Saliva oxytocin measures do not reflect peripheral plasma concentrations after intranasal oxytocin administration in men

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Abstract

Oxytocin plays an important role in social behavior. Thus, there has been significant research interest for the role of the oxytocin system in several psychiatric disorders, and the potential of intranasal oxytocin administration to treat social dysfunction. Measurement of oxytocin concentrations in saliva are sometimes used to approximate peripheral levels of oxytocin; however, the validity of this approach is unclear. In this study, saliva and plasma oxytocin was assessed after two doses of Exhalation Delivery System delivered intranasal oxytocin (8IU and 24IU), intravenous oxytocin (1IU) and placebo in a double-dummy, within-subjects design with men. We found that intranasal oxytocin (8IU and 24IU) administration increased saliva oxytocin concentrations in comparison to saliva oxytocin concentration levels after intravenous and placebo administration. Additionally, we found that saliva oxytocin concentrations were not significantly associated with plasma oxytocin concentrations after either intranasal or intravenous oxytocin administration. Altogether, we suggest that saliva oxytocin concentrations do not accurately index peripheral oxytocin after intranasal or intravenous oxytocin administration, at least in men. The data indicates that elevated oxytocin saliva levels after nasal delivery primarily reflect exogenous administered oxytocin that is cleared from the nasal cavity to the oropharynx, and is therefore a weak surrogate for peripheral blood measurements.

Keywords: oxytocin, endocrinology, saliva, neuropeptides, plasma

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Several psychiatric illnesses are characterized by dysfunction in social behavior, such as schizophrenia and autism. There has been considerable interest in the potential of the neuropeptide oxytocin to address social dysfunction problems in these disorders (Alvares et al., 2017; Shilling and Feifel, 2016). Preclinical research has shown that oxytocin gene knockout mice have deficits in social behavior, that are reversed with central oxytocin administration (Winslow and Insel, 2002). Following this work, research investigated peripherally circulating oxytocin concentrations, reporting reduced oxytocin in several psychiatric disorders (Hoge et al., 2008; Modahl et al., 1998) and negative associations with symptom severity (Rubin et al., 2010). Such results have contributed to increased efforts to boost oxytocin levels via intranasal oxytocin administration (Quintana et al., 2016). Intranasally administered oxytocin is thought to travel to the brain along ensheathed channels surrounding the olfactory and trigeminal nerve fibres (Lochhead and Thorne, 2012; Quintana et al., 2015a), which heavily innervate the upper and posterior regions of the nasal cavity (Doty and Bromley, 2007; Prasad and Galetta, 2007).

Although the sampling of blood plasma is a popular approach to collect peripheral oxytocin measures, which are often covaried with psychological variables [e.g., anxiety, relationship distress, attachment style (Carson et al., 2014; Strathearn et al., 2009; Taylor et al., 2010)], this is usually not practical as a trained phlebotomist is required to take blood. Blood collection phobias, which may discourage some individuals from participating in research, are also not uncommon with a lifetime prevalence of up to 5% (Bienvenu and Eaton, 1998). Saliva collection is an alternative approach to blood sampling that requires less technical expertise and circumvents needle phobia in research participants. Circulating molecules in blood plasma are thought to transfer to salivary glands via surrounding capillaries (Gröschl, 2009). The relative absence of proteins in saliva compared to blood plasma reduces the risk of assay interference (Leng and Sabatier, 2016). Given these

advantages, saliva measures of oxytocin concentrations are commonly used in biobehavioral research and have also been used as a biomarker of psychiatric illness (e.g., Feldman et al., 2014; Fujisawa et al., 2014).

Despite the ease of saliva collection, there are several limitations with using saliva for peripheral oxytocin concentrations. First, the concentration of hormones in saliva is much less than blood plasma (Kaufman and Lamster, 2002), which limits comparison with the more commonly reported measure of blood plasma oxytocin. The correlation between basal saliva and plasma oxytocin concentrations is also quite modest (r values from .41 to .59; McCullough et al., 2013). Although previous studies have reported saliva oxytocin concentrations after intranasal oxytocin administration (Daughters et al., 2015; Van IJzendoorn et al., 2012; Weisman et al., 2012), little is known about the relationship between saliva and plasma oxytocin after intranasal oxytocin administration. Second, the origin of the reported increases in saliva oxytocin concentrations after intranasal oxytocin administration (e.g., Van IJzendoorn et al., 2012; Weisman et al., 2012) is not clear, especially during the first 30 minutes after intranasal oxytocin administration. The mucociliary clearance (Marttin et al., 1998) of intranasally delivered oxytocin from the nasal cavity to the oropharynx (also described as “trickle-down” or “drip-down” oxytocin) is a widely acknowledged limitation of saliva oxytocin measures after intranasal oxytocin administration (Daughters et al., 2015; Weisman et al., 2012). In the absence of detailed knowledge of the clearance pattern following nasal delivery of oxytocin and without radiolabeled oxytocin, it is currently not possible to separately identify trickle-down oxytocin from endogenous oxytocin or exogenous oxytocin that has been absorbed in the circulatory system and reflected in saliva via transfer from the circulatory system. An alternative approach to help distinguish endogenous oxytocin reflected in saliva from exogenous trickle-down oxytocin would be to include an intravenous (IV) oxytocin comparator, which would eliminate the confounding impact of oxytocin cleared from the nose. However, research is yet to investigate oxytocin concentrations in saliva after IV oxytocin administration.

