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Oxytocin in young children with Prader-Willi syndrome: Results of a randomized, double-blind, placebo-controlled, crossover trial investigating 3 months of oxytocin

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Abstract

Context: Prader-Willi syndrome (PWS) is characterized by hypothalamic dysfunction, hyperphagia and a typical behavioural phenotype, with characteristics of autism spectrum disorder (ASD) like stubbornness, temper tantrums and compulsivity. It has been suggested that the oxytocin system in patients with PWS is dysfunctional. In ASD, intranasal oxytocin treatment has favourable effects on behaviour.

Objective: To evaluate the effects of 3 months of twice daily intranasal oxytocin (dose range 16-40 IU/day), compared to placebo, on behaviour and hyperphagia in children with PWS.

Design: Randomized, double-blind, placebo-controlled, crossover study in the Dutch PWS Reference Center.

Patients: Twenty-six children with PWS aged 3-11 years.

Main outcome measures: (Change in) behaviour and hyperphagia measured by Oxytocin Questionnaire and Dykens hyperphagia questionnaire.

Results: In the total group, no significant effects of oxytocin on social behaviour or hyperphagia were found. However, in boys, the Oxytocin Questionnaire scores improved significantly during oxytocin treatment, compared to a deterioration during placebo (4.5 (−0.8 to 15.3) vs. −4.0 (−11.3 to 0.8), P = .025). The Dykens hyperphagia questionnaire scores remained similar during oxytocin treatment, while there was a deterioration during placebo (0.0 (−0.8 to 4.3) vs. −3.5 (−6.0 to 0.0), P = .046). Patients with a deletion had significant improvements in both questionnaire scores during oxytocin treatment, but deteriorations during placebo. Oxytocin treatment was well tolerated, and there were no serious adverse events.

Conclusions: Intranasal oxytocin treatment has positive effects on social and eating behaviour in 3-11 years aged boys with PWS and in children with a deletion without safety concerns. Intranasal oxytocin in children with PWS might be considered, but

individual effects should be carefully evaluated and treatment discontinued if no effects are found.

KEYWORDS
behaviour, children, hyperphagia, intranasal oxytocin, Prader-Willi syndrome

1 | INTRODUCTION

Prader-Willi syndrome (PWS) is a rare, neurodevelopmental disorder caused by the lack of expression of the Prader-Willi region of the paternally derived chromosome 15, mostly caused by a paternal deletion or maternal uniparental disomy (mUPD). PWS is characterized by a distinctive phenotype with neonatal hypotonia with suckling problems often requiring tube feeding, endocrine disturbances, developmental delay, hyperphagia and reduced energy expenditure resulting in obesity when uncontrolled and a typical behavioural phenotype.[1-3] Behaviour of children with PWS is characterized by stubbornness, temper tantrums, compulsivity and difficulties in changing routines.[2,4,5] Children thereby show symptoms of autism spectrum disorder (ASD), and 36% fulfil the criteria of ASD.[6] Growth hormone (GH) therapy results in significant improvements in body composition and mental and motor development in children with PWS.[7,8] However, effects of GH on behaviour and hyperphagia are limited.[9]

One of the genes in the Prader-Willi region is Magel2. Magel2-deficient mice have been shown to reproduce some of the phenotype of PWS.[10,11] Magel2-deficient mice have a major reduction of hypothalamic oxytocin secretion and a 50% neonatal mortality due to suckling defects.[10] A single oxytocin injection in mouse pups at 3-5 hours after birth normalized suckling and feeding behaviour, resulting in survival of all pups.[10] Furthermore, in the 50% surviving adult mice, deficiencies in learning abilities and social behaviours were found.[11] In these mice, oxytocin given 1 hour before behavioural testing reversed social recognition deficits.[11] This might suggest that oxytocin could potentially influence at least some of the behavioural phenotypes of PWS. Furthermore, the number of oxytocin-expressing neurons in the hypothalamus of patients with PWS is significantly decreased[12] and both higher and lower plasma oxytocin levels were found in PWS compared with healthy controls.[13,14] Altogether, findings suggest that the oxytocin system in patients with PWS is dysfunctional and oxytocin might be involved in the hyperphagia seen in PWS.

Oxytocin is known to be involved in social skills,[15] food intake,[16-18] energy expenditure[19] and body weight,[20] all of which are seriously affected in PWS. Currently, there are no pharmacotherapeutic options to effectively reduce symptoms of hyperphagia. Most psychotropic options to alleviate severe behavioural problems have undesirable side effects. Studies of oxytocin in autism have shown favourable effects on behaviour.[21-23] There are a few studies investigating short-term oxytocin, most for a maximum of 4 weeks and one investigated 8 weeks of oxytocin, in both children and adults with PWS.[24-29]These studies show varying effects. Our previous randomized, double-blind, placebo-controlled, crossover trial investigating 4 weeks of oxytocin vs. placebo showed positive effects on social behaviour in children aged 6-11 years, but not in older children,[28] indicating that younger children might benefit more from oxytocin treatment. However, there are currently no studies investigating effects of longer-term oxytocin treatment in young children with PWS.

