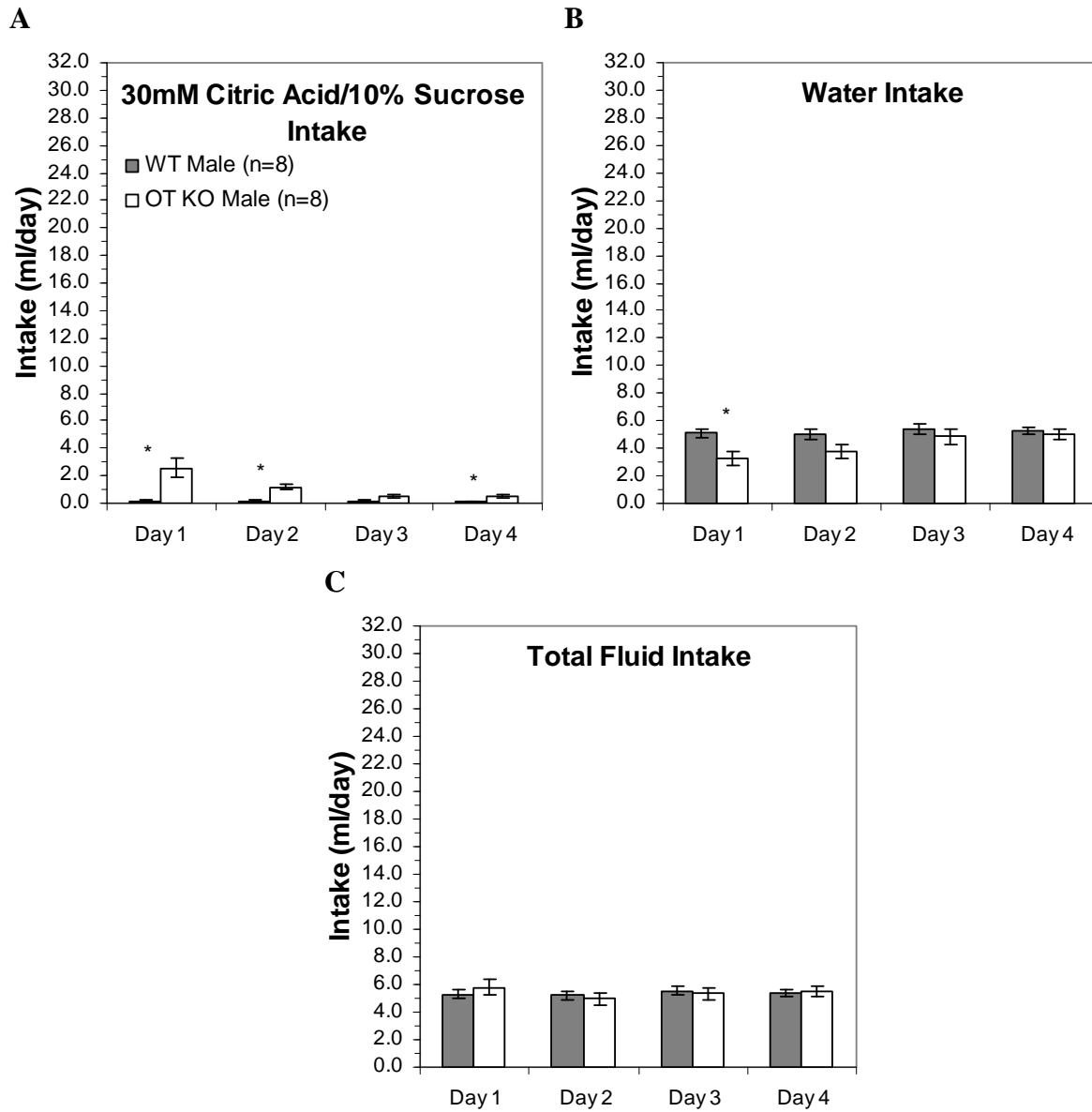


Figure 14. Daily intake of a 30mM citric acid and 10% sucrose mixture (A), water (B), and total fluid (mixture plus water) in oxytocin knockout (OT KO) and wild type (WT) male mice when presented with two-bottle access over a four day exposure period. OT KO mice consumed more of the 30mM citric acid/10% sucrose mixture (ANOVA, $P < 0.001$) on days 1, 2, and 4 as compared to WT mice. WT mice consumed more water (ANOVA, $P = 0.048$) as compared to OT KO mice. Total fluid intake did not differ between genotypes. Values represent means \pm SEM. * indicates a significant difference between genotypes ($P < 0.05$)

4.3 DISCUSSION

Building on previous findings in sucrose ingestion, the present study sought to determine whether the enhanced intake of 10% sucrose solution by OT KO mice was limited to the standard two-bottle access paradigm, or whether OT KO mice would continue to display enhanced intake following further manipulations.