Since nasal mucosa drug absorption is largely dependent on molecular weight, the absorption of oxytocin (1008 daltons) by the nasal mucosa is relatively low (less than 10%; Landgraf, 1985; McMartin et al., 1987). This suggests that much of the drug is eventually cleared from the nose. The spray deposition pattern of the delivery device, and associated clearance pattern, is likely to influence oxytocin levels in saliva after administration. Traditional spray pumps deliver approximately half of the drug to ciliated respiratory mucosa of nasal regions beyond the nasal valve (Kimbell et al., 2007; Leach et al., 2015). This fraction is rapidly cleared to the nasopharynx within 15-30 minutes by mucociliary clearance and sniffing (Batts et al., 1991; Lansley, 1993; Weisman et al., 2012). However, a large remainder is deposited on the sparsely ciliated transitional mucosa and non-ciliated epithelium in the anterior vestibule, from where it is slowly cleared to the nasopharynx over the course of several hours (Djupesland et al., 2013; Leach et al., 2015). Moreover, the deposition and clearance patterns may vary substantially in response to how the individual uses the device, physiological phenomena like the nasal cycle, and with pathological conditions (Djupesland et al., 2013; Leach et al., 2015; Soane et al., 2001).

A recently introduced Exhalation Delivery System (EDS) device has been shown to limit the anterior deposition to the non-ciliated mucosa, while consistently delivering a larger fraction of the administered dose to the upper posterior region of the nasal cavity (Djupesland et al., 2014; Djupesland and Skretting, 2012; Djupesland et al., 2006). Deposition to this area facilitates nose-to-brain transport as this region is heavily innervated by olfactory and trigeminal nerve fibers. Moreover, recent studies on regional clearance suggest that there is faster clearance from this region than lower regions of the posterior nasal cavity (Djupesland et al., 2014; Djupesland and Skretting, 2012; Djupesland et al., 2006). Indeed, substances administered by the EDS device tend to be absorbed or cleared to a greater extent at 30 minutes after administration (Djupesland et al., 2014; Djupesland and Skretting, 2012; Djupesland et al., 2006) whereas drugs administered with traditional devices would clear over longer time periods, as more of the drug is deposited on non-ciliated nasal

cavity surface regions. However, the clearance of oxytocin delivered by the EDS from the nasal to oral cavity has yet to be evaluated.

In summary, there is uncertainty surrounding whether saliva oxytocin concentrations correspond to plasma oxytocin concentrations after intranasal oxytocin administration and the degree of clearance of intranasally delivered oxytocin from the nasal to oral cavity. Thus, the aim of this study was to examine the effects of EDS delivered intranasal oxytocin and IV oxytocin administration on salivary oxytocin concentrations in men, over the course of two hours. Saliva oxytocin concentrations will also be compared with previously reported plasma concentrations from the same experiment (Quintana et al., 2015b).

Methods

Participants

Participants were recruited through advertisements at the University of Oslo, and were eligible to participate if male, aged 18 to 35 (inclusive), and in good physical and mental health. Exclusion criteria included use of any medications within the last 14 days, history of physical or psychiatric disease, and $IQ < 75$. A screening visit occurred between 3-21 days prior to the first treatment session. The Wechsler Abbreviated Scale of Intelligence (Wechsler, 1999) and the Mini-International Neuropsychiatric Interview (Lecrubier et al., 1997) were administered to index IQ and confirm the absence of psychiatric illness, respectively. A physical examination was performed by study physicians and nurses, which included 12-lead ECG and the collection of routine blood samples, to confirm the absence of physical illness. Fifty-seven male volunteers were assessed for study eligibility, with 18 participants aged 20-30 years ($M = 23.81$, $SD = 3.33$) included (Fig. 1; Quintana et al., 2015b). Two participants withdrew after study enrollment, thus saliva oxytocin concentration data from sixteen participants were included in the analysis. This trial was approved by the Regional Committee for Medical and Health Research Ethics (REC South East) and participants provided written informed consent before they participated. The

study is registered at <http://clinicaltrials.gov> (NCT01983514).

