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ORIGINAL ARTICLE

# Promising effects of oxytocin on social and food-related behaviour in young children with Prader–Willi syndrome: a randomized, double-blind, controlled crossover trial

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## Summary

**Background** Prader–Willi syndrome (PWS) is known for hyperphagia with impaired satiety and a specific behavioural phenotype with stubbornness, temper tantrums, manipulative and controlling behaviour and obsessive–compulsive features. PWS is associated with hypothalamic and oxytocinergic dysfunction. In humans without PWS, intranasal oxytocin administration had positive effects on social and eating behaviour, and weight balance.

**Objective and hypotheses** To evaluate the effects of intranasal oxytocin compared to placebo administration on social behaviour and hyperphagia in children with PWS.

**Design** Randomized, double-blind, placebo-controlled, crossover study in a PWS Reference Center in the Netherlands.

**Method** Crossover intervention with twice daily intranasal oxytocin (dose range 24-48 IU/day) and placebo administration, both during 4 weeks, in 25 children with PWS (aged 6 to 14 years).

**Results** In the total group, no significant effects of oxytocin on social behaviour or hyperphagia were found, but in the 17 children younger than 11 years, parents reported significantly less anger ($P = 0{\cdot}001$), sadness ($P = 0{\cdot}005$), conflicts ($P = 0{\cdot}010$) and food-related behaviour ($P = 0{\cdot}011$), and improvement of social behaviour ($P = 0{\cdot}018$) during oxytocin treatment compared with placebo. In the eight children older than 11 years, the items happiness ($P = 0{\cdot}039$), anger ($P = 0{\cdot}042$) and sadness ($P = 0{\cdot}042$) were negatively influenced by oxytocin treatment compared to placebo. There were no side effects or adverse events.

**Conclusions** This randomized, double-blind, placebo-controlled study suggests that intranasal oxytocin administration has beneficial effects on social behaviour and food-related behaviour in children with PWS younger than 11 years of age, but not in those older than 11 years of age.

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## Introduction

Prader–Willi syndrome (PWS) is characterized by neonatal hypotonia with suckling problems, early onset of hyperphagia with impaired satiety, endocrine disturbances and a specific behavioural phenotype with stubbornness, temper tantrums, manipulative and controlling behaviour, obsessive–compulsive features and difficulties in changing routines.[1–3] This results from the absence of expression of the paternally derived genes located on chromosome 15 at the locus q11·2-13, caused by a paternal deletion, maternal uniparental disomy, imprinting centre disorder or paternal chromosomal translocation.[1] One of the nonexpressed genes in this region is MAGEL2.

MAGEL2-deficient mice have a major reduction of oxytocin in the hypothalamus and an altered onset of suckling activity resulting in impaired feeding and 50% mortality.[4] Injection of a specific oxytocin receptor antagonist in wild-type mouse pups resulted in a similar feeding deficiency as seen in MAGEL2 mutants.[4] Administration of oxytocin to MAGEL2-deficient mouse pups 3–5 h after birth normalized suckling and feeding behaviour and rescued all of them.[4] Human newborns with PWS show similar suckling problems as found in the MAGEL2-deficient mice, which suggests that the lack of MAGEL2 gene might play a role in the suckling deficit seen in PWS newborns.

In adult patients with PWS, the number of oxytocin-expressing neurons in the hypothalamus was significantly decreased by 42% and plasma levels of oxytocin were relatively low in relation to their obesity.[5,6] However, in 23 children with PWS between 5 and 11 years of age, high plasma levels of oxytocin were reported.[7] Altogether, the oxytocin system in patients with PWS appears to be dysfunctional.

Oxytocin is known to be involved in food intake,[8,9] body weight[10,11] and social skills,[12] all of which are seriously affected

in patients with PWS. The majority of patients with PWS have hyperphagia with impaired satiety, and they are severely at risk to become obese. They show symptoms of autistic spectrum disorder (ASD) and 36% of them fulfil the criteria of ASD.[13] Social cognitive functioning is markedly reduced, which has major consequences for the family and surrounding and for the approach of patients with PWS.[14,15] Currently, there are no treatment options for the hyperphagia and social behavioural problems of patients with PWS, but the oxytocin system is a promising target. Studies on intranasal oxytocin administration showed that oxytocin reduced body weight of obese non-PWS patients.[10] Also, a single oxytocin gift improved emotion recognition in healthy and autistic adults[16,17] and reduced repetitive behaviours in those with ASD.[18]

Only two studies have investigated the effects of oxytocin treatment in PWS. Tauber *et al.* administrated a single gift of 24 IU intranasal oxytocin to 24 adults with PWS. After two days, they showed increased trust in others and decreased sadness tendencies with less disruptive behaviour.[19] In the placebo-controlled crossover study by Einfeld *et al.*, 22 individuals with PWS aged 12–30 years received 18–40 IU intranasal oxytocin twice daily during 8 weeks, but they showed no benefit in the target behaviours or weight.[20]

Given the possible dysfunction of the oxytocin system in PWS and the involvement of oxytocin in socials skills, food intake and body weight, we hypothesized that oxytocin supplementation in children with PWS would improve social behaviour and hyperphagia. We therefore investigated the effects of intranasal oxytocin administration on social behaviour, food intake and satiety in children with PWS in a randomized, double-blind, placebo-controlled, crossover study.