Given the dysfunctional oxytocin system in PWS and involvement of oxytocin in social skills, food intake and body weight, we hypothesized that intranasal oxytocin in young children with PWS would improve social behaviour and hyperphagia. We, therefore, conducted a randomized, double-blind, placebo-controlled crossover study on the effects of 3 months of oxytocin versus 3 months of placebo. The primary endpoints were changes in social behaviour and hyperphagia. Furthermore, we investigated if there was a difference in effects between boys or girls or between the genetic subtypes.

2 | METHODS

2.1 | Subjects

Inclusion criteria were as follows: (a) genetically confirmed diagnosis of PWS; (b) age 3-11 years; (c) behavioural problems such as reduced social reciprocity, repetitive behaviour or temper tantrums, with or without hyperphagia defined as being in nutritional phase 2b or 3 according to Miller[30]; and (d) using GH treatment for at least 1 year. Exclusion criteria were as follows: (a) severe psychiatric problems; (b) non-cooperative behaviour; (c) allergic reactions or hypersensitivity to oxytocin; (d) cardiac abnormalities; (e) extremely low dietary intake or less than minimal required intake according to WHO; and (f) using medication to reduce weight (fat).

Forty-six children with PWS were eligible. Parents of 3 children did not want to participate because they did not find their child's behaviour to be problematic, parents of 16 children did not want to participate due to too large a burden or practical issues concerning visits to study centre. One patient was scheduled to participate but had to withdraw due to scoliosis correction just before study start. The study group consisted of 26 children with PWS. All children were on GH therapy. Median daily GH dose was 0.67 mg/m^2/day (~0.023 mg/kg/day).

The study protocol was approved by the Medical Ethics Committee of Erasmus University Medical Center, Rotterdam, The

Netherlands. Written informed consent was obtained from parents and assent was obtained from children.

2.2 | Design

Randomized, double-blind, placebo-controlled, crossover design was used to investigate effects of intranasal oxytocin on social behaviour and food intake. Children received either oxytocin or placebo for 3 months, followed by a 1-month wash-out period. Following the wash-out period, patients crossed over to the alternative treatment for a further 3 months. An independent statistician generated the random allocation sequence, and only the statistician and independent pharmacist were unblinded. Because of possible age and sex effects, children were stratified according to age (3-6.99 years and 7-10.99 years) and sex, and then randomly and blindly assigned to receive twice daily, before breakfast and dinner, intranasal administration of either oxytocin (Syntocinon®, 2 IU/puff, Defiante Farmaceutica Portugal) or identical appearing placebo (placebo, 0 IU/puff, UniversitätsKlinikum Heidelberg Apotheke, Germany). Placebo consisted of aqua conservans, sodium chloride, chlorobutanol and glycerine. Both oxytocin and placebo nasal sprays were packaged and labelled by the UniversitätsKlinikum Heidelberg Apotheke, Germany. Nasal spray bottles were collected at the end of a study phase and it was recorded if they were empty or full at the end of a study phase. Compliance was assessed at the study visits by asking about missed doses. Dose was based on doses used in other trials[15,26,28] and calculated according to body surface area; <0.8 m^2 b.i.d. 8IU; 0.8-1.15 m^2 b.i.d. 12IU; 1.15-1.45 m^2 b.i.d. 16IU; and 1.45-1.75 m^2 b.i.d. 20IU.

2.3 | Measurements

Children were examined at the outpatient clinic four times during the study: baseline visit before start of nasal spray (Visit 1); after 3 months, end of first phase (Visit 2); after 4 months, end of wash-out period (Visit 3); and after 7 months, end of second phase (Visit 4). Standing height was measured with a calibrated Harpenden stadiometer and weight on a calibrated scale (Servo Balance). Height, weight and body mass index (BMI) standard deviation scores (SDS) were calculated with Growth Analyser 4.0 (available at www.growthanalyser.org) and were adjusted for sex and age according to Dutch reference values.[31,32] DXA (Lunar Prodigy; GE Healthcare) was used to measure fat percentage. All scans were made on the same machine with daily quality assurance.