Study design

One of four treatments were administered in double-blind fashion using one of four randomized sequences [treatments were 8 international units (IU) intranasal oxytocin, 24IU intranasal oxytocin, 1IU IV oxytocin, or placebo]. A double-dummy design was adopted, whereby a nasal spray solution and IV solution was administered at every treatment session, with solution contents depending on treatment condition. The oxytocin and placebo nasal spray solutions were supplied by Sigma-Tau Industrie Farmaceutiche Riunite (Rome, Italy) to a local pharmaceutical service provider (Farma Holding, Oslo, Norway) for the filling of the nasal spray devices. The IV oxytocin (10 IU/ml; Grindeks, Riga, Latvia) and placebo formulations (0.9% sodium chloride) were added to a 0.9% sodium chloride solution. This solution was infused at a rate of 600 mL/h over a 20-minute period. The nasal spray solution was self-administered shortly after the completion of the IV infusion. Notably, the same volume of nasal spray was used for each condition so the potential trickle-down volume would be equivalent between conditions. Bottles of oxytocin contained a total of 40IU of OT per ml, with each spray providing a 4IU dose. Each ml of solution contained 0.2mg of propyl parahydroxybenzoate and 0.4 mg of methyl parahydroxybenzoate. Other excipients included chlorobutanol, disodium phosphate anhydrous, citric acid anhydrous, sodium chloride, glycerol, sorbitol solution 70%, and purified water. The placebo intranasal formulation contained all excipients except the active ingredient.

To ensure the same volume was administered into the nasal cavity for each treatment arm, three puffs were always administered per nostril (alternating between each nostril). For each treatment arm, the first two puffs were self-administered from bottle “A2”, and the next four puffs were self-administered from bottle “B4”, respectively. Depending on the randomization sequence, participants received either 8IU intranasal oxytocin [two, 4IU OT puffs in each nostril from bottle A2 and four placebo puffs (two in each nostril) from bottle

B4] with placebo IV, 24IU intranasal oxytocin [two, 4IU OT puffs in each nostril from bottle A2 and four, 4IU OT puffs (two in each nostril) from bottle B4] with placebo IV, placebo nasal treatment [two placebo puffs in each nostril from bottle A2 and four placebo puffs (two in each nostril) from bottle B4] with 1IU OT IV, or intranasal placebo [two, placebo OT puffs in each nostril from bottle A2 and four placebo puffs (two in each nostril) from bottle B4] with placebo IV. A pilot study, described by Quintana and colleagues, (2015b), guided the selection of the 1IU IV OT dosage and infusion rate. To appropriately compare the effects of administration route (intranasal vs. intravenous) on oxytocin concentrations, we used an IV dose and infusion rate intended to elicit equivalent peripheral blood concentrations to intranasally administered oxytocin. Data from the study confirmed that there was no significant difference in peripheral blood concentrations between 1IU OT, 8IU intranasal OT, and 24IU intranasal OT conditions (Quintana et al., 2015).

Saliva and blood samples were collected at the following temporal interval relative to the completion of IV and nasal spray administration: baseline (approximately 25 minutes before the completion of IV and nasal spray administration), 0 minutes (immediately after the completion of IV and nasal spray administration), 10 minutes, 30 minutes, 60 minutes, and 120 minutes. Saliva samples were collected using the Salivette Citric Acid Cotton Swab (Sarstedt AG & Co, Nümbrecht, Germany). For each sample, participants placed a cotton roll in their mouths, and were instructed to think of their favourite food until the cotton was saturated (approximately 1-2 mins). Blood samples were collected via IV catheter and centrifuged at 4°C within 5 minutes of blood draw. Both blood and saliva samples were frozen at -80°C until enzyme-linked immunosorbent assay (ELISA) using commercially available kits (Enzo Life Sciences, Farmingdale, NY). According to the manufacturer (Enzo Life Sciences, 2017), the ELISA assays have an intra-assay reliability of 12.6-13.3%, an inter-assay reliability of 11.8-20.9%, and less than 0.02% cross-reactivity with mammalian peptides (e.g., vasopressin). Analysis was performed by the Oslo University Hospital hormone laboratory following manufacturer specifications (Enzo Life Sciences, 2017). As per

these specifications, the samples were extracted, evaporated to dryness, and then rehydrated before measurement. As extraction concentrated the samples to increase precision and reduce matrix interference, the corrected lower limit of sensitivity for the samples was 2.61 pg/mL. Any values below the detectable limit were conservatively replaced with a value of 2.61 pg/mL. The number of saliva samples below the detectable limit at each temporal interval are presented in Table S1. Notably, 19 out of the 37 collected basal samples were below the assay's detectable limit. The blood plasma oxytocin concentration data have been previously published (Quintana et al., 2015b), but will be presented again here for easy comparison with the saliva oxytocin concentration data.