Results shown here demonstrate that OT KO animals consume significantly greater amounts of sucrose solutions in concentrations between 0.625% and 10% as compared to WT animals. These studies support previous findings with sucrose and saccharin (Amico et al, 2005; Billings et al, 2006) and further expand results of sucrose ingestion studies by demonstrating that OT reduces the ingestion of sucrose solution over a two-week time period, over a range of ascending concentrations, following overnight dehydration, and when combined with aversive substances.

Upon initial exposure, OT KO mice show no delay in consuming sucrose solutions even at concentrations as low as 0.625%, following overnight fluid deprivation, and when combined with 0.5M NaCl, in support of previous results found with 10% sucrose (Amico et al, 2005). Although OT KO mice did not show a preference for sucrose over water when 10% sucrose was combined with 30mM citric acid, OT KO mice consumed significantly more of the mixture than WT mice.

As shown in Experiment 4, OT also attenuates the consumption of aversive NaCl (salt) and citric acid (sour) solutions when mixed with sucrose. Both WT and OT KO animals will not freely drink measurable amounts of 0.5M NaCl (Vollmer et al, 2006) or 30mM citric acid (present work) under baseline conditions. However, when these aversive solutions were combined with sucrose, OT KO animals consumed significantly more of the 0.5M NaCl/10%

sucrose mixture and the 30mM citric acid/10% sucrose mixture as compared to WT animals. Both genotypes significantly increased ingestion of 0.5M NaCl beyond the 0.5 ml reported by Vollmer et al when the salt solution was combined with 10% sucrose. Animals responded to the increased osmotic load by also increasing water intake, yet OT KO animals still consumed more of the NaCl/sucrose mixture than water on days 1 and 3. The alternating preference for the NaCl/sucrose mixture or water is likely due to the osmotic load of the mixture. At the same time it is difficult to determine if intake is driven by the need for osmotic balance, as it is impossible to calculate the total osmotic load of each animal without also measuring food intake during fluid consumption.

OT KO animals consumed over 50% of their total calories from sucrose solution (Experiment 1), yet there were no differences in total caloric consumption between the genotypes. Consistent with previous findings, the absence of OT does not interfere with the ability of animals to regulate daily caloric intake. Both genotypes maintained normal levels of total caloric intake even when animals were introduced to palatable solutions. Further, there were no genotypic or treatment differences in metabolic parameters measured after WT and OT KO mice were exposed to eighteen days of sucrose and water or two bottles of water. Bottle placement had no effect on sucrose or water consumption proving that increased ingestion of sucrose solutions is not due to place preference.

Independent of previous findings, these results indicate that despite the addition of aversive solutions such as salt or citric acid to a 10% sucrose solution, OT KO mice consume more sucrose than WT mice. OT KO mice also consume more sucrose solution than WT mice at concentrations as low as 0.625%. However, both genotypes maintained consistent total caloric intake independent of the amount of sucrose consumed by proportionately decreasing chow

intake. These collective results support the view that the absence of OT blunts processes related to the termination of sweet solution intake, but does not affect processes related to total caloric intake when animals are presented with normal laboratory chow.

5.0 CONCLUSION

The present work supports the view that the congenital absence of OT in OT KO mice is associated with enhanced intake of saccharin and carbohydrate-containing liquids, but is not associated with enhanced intake of a palatable fat-enriched emulsion (Intralipid). OT gene deletion has not been shown to disrupt food or water intake under basal conditions or after overnight fluid deprivation, therefore the immediate and sustained excessive intake of sucrose by OT KO mice at a variety of concentrations, following overnight fluid deprivation, and when combined with aversive solutions is significant. Findings thus far suggest that place preference plays no role in the consumption of palatable solutions.

The absence of OT does not interfere with the ability of mice to regulate their total daily caloric intake. Mice maintained an unchanged total daily caloric intake by proportionately reducing chow intake as calories derived from consumption of Intralipid increased. The absence of OT seems to selectively blunt processes related to the termination of non-sweet carbohydrate intake as well as the intake of sweet solutions, but does not affect processes related to total caloric intake when animals are presented with normal laboratory chow.

APPENDIX A

CALORIC CONTENT OF PALATABLE SOLUTIONS

% Intralipid		% Sucrose	
	Caloric content/ml		Caloric content/ml
0.3%	0.03 kcal/ml	0.625%	0.03 kcal/ml
0.5%	0.05 kcal/ml	1.25%	0.05 kcal/ml
1%	0.10 kcal/ml	2.5%	0.10 kcal/ml
2.1%	0.21 kcal/ml	5%	0.21 kcal/ml
2.5%	0.25 kcal/ml		
4.1%	0.41 kcal/ml	10%	0.41 kcal/ml
5%	0.50 kcal/ml		
8.2%	0.82 kcal/ml	20%	0.82 kcal/ml
10%	1.0 kcal/ml		

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