Fasting glucose and insulin were collected after an overnight fast and assayed in the Biochemical and Endocrine laboratories of Erasmus Medical Center, Rotterdam. The last dose of oxytocin/placebo nasal spray was given the evening before collection. Blood samples for acylated ghrelin (AG) and unacylated ghrelin (UAG) were collected in EDTA tubes, and 4-(2-aminoethyl)benzenesulphonyl fluoride hydrochloride (AEBSF, Sigma-Aldrich Chemicals) was added to a concentration of 2 mg/ml at time of collection. Blood was centrifuged at 4℃ to prepare plasma, which was quickly frozen and stored at −80℃ until assayed. Assays were performed as described previously.[33] Samples with an extremely low AG/UAG level (<0.1) were excluded due to stabilization concerns. Oxytocin levels in blood samples were measured in duplicate with an oxytocin ELISA kit (Enzo Life Sciences).

2.4 | Recording of safety and adverse events

The first nasal spray of each phase was administered at the study centre. Patients were observed after administration for 60 minutes with a standard checklist for possible adverse events (eg nausea, skin rash). Furthermore, blood pressure and oxygen saturation were measured before administration of the nasal spray, and ten and 60 minutes thereafter. No adverse effects occurred during observation. After the observation, patients were allowed to leave the study centre and parents were carefully instructed to call our special study mobile phone 24/7 in case they had any concerns regarding adverse effects. Adverse events were assessed during the study visits. Furthermore, 4 weeks after the start of a nasal spray parents were contacted by phone for evaluation of possible adverse effects.

2.5 | Questionnaires

Parents completed questionnaires at all visits. Parents completed Oxytocin Questionnaire[28] and Dykens Hyperphagia Questionnaire measuring changes in eating behaviour.[34] Oxytocin Questionnaire unravels changes in emotions and social and eating behaviour, and was also used in our previous study.[28] Parents were asked to score the change from −3 (much less frequently) to +3 (much more frequently) and a positive score represented an improvement. Furthermore, parents were asked to complete the Repetitive Behavior Scale-Revised (RBS-R)[35-37] and Social Responsiveness Scale (SRS-P).[38]

Parents also completed standardized diaries about behaviour during 5 days prior to a visit. In the behavioural diary, parents were asked to record the number of events (eg temper tantrums or happy episodes) happening during a day.

2.6 | Statistics

Castor EDC was used for data capture.[39] Statistical analyses were performed with SPSS version 24.0 (SPSS Inc, Chicago, IL). For calculating sample size, SD and effect size were based on a previous study in young children with autism using SRS-P.[23] We used the standard deviation of the difference between two values for the same patient of 17 and minimal detectable difference of 10. Power was set at 0.8 and significance level of 0.05. Based on these values, a total of 25 patients had to enter the two-treatment crossover study. To avoid carry-over effects between phases 1 and 2, a

TABLE 1 Baseline characteristics of total group and per treatment schedule

	Total group of patients						
	PWS (n = 26)		**Oxytocin / Placebo (n = 13)**		**Placebo / Oxytocin (n = 13)**		P *
Gender	13 boys, 13 girls		7 boys, 6 girls		6 boys, 7 girls		
Genetic subtype (DEL/mUPD)	14 / 12		9 / 4		5 / 8		
Age (yrs)	7.5	(5.6-9.1)	7.3	(4.8-9.7)	7.7	(6.3-9.2)	0.69
BMI for age (SDS)	1.8	(0.3-3.5)	2.3	(1.8-4.7)	0.4	(−0.3 to 2.0)	**0.005**
Age at start GH treatment (yrs)	0.7	(0.7-1.0)	0.7	(0.7-1.3)	0.7	(0.7-0.8)	0.45
RBS-R	17.0	(11.0-28.0)	18.0	(11.0-31.0)	17.0	(9.5-27.0)	0.61
SRS-P	71.0	(50.0-89.0)	64.0	(56.0-92.0)	72.5	(49.3-87.0)	0.93
	Boys						
	PWS (n = 13)		**Oxytocin / Placebo (n = 7)**		**Placebo / Oxytocin (n = 6)**		***P****
Genetic subtype (DEL/mUPD)	7 / 6		5 / 2		2 / 4		
Age (yrs)	7.3	(5.3-9.2)	7.3	(5.0-10.4)	7.3	(5.5-8.2)	1.0
BMI for age (SDS)	2.2	(0.3-4.7)	3.9	(1.9-5.3)	0.2	(−0.9 to 2.0)	**0.018**
RBS-R	18.5	(11.5-27.8)	15.0	(8.5-33.8)	22.0	(13.0-33.3)	0.49
SRS-P	81.0	(56.0-97.0)	61.0	(42.0-97.5)	85.0	(58.8-99.8)	0.54
	Girls						
	PWS (n = 13)		**Oxytocin / Placebo (n = 6)**		**Placebo / Oxytocin (n = 7)**		***P****
Genetic subtype (DEL/mUPD)	7 / 6		4 / 2		3 / 4		
Age (yrs)	8.0	(6.1-9.2)	6.9	(4.4-9.3)	8.6	(6.4-9.6)	0.45
BMI for age (SDS)	1.2	(0.2-2.3)	2.2	(1.0-2.7)	0.6	(−0.1 to 2.4)	0.27
RBS-R	17.0	(9.0-30.0)	22.5	(13.8-39.0)	11.0	(5.0-25.0)	0.18
SRS-P	67.5	(49.3-79.3)	67.5	(53.8-87.5)	60.5	(44.0-75.8)	0.49

Note: Data expressed as median with interquartile range.