Exhalation Delivery System intranasal drug delivery

The exhalation delivery system (EDS) (also known as a Breath Powered device; OptiNose AS, Oslo, Norway) has been designed to improve deposition to sites in the upper and posterior regions of the nasal cavity beyond the narrow nasal valve by taking advantage of nasal physiology (Djupestrand et al., 2013; Djupestrand et al., 2014). The EDS has a mouthpiece and a sealing nosepiece. When administering the drug, the user exhales into the mouthpiece of the device through their mouth against resistance, which closes the soft palate and isolates the nasal cavity from the oral cavity. In addition to reducing loss of drug to drip-out or swallowing, the EDS mechanism of delivery is designed to improve high and deep drug deposition. The standard asymmetrical and sealing EDS nosepiece helps expand the superior nasal valve, balance nasal/oral pressures, and facilitate an airflow pattern that carries drug to the upper posterior nasal cavity region (Djupestrand and Skretting, 2012). The "N2B" nosepiece has been further optimized to enhance delivery to the uppermost parts of the nose relative to traditional delivery devices (Djupestrand et al., 2014; Djupestrand and Skretting, 2012; Djupestrand et al., 2006). Studies with radiolabeled drug have demonstrated a significantly different deposition pattern with EDS compared to conventional nasal sprays and have shown improved pharmacokinetics and/or enhanced clinical activity with studied

drugs (Djupesland et al., 2013; Djupesland et al., 2014; Djupesland et al., 2006).

Statistical analysis

Statistical analysis was conducted using the R statistical environment (R Development Core Team, 2014). To examine the effects of condition and time on saliva concentrations, a multilevel approach was used to compare nested linear mixed models. Using this approach, main effect and interaction models are compared against a baseline model (i.e., a model where saliva concentration is predicted by its overall mean). A linear mixed model is an attractive alternative to a repeated measures ANOVA, which requires listwise deletion for any missing data. Robust mixed ANOVAs were also performed, which can account for any violations of non-normally distributed data (Wilcox, 2011). Bayes factor linear models were also fit to compare models of interest against a baseline model using a Jeffrey-Zellner-Siow prior. Bayes factors quantify relative evidence for the null and alternative models, which is described as an odds ratio. Bayes factors values greater than 10, 30, and 100 provide strong, very strong and extreme evidence for the alternative hypotheses, respectively (Lee and Wagenmakers, 2014). Likelihood ratios and corresponding p -values were computed to assess if main effect and interaction models were better fits of the data than the baseline model. To control the Type I error rate, p -value thresholds for the test of simple effects at each temporal interval were adjusted using a false discovery rate (FDR). Additionally, follow up tests of simple effects were performed using Tukey-corrected comparisons. Given the low sample sizes and presence of outliers, Kendall's rank correlation tau was calculated to examine the rank correlations between saliva and blood concentrations at each temporal interval for each condition. P -values ≥ 0.05 and < 0.1 were considered on the border of statistical significance for all analyses.

Missing values

To assess differences in total samples provided per condition and each temporal interval (Table 1), a multilevel approach was used to compare nested linear mixed models.

There was no main effect of treatment on saliva samples available for analysis [$\chi^2(3) = 1.9$, $p = 0.59$], but there was a main effect of time [$\chi^2(5) = 24.12$, $p = 0.002$]. Follow up pairwise comparison tests with Tukey adjusted p values indicated that there were significantly more saliva samples available for analysis from baseline compared to 10 minutes ($p = 0.03$), 30 minutes ($p < 0.001$), and 60 minutes ($p = 0.03$). There was no statistically significant treatment $\times$ time interaction [$\chi^2(15) = 13.19$, $p = 0.59$]. Regarding missing plasma samples (Table 1), there was no main effect of treatment [$\chi^2(5) = 2.13$, $p = 0.55$], time [$\chi^2(5) = 5.38$, $p = 0.37$], or a significant treatment and time interaction [$\chi^2(15) = 14.36$, $p = 0.5$].

Results

Mean baseline measures of plasma and saliva oxytocin (Table S2; Fig. 2, Fig. 3) were within the expected range (Leng and Ludwig, 2016). There was a main effect of treatment and time on saliva oxytocin concentrations, as shown by significantly better fits for treatment [$\chi^2(3) = 14.95$, $p = 0.002$; BF > 100000] and time models [$\chi^2(5) = 27.24$, $p = 0.001$; BF = 54.5] than the null model (Fig. 2). Additionally, the treatment $\times$ time interaction was a statistically significant better fit than the main effects model [$\chi^2(15) = 47.16$, $p < 0.001$; BF = 13.4]. In other words, the observed data is 13.4 times more likely under the alternative hypothesis (i.e., a treatment $\times$ time interaction) than the null model. Tukey adjusted pairwise comparisons revealed that the saliva oxytocin concentrations were significantly higher after 24IU administration compared to placebo ($p = 0.002$) and IV ($p = 0.003$) oxytocin. Saliva oxytocin concentrations after 8IU oxytocin were higher than placebo ($p = 0.043$). There was also an increase after 8IU oxytocin compared to IV, but this difference was on the border of statistical significance ($p = 0.071$). A robust mixed ANOVA (Wilcox, 2011) revealed similar results, with a statistically significant main effect of treatment ($p = 0.001$) and time ($p = 0.004$), and a statistically significant treatment $\times$ time interaction ($p < 0.001$). Pairwise robust t-tests revealed significantly higher saliva oxytocin concentrations after 24IU administration compared to placebo ($p = 0.01$), but not IV

administration ($p = 0.16$). Saliva oxytocin concentrations were higher after 8IU administration compared to placebo ($p = 0.036$), but not IV administration ($p = 0.12$).

Follow up analysis for treatment effects at each temporal interval are presented in Table 2. Of note, there was no significant difference between the treatment and null models at baseline. However, there were significant differences between treatment and null models at 0 minutes ($p < .0001$; FDR adjusted p -value threshold, $p = 0.008$), 10 minutes ($p = .01$; FDR adjusted p -value threshold, $p = 0.016$), and 120 minutes ($p = .021$; FDR adjusted p -value threshold, $p = 0.025$). Pairwise comparisons (Tukey corrected) revealed that saliva oxytocin concentrations after 24IU treatment were significantly higher than both IV oxytocin and placebo at 0 minutes and 120 minutes. Saliva oxytocin concentrations after 8IU was significantly higher compared to placebo and IV after 0 minutes and 10 minutes. The difference between saliva concentrations after 24IU and placebo after 10 minutes was on the border of Tukey-corrected statistical significance ($p = 0.09$). There were no significant differences in saliva oxytocin concentrations between 8IU and 24IU at any temporal interval.

For the relationship between saliva and plasma (Fig. 2 and Fig. 3), there was only one statistically significant rank correlation test out of a potential 24 tests (Table 3), which indicated an association between saliva and plasma oxytocin at 10 minutes after 24IU oxytocin administration ($\tau = 0.75$, $p = 0.04$). However, this result did not survive correction for multiple tests. Only one Bayesian Kendall correlation yielded a Bayes factor greater than 3 (24IU oxytocin baseline condition, $BF = 4.28$). However, none of the Bayes Factors that were below 1, which is indicative of support for the null hypothesis that variables are related, were less than 0.33, suggesting that these analyses were underpowered. Notably, there was no statistically significant correlation at any temporal interval after IV administration, however, this relationship was on the border of statistical significance ($\tau = 0.64$, $p = 0.08$) 10 minutes after administration.

Discussion

Our results indicate that saliva oxytocin concentrations after 8IU and 24IU intranasal oxytocin are markedly inflated compared to saliva oxytocin concentrations after IV oxytocin and placebo administration. This increase begins almost immediately after intranasal oxytocin administration and persists for at least 2 hours. If saliva oxytocin were representative of peripherally circulating oxytocin, then one would expect saliva levels to be equivalent after intranasal and IV oxytocin administration. However, as saliva oxytocin concentrations after intranasal oxytocin were larger compared to IV administration, we suggest that cleared exogenous oxytocin from the nasal cavity into the oropharynx largely contributes to these increases. Altogether, these results in men suggest that saliva concentrations should not be used as a proxy measure of peripherally circulating oxytocin after intranasal exogenous oxytocin administration, as the exogenous oxytocin likely interferes with these measures.

Comparing the present data obtained with the novel EDS device to previous studies measuring saliva oxytocin after nasal delivery with traditional spray pumps offers some intriguing observations. There was a striking increase in saliva oxytocin 10 minutes after intranasal oxytocin administration (8IU, 258.8 pg/ml, 24IU, 205 pg/ml). However, these observed levels were only approximately a quarter of what has previously been reported at a similar temporal interval after traditional pump-actuated 24IU intranasal oxytocin administration (15 minutes after administration, 1265.5 pg/ml; Weisman et al., 2012). This rapid clearance pattern is consistent with greater initial absorption at targeted ciliated intranasal target sites (Marttin et al., 1998), which is in line with the hypothesis that oxytocin is transported to the brain following oxytocin delivery with the EDS device. However, this is currently speculative without measures of oxytocin in the brain. It is not clear why the mean saliva concentration after 8IU administration was higher than 24IU administration after 10 minutes. However, a similar pattern of increased salivary oxytocin after a lower dose of intranasal oxytocin compared to a higher dose has been reported

previously by van IJzendoorn and co-workers (2012). This study reported elevated levels of saliva oxytocin one hour after 16IU (446.6 pg/mL) and 24IU (157.4 pg/mL) pump actuated intranasal oxytocin (Van IJzendoorn et al., 2012). Of note, these levels of saliva oxytocin reported by van IJzendoorn and co-workers (2012) are also higher than what we observed in the present study at the same temporal interval after administration (1 hour). The nasal clearance pattern with time after spray pump delivery of a topical steroid over the course of 4-6 hours (Shah et al., 2015) also appear to correlate well with the pattern of saliva oxytocin levels during the 2 hours observation time in the present study, and for longer time periods in previous studies (Leach et al., 2015). It is not clear why levels of oxytocin in saliva were similar after 8IU and 24IU intranasal oxytocin during most time points, however, this is consistent with the equivalent measures in plasma after 8IU, 24IU and IV oxytocin administration previously reported (Quintana et al., 2015b).