Abbreviation: SRS-P social responsiveness scale. RBS-R: Repetitive Behavior Scale-Revised.

**P*-value at baseline between the two treatment schedules.

TABLE 2 Effects on hyperphagia and social behaviour of total group

	After 12 weeks of oxytocin			After 12 weeks of placebo			
Items investigated	**Median**		**IQR**	**Median**		**IQR**	***P****
Oxytocin study questionnaire[b]							
Total score	1.0		(−5.5 to 11.0)	-2.0		(−8.5 to 5.5)	0.20
	Better	Same	Worse	Better	Same	Worse	
Total score	13	3	9	8	3	14	0.30
Happiness	10	15	0	5	19	1	0.27
Anxious	6	17	2	1	22	2	0.06
Food seeking behaviour	5	18	2	0	21	4	0.06
Dykens change in hyperphagia[b]							
Total score	0.0		(−1.0 to 3.0)	0.0		(−6.0 to 0.0)	0.06
	Better	Same	Worse	Better	Same		
Total score	8	9	8	3	11	11	0.18
Improvement social behaviour	14			6			0.12
Improvement eating behaviour	7			1			0.07
RBS-R total score[a]	11.0		(5.5-16.5)	14.0		(5.0-22.5)	0.20
SRS-P total score[a]	68.0		(55.0-80.5)	68.0		(50.0-78.5)	0.52
Measurements							
Δweight (kg)	1.0		(0.4-2.1)	1.0		(0.6-1.4)	0.70
ΔBMI (kg/m^2)	0.3		(−0.3 to 0.7)	0.3		(−0.1 to 0.6)	0.65
Δfat percentage	0.3		(−1.0 to 1.4)	0.3		(−0.9 to 1.7)	0.59
Δlean body mass (gram)	552.0		(115.8-913.0)	357.5		(−315.5 to 869.5)	0.21
Safety							
Systolic blood pressure	105.0		(100.0-112.0)	108.0		(97.8-116.3)	0.55
Diastolic blood pressure	66.0		(61.0-69.0)	65.0		(57.0-76.5)	0.87
Fasting glucose	5.0		(4.6-5.1)	4.8		(4.5-5.1)	0.08
Fasting insulin	58.0		(41.0-101.8)	64.5		(26.0-92.0)	0.90

Note: Data expressed in median (IQR) unless otherwise specified;

[b]a positive score indicates improvement.

[a]a higher score indicates more problems in behaviour.

**P*-value between oxytocin and placebo phase. *P*-value for variables expressed in better, same, worse, is the *P*-value for better versus same and worse.

wash-out period was implemented. Statistical analyses appropriate for crossover trials were used, taking into account any carry-over or period effect, but these were not found. Results after 3 months of either oxytocin or placebo were primary outcome measures. Results of visits (ie questions about changes or total score on questionnaire) or differences (Δ) between visits 1 and 2, and between visits 3 and 4 were assessed. Effect of oxytocin versus placebo was tested by paired sample t-test or Wilcoxon tests, in case of continuous data and McNemar tests in case of binary data. Primary endpoint was the effect of oxytocin on social behaviour assessed by the Dykens Questionnaire, Oxytocin Study Questionnaire, SRS-P and RBS-R. Because of possible age, sex or genotype effects, we investigated correlations between age and effects and performed subanalyses in boys vs. girls and deletion vs. mUPD. Results were considered significant if *P*-value was <0.05.

3 | RESULTS

3.1 | Baseline Characteristics

No carry-over or period effect was found. Table 1 shows baseline characteristics of 26 children with PWS who were included between May 2018 and May 2019. Median age at inclusion was 7.5 (5.6-9.1) years, and BMI was 1.8 (0.3-3.5) SDS. Fourteen (53.8%) patients had