The sharp increase of oxytocin concentration in saliva after intranasal oxytocin administration is obvious, and even if a significant fraction originates from the cleared exogenous oxytocin other sources might contribute. It has been suggested that increases in salivary oxytocin hours after administration might be due to feedforward mechanisms (Van IJzendoorn et al., 2012), whereby oxytocin stimulates its own dendritic release (Rossoni et al., 2008). However, this “feedforward” hypothesis is now less certain, with recent work in macaques demonstrating that the intranasal administration of d5-denatured oxytocin (which can be distinguished from endogenous oxytocin via mass spectrometry) does not increase central levels of endogenous oxytocin (Lee et al., 2017). Therefore, it is more likely that increased levels of oxytocin in saliva and blood simply reflects exogenous oxytocin.

Small lipid-soluble hormones like cortisol enter saliva from peripheral circulation via ultrafiltration and passive diffusion (Kaufman and Lamster, 2002). Contribution to oxytocin in saliva from diffusion from the blood is also possible, but as oxytocin is a relatively large non-lipid soluble peptide, its contribution to saliva oxytocin after nasal oxytocin delivery is probably limited, which is consistent with our data. Moreover, using the same dataset we

previously reported a sharp increase in plasma oxytocin shortly after IV OT administration (0 minutes temporal interval; Quintana et al., 2015b; Fig. 3; Table 2). As there was no corresponding increase in saliva OT levels at this same temporal interval (Fig. 2, Table S2), this does not support oxytocin diffusion from circulating blood. While a delay in the appearance of oxytocin in saliva via diffusion from blood may occur, our data demonstrated almost equivalent oxytocin measures at each temporal interval after IV administration (Fig. 2, Table S2).

Differences in the detection of oxytocin in plasma and saliva may also be due to different breakdown rates of oxytocin. Oxytocinase (also known as leucyl/cystinyl aminopeptidase) metabolizes oxytocin and is expressed throughout the brain and body (Fagerberg et al., 2014; Matsumoto et al., 2001; Tsujimoto and Hattori, 2005). Oxytocinase levels are thought to contribute to the prevention of premature delivery (Kozaki et al., 2001) via increased concentrations during the later stages of pregnancy (Yamahara et al., 2000), but also appear to play a role in regulating central oxytocin availability in rodents (Tobin et al., 2014). It is not known whether oxytocinase metabolizes oxytocin differently in saliva compared to blood, which may have influenced our observations and led to non-significant correlations between saliva and blood oxytocin. Relatedly, the time taken for oxytocin molecules to be transported between circulating blood and saliva may have also contributed to non-significant correlations at each time point. Although we extracted our samples, it is also possible that the high levels of proteins present in blood plasma (compared to saliva oxytocin), which can interfere with ELISA measures as oxytocin can bind to these proteins, may have also contributed to the low correlations between blood and saliva oxytocin.

We assessed whether peripherally circulating oxytocin enters saliva by assessing the relationship between saliva and blood plasma oxytocin after IV oxytocin administration using a dosage that provided equivalent peripheral plasma concentrations to intranasal oxytocin administration (Quintana et al., 2015b). Lack of significant relationships between saliva and plasma oxytocin levels after nasal and IV oxytocin delivery suggests that saliva

concentrations of oxytocin do not reflect peripherally circulating oxytocin after exogenous administration. However, this particular analysis should be considered preliminary, considering the low number of samples available for comparison. While saliva oxytocin measures may have some merit as a marker of endogenous oxytocin system activity, future investigations of peripheral oxytocin concentrations after oxytocin administration should sample plasma rather than saliva. Moreover, past research using saliva concentrations as a correlate of behavior or disease biomarker should be replicated using plasma oxytocin concentrations.

This study had some limitations worth noting. First, this was part of a larger study (Quintana et al., 2015b), which included a social cognition task and brain imaging for all treatment conditions. Due to the experimental design constraining saliva collection to specific temporal interval, we may have missed the peak saliva concentration. The completion of tasks may have also influenced circulating oxytocin, however, the same tasks occurred after all treatment administrations and it is unlikely that there was any systematic confounding effect. Second, many of the saliva and plasma oxytocin measurements were below the assay's detectable limit, which may have restricted the lower bound precision of the measures. Of note, 19 out of the 37 collected basal samples were below the assay's detectable limit, which limits the ability to replicate prior work reporting low-to-modest correlations between basal saliva and plasma oxytocin concentrations (McCullough et al., 2013). Regardless, this does not change the conclusions of the study, as we were primarily interested in changes in oxytocin saliva concentrations after intranasal oxytocin administration, which was well above the lower bound limit. As the main effects of treatment and time were driven by oxytocin concentration increases after intranasal oxytocin administration, data below the assay's detectable limit, which mostly occurred at baseline, were unlikely to have changed the conclusions of the analysis as these values were conservatively replaced with the lowest detectable limit. Third, there were relatively high levels of missing data. This was due to difficulties in saliva collection due to time constraints, as this was part of a larger study in

which timing of the social cognition tasks and brain image acquisition after intranasal oxytocin administration was crucial. However, there was no difference in missing data between conditions. There was a greater availability of baseline saliva samples, which can be attributed to fewer time constraints of this temporal interval compared to the other periods. Periods after treatment administration had more time constraints due to other experimental tasks, which may have led to the insufficient saliva volume collection preventing analysis. Fourth, it is unclear whether 1IU was the most appropriate dose for the IV comparator. While our intention was use an IV dose that matched blood plasma levels after 24IU intranasal oxytocin, which we have shown previously (Quintana et al., 2015), a dose-ranging study may have provided a dose that better matched peripheral levels after intranasal administration. Fifth, as the collection of fluids takes place close to (intranasal) or at the site of (intravenous) exogenous administration, this will bias towards high concentrations at the collection site, thus measures taken directly after administration may provide inaccurate correlation measures between plasma and saliva oxytocin, especially during the period directly after administration. Finally, given the study population these conclusions can only be extrapolated to men. Although these patterns of nasal clearance would not be expected to be different in females, future research in females is needed for confirmation.

By comparing saliva and plasma oxytocin concentrations after both intranasal and intravenous oxytocin administration, the current findings provide novel insights in oxytocin pharmacokinetics. Namely, the data suggest that saliva oxytocin measures may not reflect blood plasma concentrations, at least in men. Further, the enhanced oxytocin saliva levels after administration of oxytocin with EDS device compared to IV suggests that exogenous oxytocin has been cleared from the nasal cavity to the oropharynx, possibly due to mucociliary clearance.

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Figure captions

Figure 1. **Study CONSORT diagram.** A = 8IU intranasal oxytocin, B = 24IU intranasal oxytocin, C = 1 IU IV oxytocin, D = Placebo. Five participants also participated in an open-label pilot study to pilot the intravenous oxytocin dose. Four of these participants were enrolled into the randomized study.

Figure 2. **Mean saliva oxytocin concentrations at each temporal interval.** Error bars represent standard errors. * = $p < 0.05$ compared to both placebo and 1IU IV oxytocin. ‡ = $p < 0.1$ compared to placebo.

Figure 3. **Mean plasma oxytocin concentrations at each temporal interval.** Error bars represent standard errors. Note that this data and its statistical inference have been presented previously in Quintana et al., (2015b), but the data presented here for comparison with saliva oxytocin concentrations.

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Table 1. Differences in saliva and plasma sample numbers between treatment conditions at each temporal interval.

	8IU IN OT	24IU IN OT	1IU IV OT	PL
Baseline				
Saliva samples	8	8	11	10
Plasma samples	16	15	16	16
0 min				
Saliva samples	5	8	5	8
Plasma samples	15	14	16	15
10 min				
Saliva samples	5	6	7	7
Plasma samples	15	15	15	16
30 min				
Saliva samples	3	5	2	5
Plasma samples	16	15	16	15
60 min				
Saliva samples	6	8	5	5
Plasma samples	16	16	14	16
120 mins				
Saliva samples	5	6	9	8
Plasma samples	16	16	16	16

Note: Maximum samples per treatment session = 16. 8IU IN OT = 8 international units of intranasal oxytocin; 24IU IN OT = 24 international units of intranasal oxytocin; 1IU IV OT = 1 international unit of intravenous oxytocin; PL=placebo.

Table 2. Simple effects of treatment on oxytocin saliva concentrations at each temporal interval

	LMM comparisons		8IU IN OT vs. placebo		24IU IN OT vs. placebo		1IU IV OT vs. placebo		24IU IN OT vs. 1 IU IV OT		8IU IN OT vs. 1IU IV OT		8IU IN OT vs. 24IU IN OT	
	Likelihood ratio	<i>p</i>	Estimate (se)	<i>p</i>	Estimate (se)	<i>p</i>	Estimate (se)	<i>p</i>	Estimate (se)	<i>p</i>	Estimate (se)	<i>p</i>	Estimate (se)	<i>p</i>
Time point														
Baseline	$\chi^2(7) = 2.25$	0.523	-1.8 (1.5)	0.638	0.3 (1.4)	0.997	-0.9 (1.4)	0.921	1.2 (1.4)	0.836	-0.9 (1.4)	0.925	2 (1.5)	0.518
0 min	$\chi^2(7) = 18.34$	< 0.001	-291.3 (99.7)	0.018	-387.2 (83.7)	< 0.001	6.1 (91.7)	0.999	-393.2 (91.8)	<0.001	-291.3 (99.7)	0.025	-95.9 (98.5)	0.763
10 min	$\chi^2(7) = 11.29$	0.01	-200.2 (59.9)	0.005	-135.9 (57.8)	0.086	-18.8 (52)	0.984	-117.1 (56.5)	0.162	-181.4 (56.5)	0.007	64.3 (56)	0.659
30 min	$\chi^2(7) = 3.44$	0.329	-180.7 (115.6)	0.393	-159.3 (91.4)	0.296	-15.4 (132.1)	0.999	-143.9 (129.2)	0.676	-165.3 (142.7)	0.648	21.4 (109.8)	0.997
60 min	$\chi^2(7) = 2.56$	0.434	-50.7 (85.8)	0.935	-118.5 (80.5)	0.453	-16.9 (91.6)	0.998	-101.6 (81.4)	0.595	-33.8 (88)	0.981	-67.8 (76.6)	0.812
120 min	$\chi^2(7) = 9.78$	0.021	-72.4 (60.7)	0.63	-174.2 (57.6)	0.013	-0.55 (51.7)	0.999	-173.7 (56.2)	0.011	-71.9 (59.5)	0.619	-101.8 (64.7)	0.392