TABLE 3 Effects on hyperphagia and social behaviour in boys and girls separately

	BOYS (N = 12)							**GIRLS (N = 13)**						
	Oxytocin phase			Placebo phase				Oxytocin phase			Placebo phase			
Items investigated	Median		IQR	Median		IQR	P^*	Median		IQR	Median		IQR	P^*
Oxytocin study questionnaire														
Total score	4.5		(−0.8 to 15.3)	-4.0		(−11.3 to 0.8)	**0.025**	0.0		(−12.5 to 5.5)	0.0		(−6.5 to 10.0)	0.75
	Better	Same	Worse	Better	Same	Worse		Better	Same	Worse	Better	Same	Worse	
Total score	7	2	3	3	1	8	0.29	6	1	6	5	2	6	1.0
Happiness	5	7	0	0	12	0	0.06	5	8	0	5	7	1	1.0
Anxious	4	8	0	1	10	1	0.25	2	9	2	0	12	1	0.5
Food seeking behaviour	4	7	1	0	8	4	0.13	1	11	1	0	13	0	1.0
Dykens change in hyperphagia														
Total score	0.0		(−0.8 to 4.3)	-3.5		(−6.0 to 0.0)	**0.046**	0.0		(−1.0 to 2.0)	0.0		(−2.0 to 0.0)	0.57
	Better	Same	Worse	Better	Same	Worse		Better	Same	Worse	Better	Same	Worse	
Total score	5	4	3	1	4	7	0.22	3	5	5	2	7	4	1.0
Improvement social behaviour	7			2			0.18	7			4			0.55
Improvement eating behaviour	5			1			0.22	2			0			0.5

Note: Data expressed in median (IQR) unless otherwise specified.

[a]a higher score indicates more problems in behaviour.

[b]a positive score indicates improvement.

*p-value between oxytocin and placebo phase. p-value for variables expressed in betters, same worse, is the p-value for better versus no difference.

Significant differences are indicated in bold.

a deletion and twelve (46.2%) an mUPD. Weight for height SDS and BMI SDS were significantly higher in patients who received oxytocin in the first study phase, than in those who received oxytocin in the second study phase. Other characteristics did not differ. There were no differences between boys and girls at study start. One patient dropped out during the second study phase, while receiving oxytocin, due to serious family health problems. Results from this patient were not used in analyses.

3.2 | Effects of oxytocin on social behaviour and food behaviour

3.2.1 | Total group

Table 2 shows questionnaire results after 3 months of oxytocin or placebo. Median (IQR) Oxytocin Questionnaire score was 1.0 (−5.5 to 11.0) after oxytocin and −2.0 (−8.5 to5.5) after placebo, (P = .20), with positive scores reflecting improvement in behaviour and negative scores reflecting deterioration. Change in hyperphagia measured by Dykens questionnaire showed a trend to being less hyperphagic after oxytocin (P = .06). Total score (IQR) on RBS-R was 11.0 (5.5-16.5) after oxytocin and 14.0 (5.0-22.5) after placebo (P = .20), with lower scores reflecting less repetitive behaviour. There was no difference in total score on SRS-P between oxytocin and placebo. Fourteen parents reported improvement in social behaviour of their child during oxytocin, while six reported improvement during placebo, indicating a trend (P = .12). Seven parents reported improvement in eating behaviour during oxytocin versus one during placebo, indicating a trend (P = .07). Fourteen parents were able to correctly tell in which phase their child received oxytocin, while five thought their child was receiving oxytocin when their child was in fact receiving placebo. Children of the latter five parents were all <6 years. Parents in the older age group either did experience positive effects during oxytocin (N = 9) or did not experience a difference (N = 5). Parents were given the option to continue open-label oxytocin nasal spray. Fifteen (57.7%) parents chose to continue open-label oxytocin because they felt that their child had experienced substantial beneficial effects.

In the diary about behaviour, parents reported a trend to more positive behaviour during oxytocin compared to placebo (P = .06) and reported a trend to fewer episodes of self-injurious behaviour during oxytocin compared to placebo (P = .09). There was no difference in reported temper tantrums or conflicts during oxytocin vs. placebo.

3.2.2 | Sex-dependent effects of oxytocin treatment

We anticipated, before study start, that there might be a difference in oxytocin effects between boys and girls, and patients were, therefore, stratified according to sex. Indeed, our results showed a difference in effects between boys and girls (Table 3).

In boys, median (IQR) Oxytocin Questionnaire score after oxytocin was 4.5 (−0.8 to 15.3), indicating improvement, while it was −4.0 (−11.3 to 0.8) after placebo, indicating deterioration of behaviour, which was a significant difference (P = .025). After 3 months of oxytocin, change in Dykens hyperphagia score was 0.0 (−0.8 to 4.3), while placebo resulted in a worsening of hyperphagia of −3.5 (−6.0 to 0.0) (P = .046).

In girls, there were no significant differences between oxytocin or placebo in any of the questionnaires.

3.2.3 | Effects in genetic subtypes

We next investigated if there was a difference in effects of oxytocin between patients with a deletion or mUPD (Table 4).