Note: Tukey adjusted p-values reported for each temporal interval. 8IU IN OT = 8 international units of intranasal oxytocin; 24IU IN OT = 24 international units of intranasal oxytocin, 1IU IV = 1 international unit of intravenous oxytocin.

Table 3. Kendall's rank correlation tests for the relationship between saliva and plasma oxytocin concentrations

		Baseline	0 min	10 min	30 min	60 min	120 min
8IU	Kendall's Tau	-0.48	-0.11	-0.67	-	0.58	-0.6
	p-value	0.16	0.8	0.17	-	0.14	0.17
	Bayes factor	1.44	0.54	1.11	-	1.42	1.2
	n	8	5	5	3	6	5
24IU	Kendall's Tau	0.67	-0.04	0.75	0.71	0.36	-0.07
	p-value	0.06	0.9	0.04	0.18	0.25	0.85
	Bayes factor	4.28	0.44	2.82	1.2	0.83	0.5
	n	8	8	6	4	8	6
IV	Kendall's Tau	0.29	-0.07	0.64	-	0.33	0.28
	p-value	0.28	0.85	0.08	-	0.56	0.4
	Bayes factor	0.75	0.5	2.41	-	0.68	0.66
	n	11	6	7	2	4	9
Placebo	Kendall's Tau	-0.26	0.3	0.36	1	0.33	-0.43
	p-value	0.43	0.36	0.32	0.06	0.47	0.24
	Bayes factor	0.63	0.69	0.78	2.44	0.68	1.12
	n	10	8	7	4	5	8

Supplementary material

Saliva oxytocin measures do not reflect peripheral plasma concentrations after intranasal oxytocin administration in men

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Table S1. Number of saliva samples below the detectable limit at each temporal interval

	Baseline				0 mins				10 mins				30 mins				60 mins				120 mins			
	8IU	24IU	IV	PL	8IU	24IU	IV	PL	8IU	24IU	IV	PL	8IU	24IU	IV	PL	8IU	24IU	IV	PL	8IU	24IU	IV	PL
Number of samples	4	5	4	6	0	0	2	3	0	0	3	1	0	1	2	3	0	1	1	2	0	0	6	5
Total samples	8	8	11	10	5	8	5	8	5	6	7	7	3	5	2	5	6	8	5	5	5	6	9	8

Note: Maximum samples per treatment session = 16. Maximum total samples per temporal interval across all treatments = 64. 8IU = 8 international units of intranasal oxytocin; 24IU = 24 international units of intranasal oxytocin, IV = 1 international unit of intravenous oxytocin; PL = placebo.

Table S2. Plasma and saliva oxytocin concentrations at each temporal interval after all treatments

		Baseline	0 mins	10 mins	30 mins	60 mins	120 mins
8IU IN OT	Plasma	4.6 (1.1)	6.9 (1.4)	8 (2.7)	10.1 (3.6)	9.6 (2.7)	8.8 (2.2)
	Saliva	4.8 (2)	277.4 (126)	258.8 (126)	171.1 (130.8)	55.5 (21.4)	73.1 (28.6)
24IU IN OT	Plasma	7.7 (2.4)	14.2 (3.6)	12 (2.8)	8.5 (2)	7.7 (2)	6.3 (1.2)
	Saliva	2.78 (0.1)	389.3 (109.4)	205 (65.7)	150.8 (115.1)	116.8 (97.3)	175.5 (101.2)
1 IU IV	Plasma	6 (1.6)	25.6 (4)	10.8 (2.7)	7 (1.8)	9.5 (2.4)	6.1 (1.1)
	Saliva	3.9 (1)	4.2 (0.7)	3.6 (0.7)	2.6 (0)	2.9 (0.1)	2.8 (0.1)
Placebo	Plasma	3.9 (0.4)	6 (1.3)	4.2 (0.6)	5.1 (1.3)	4.9 (1)	5.3 (1.5)
	Saliva	3.1 (0.3)	3.1 (0.2)	4.8 (1.5)	2.8 (0.1)	7.1 (2.6)	2.8 (0.1)

Note: Means are presented with standard errors in parentheses. 8IU IN OT = 8 international units of intranasal oxytocin; 24IU IN OT = 24 international units of intranasal oxytocin, 1IU IV = 1 international unit of intravenous oxytocin. Plasma oxytocin concentration data has been presented previously by Quintana and colleagues (2015b).