In patients with a deletion, Oxytocin Questionnaire score significantly improved during oxytocin (5.5 (−0.3 to 15.0)), compared to deterioration (−1.5 (−12.8 to 3.0)) during placebo (P = .03). Dykens hyperphagia questionnaire score also significantly improved during oxytocin, compared to placebo (P = .03).

In boys with a deletion, although this group was small, there was a significant difference between oxytocin and placebo in Oxytocin Questionnaire scores, being 9.0 (0.0-18.0) after oxytocin versus −2.0 (−12.0 to 0.0) after placebo (P = .04). Change in the Dykens hyperphagia score showed a trend, being 0.0 (0.0-2.0) after oxytocin versus −6.0 (−6.5 to 0.0) after placebo (P = .08).

In patients with an mUPD, no differences were found between oxytocin and placebo.

3.2.4 | Effects of oxytocin on other parameters in the total group

There was no difference between oxytocin and placebo in Δweight, ΔBMI or Δfat percentage after 3 months. During oxytocin, AG had a median decrease of −10.6 (−88.1 to 34.2) pg/ml, while during placebo, there was an increase of 17.4 (−53.6 to 47.9) pg/ml, but this difference was not significant (P = .27) (Table 5). UAG increased during both oxytocin and placebo. AG/UAG ratio decreased during oxytocin, while remaining similar during placebo, however this difference did not reach statistical significance in the eleven subjects available for analyses (P = .25). There was a higher increase in serum oxytocin levels during oxytocin than during placebo, but not significant (786 (−168 to 2896) pg/l and 363 (−475 to 1775) pg/l, respectively P = .27).

3.2.5 | Safety parameters

Intranasal administration of oxytocin was well tolerated. Compliance was remarkably high, and only few parents reported to occasionally

TABLE 4 Effects on hyperphagia and social behaviour according to genetic subtype

	Patients with a deletion (N = 14)							Patients with a mUPD (N = 11)						
	Oxytocin phase			Placebo phase				Oxytocin phase			Placebo phase			
Items investigated	Median		IQR	Median		IQR	*P**	Median		IQR	Median		IQR	*P**
Oxytocin study questionnaire[b]														
Total score	5.5		(−0.3 to 15.0)	-1.5		(−12.8 to 3.0)	**0.03**	-1.0		(−14.0 to 3.0)	-2.0		(−6.0 to 10.0)	0.62
	Better	Same	Worse	Better	Same	Worse		Better	Same	Worse	Better	Same	Worse	
Total score	9	2	3	4	2	8	0.18	4	1	6	4	1	6	1.0
Dykens change in hyperphagia[b]														
Total score	0.0		(−1.0 to 2.5)	0.0		(−6.0 to 0.0)	**0.03**	0.0		(−1.0 to 4.0)	0.0		(−3.0 to 0.0)	0.78
	Better	Same	Worse	Better	Same	Worse		Better	Same	Worse	Better	Same	Worse	
Total score	5	5	4	1	7	6	0.13	3	4	4	2	4	5	1.0
Improvement social behaviour	10			3			0.09	4			3			1.0
Improvement eating behaviour	5			0			0.06	2			1			1.0
	Boys with a deletion (N = 7)							Boys with an mUPD (N = 5)						
	Oxytocin phase			Placebo phase				Oxytocin phase			Placebo phase			
Items investigated	Median		IQR	Median		IQR	*P**	Median		IQR	Median		IQR	*P**
Oxytocin study questionnaire[b]														
Total score	9.0		(0.0-18.0)	-2.0		(−12.0 to 0.0)	**0.04**	1.0		(−4.0 to 12.0)	-6.0		(−12.5 to 6.5)	0.39
	Better	Same	Worse	Better	Same	Worse		Better	Same	Worse	Better	Same	Worse	
Total score	4	2	1	1	5	0.38	3	0	2	2	0	3		1.0
Dykens change in hyperphagia[b]														
Total score	0.0		(0.0-2.0)	-6.0		(−6.5 to 0.0)	0.08	0.0		(−1.0 to 5.0)	-1.0		(−6.5 to 1.5)	0.42
	Better	Same	Worse	Better	Same	Worse		Better	Same	Worse	Better	Same	Worse	
Total score	3	3	1	0	3	4	0.25	2	1	2	1	1	3	1.0
Improvement social behaviour	5			1			0.22	2			1			1.0
Improvement eating behaviour	3			0			0.25	2			1			1.0

Note: Data expressed in median (IQR) unless otherwise specified.

[a]a higher score indicates more problems in behaviour.

[b]a positive score indicates improvement.

**P*-value between oxytocin and placebo phase. *P*-value for variables expressed in better, same, worse, is the *P*-value for better versus same and worse.

TABLE 5 Other parameters

	After 12 weeks of oxytocin		After 12 weeks of placebo		
Items investigated	**Median**	**IQR**	**Median**	**IQR**	***P****
ΔAG (pg/ml)[a] (N = 12)	-10.6	(−88.1 to 34.2)	17.4	(−53.6 to 47.9)	0.27
ΔUAG (pg/ml)[a] (N = 11)	13.0	(−34.6 to 32.8)	12.7	(−123.2 to 95.3)	0.42
ΔAG/UAG ratio (pg/ml)[a] (N = 11)	-0.14	(−0.52 to 0.26)	0.0	(−0.29 to 0.63)	0.25
Oxytocin in serum (pg/ml) (N = 13)	4106	(3065-8027)	4264	(3371-6381)	0.31
Δ Oxytocin in serum (pg/ml)	786	(−168 to 2896)	363	(−475 to 1775)	0.27

[a]a positive score indicates a higher AG or UAG or AG/UAG ratio at the end of the OX/PL period.

* *P*-value between oxytocin and placebo phase.

have missed a dose (eg on a day out during the weekend). There was one serious adverse event during the study: one patient developed gastroparesis while receiving placebo, probably caused by an infection for which hospitalization was required. This patient recovered without sequelae. Five patients reported headache, four during placebo and one during oxytocin. Nasal irritation was reported by three patients, two during placebo and one during oxytocin. One patient reported a skin rash during placebo. There was no difference in blood pressure and fasting glucose or insulin levels between oxytocin and placebo.

3.2.6 | Applicability of questionnaires

We noticed that the four questionnaires showed conflicting results in some children, as one questionnaire could indicate an improvement in behaviour, while another questionnaire indicated a deterioration. To assess if questionnaires adequately reflected the subjective feeling of parents, that there was an improvement in behaviour during one study phase, we investigated the scores of questionnaires completed by parents who felt there was a difference in behaviour between the two phases. In the fourteen parents who correctly pointed out in which phase their child received oxytocin, Oxytocin Questionnaire showed significant improvement in behaviour during oxytocin compared to placebo, with score of 8.5 (3.8-16.5) after oxytocin and −6.5 (−15.5 to 2.3) after placebo ($P < .001$). Dykens questionnaire also showed significant improvement during oxytocin compared to placebo, with a score of 2.0 (−0.3 to 5.0) vs. −3.0 (−6.3 to 0.0), resp. ($P = .007$). RBS-R and SRS-R did not show significant differences in scores between oxytocin and placebo. In the five parents who thought their child received oxytocin, while in fact receiving placebo, there was a trend for the Oxytocin Questionnaire score to favour placebo over oxytocin, with a score of 10.0 (−3.5 to 15.5) after placebo and −11.0 (−17.0 to −4.5) after oxytocin ($P = .07$), while there was no difference between the two phases regarding the other questionnaires.

4 | DISCUSSION

Our current randomized, double-blind, placebo-controlled, crossover study is the first study to investigate effects of 3 months of oxytocin in children with PWS aged 3-11 years. In the total group, there were no significant differences between oxytocin and placebo, although more parents reported improvement in social and eating behaviour during oxytocin than during placebo. However, when analysed separately, boys showed significant positive effects of oxytocin on hyperphagia, and social and eating behaviour. In girls, no significant beneficial effects were found. Patients with a deletion had significant positive effects, but patients with an mUPD did not.

In the current study, oxytocin treatment had a positive (suppressive) effect on hyperphagia in boys and in patients with a deletion, and there was a trend to a positive effect in the total group. When looking at the scores of Dykens questionnaire, it seems that oxytocin prevents worsening of hyperphagia, since score was 0 during oxytocin and negative during placebo. This might be explained by the age range during which hyperphagia becomes more prominent according to Miller.[30] Oxytocin might thus be able to prevent progression to higher nutritional phases.

There are currently a few studies describing the effects of short-term oxytocin nasal spray in children with PWS.[29] Our previous randomized, placebo-controlled, crossover study found positive effects of 4 weeks of oxytocin nasal spray versus placebo on social behaviour and hyperphagia in children aged 6-11 years,[28] suggesting that younger children might benefit more from oxytocin. We, therefore, included children aged 3-11 years in the current study. We could not confirm an age association, which might be due to the less problematic behaviour in very young children, which cannot be detected by questionnaires. Alternatively, there may be no age association <11 years.

Most studies investigating effects of oxytocin in PWS have reported some potential effects of oxytocin, but no study has shown convincing evidence for beneficial effects.[25-29,40] This is probably caused by the nature of the studied outcomes, being behaviour, and the fact that they are assessed by questionnaires. There are currently no questionnaires assessing the specific behavioural type seen in PWS. We, therefore, chose to use questionnaires often used in ASD, since children with PWS show symptoms of ASD.[6] However, these questionnaires probably do not fully capture the specific behavioural type seen in PWS. Furthermore, the four questionnaires used in our study sometimes resulted in conflicting outcomes in one child. We tried to investigate which questionnaire best reflected the subjective

feelings of parents regarding behaviour. Oxytocin Questionnaire and Dykens hyperphagia questionnaire showed significant improvements during oxytocin when parents reported improvements in (eating) behaviour during oxytocin, while RBS-R and especially SRS-P did not. During this study, we found that the SRS-P questionnaire was not suitable for our study population and we therefore did not analyse this questionnaire further in subanalyses. Due to a lack of validated behaviour questionnaires for PWS, it will remain hard to find objective evidence for interventions. Even trends to improvements on currently available questionnaires might, therefore, be an important indication that an intervention such as oxytocin influences behaviour.

In the current study, we found positive effects of oxytocin in boys, but not in girls. This difference between boys and girls has not been described.[25-28] There are some possible explanations for this observed sex difference. First, effects of oxytocin were measured by questionnaires. It is possible that the problematic behaviour measured by these questionnaires is better captured in boys than in girls. Second, it could be that there is a difference in oxytocin system between boys and girls, making boys more sensitive to oxytocin effects. A study in young children with ASD showed positive effects of oxytocin on social and repetitive behaviour. In that study, however, 87.1% of participants were male, which might explain why they found significant differences between oxytocin and placebo.[23] Also, other studies in ASD showing positive effects of oxytocin investigated mainly or only males.[21,22,41] Future studies should investigate this sex-dependent difference in oxytocin effects in more detail.

We also found that patients with a deletion had significant beneficial effects of oxytocin compared to placebo, while patients with an mUPD did not. This is in contrast to what we expected, since there is a higher prevalence of ASD in patients with an mUPD than in patients with a deletion.[6,42] However, other studies reported that the deletion subtype might be associated with more maladaptive behaviour, more skin-picking and more overeating,[43,44] all symptoms on which oxytocin might have positive effects. Previous studies either did not find differences between genetic subtype[28] or did not investigate if there was a difference.[5,27] However, a study by Tauber et al found that after a single intranasal administration of oxytocin, there was an increased trust in others, decreased sadness tendencies with less disruptive behaviour in the two days following administration and the majority of that study population (79%) consisted of patients with a deletion.[25]

We found a decrease in AG during oxytocin and an increase in AG during placebo, while UAG increased during both oxytocin and placebo, resulting in a lower AG/UAG ratio after oxytocin and a stable AG/UAG ratio after placebo. A lower AG/UAG ratio suggests a decrease in orexigenic drive. Although differences between oxytocin and placebo were not significant, there seems to be a trend. We showed previously that children with PWS had a higher AG/UAG ratio than healthy controls and that the switch to excessive weight gain in PWS seemed to coincide with an increase in AG/UAG ratio.[33] Although non-significant, the decrease in AG/UAG ratio during oxytocin could be the underlying mechanism explaining the effect of oxytocin on eating behaviour.

Oxytocin treatment was very well tolerated and no serious adverse events were reported, indicating that oxytocin treatment during 3 months is safe. Compliance was high. However, before definite conclusions can be drawn, studies are needed to investigate long-term safety.

Altogether, it appears that oxytocin has beneficial effects in some, but not all patients with PWS. It is important for future studies to investigate which patients might benefit from oxytocin treatment and confirm our findings. Furthermore, it could be that intranasal oxytocin has different effects at different ages. A study in infants with PWS aged <6 months showed positive effects of a short course of oxytocin on feeding and social skills.[24] Furthermore, these oxytocin-treated children displayed higher social skills at a median age of 26.5 months and were more engaged in relationships than children of comparable age who did not receive early oxytocin.[24]

The strength of current study is the placebo-controlled, double-blind, crossover design with a duration of 3 months. Furthermore, there was a high compliance during the study. A limitation of current study is the small sample size, particularly in the subanalyses, although a total sample size of 25 is relatively large for a rare disorder like PWS. Another limitation is the suboptimal questionnaires available for patients with PWS to identify changes in eating and social behaviour.

In conclusion, intranasal oxytocin treatment has positive effects on social and eating behaviour in boys with PWS and in those with a deletion. It is difficult to objectively measure effects of intranasal oxytocin treatment on social behaviour, but more parents reported an improvement in social and eating behaviour during oxytocin than during placebo and more than half of the parents wanted their child to continue off-label oxytocin treatment because of this. These positive effects have the potential to significantly improve quality of life of the children and their families. Intranasal oxytocin treatment in children with PWS might be considered, but on individual basis as the positive effects are quite variable among children with PWS. Therefore, effects should be evaluated and treatment discontinued if no beneficial effects are found.

DISCLOSURE SUMMARY

Investigator-initiated study.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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