## Methods

### *Subjects*

To be eligible to participate in this study, subjects (1) had a genetically confirmed diagnosis of PWS, (2) were aged 6 to 14 years, (3) had social behavioural problems and/or a preoccupation with food, (4) were naïve for oxytocin treatment at time of enrolment and (5) used growth hormone therapy for at least 1 year and were still receiving it. Exclusion criteria were (1) severe psychiatric problems such as psychosis, serious illness or cardiac abnormalities; (2) allergic reactions or hypersensitivity to oxytocin; (3) medication to reduce weight (fat) other than GH; and (4) noncooperative behaviour resulting in inability to comply with intranasal administration and/or hospital visits.

Forty-two children with PWS were eligible. Parents of 17 children refused to participate; ten due to too large burden, five due to practical issues and two because the children themselves did not want to participate. The study group consisted of 25 children (14 boys, 11 girls) with PWS, aged 6–14 years. GH therapy was prescribed at an initial dose of 1 mg/m$^2$/day, and dose was lowered in case of high IGF-I levels. One child used levothyroxine and another used citalopram and aripiprazole.

### *Design*

A randomized, double-blind, placebo-controlled, crossover study was conducted to investigate the effects of intranasal oxytocin administration on social behaviour, food intake and satiety. Children received either oxytocin or placebo for 4 weeks, after which they crossed over to the alternative treatment for a further 4 weeks. No washout period was implemented, as the half-life time of oxytocin is only 3–20 min. An independent statistician generated the random allocation sequence and only he and the independent pharmacist were unblinded. The children were stratified according to gender and age (6–10·99 or 11–14·99 years) and then randomly and blindly assigned to receive intranasal administration twice daily, before breakfast and dinner, of either oxytocin (Syntocinon®, 4 IU/puff, Sigma Tau) or identical appearing placebo (placebo, 0 IU/puff, Sigma Tau). The dose was based on doses used in other trials[12,20] and calculated according to body surface: a child of 0·8–1·15 m$^2$ received 2dd3 puffs (12 IU twice daily); 1·15–1·45 m$^2$ had 2dd4 puffs (16 IU twice daily); 1·45–1·75 m$^2$ had 2dd5 puffs (20 IU twice daily); and of >1·75 m$^2$ had 2dd6 puffs (24 IU twice daily).

### *Measurements*

Children were examined at outpatient clinic, at baseline, after 4 weeks and after 8 weeks. Standing height was measured with a calibrated Harpenden stadiometer, and weight was determined on a calibrated scale (Servo Balance). Height, weight and BMI were expressed as SDS according to Dutch reference data, adjusted for age and sex.[21,22] Percentage fat was measured by DXA (Lunar Prodigy; GE Healthcare). All scans were made on the same machine, and daily quality assurance was performed.

Blood samples were collected in the morning after 12-h overnight fast. Samples were quickly frozen on dry ice and stored at −80 °C until assayed. A breakfast meal consisting of 270 grams (560 kcal) industrially produced multigrain pancakes cut into pieces was used to examine the food intake and satiety. This amount was determined in collaboration with a dietitian; weight was 150%, and calorie-intake 185% of a normal healthy breakfast for a child with PWS. After blood collection, the fasted children were instructed to eat as much as they wished of the meal. The weight of the plate with pancakes was measured at baseline and after the child finished eating. Besides, the duration of eating was determined. The child stayed in the room together with the investigator, while their parents waited elsewhere.

Social and eating behaviour were carefully monitored by the parents at home and investigated by two parent questionnaires. Dykens Hyperphagia Questionnaire was used to determine (changes in) eating behaviour and hyperphagia.[23] Although the Dykens Hyperphagia Questionnaire is validated, we have experience that not all items of this questionnaire are applicable nowadays. The new generation of children with PWS had an early diagnosis and received intensive support from (para)medics, and started with GH treatment at a young age, which has markedly changed the phenotype, but they are still pre-occupied with

food. Therefore, the Oxytocin Study Questionnaire was developed by three physicians and a psychologist, all very experienced in PWS. The questionnaire unravels (changes in) emotions, social and eating behaviour and possible side effects. Parents were asked to fill in the applicable change from −3 (much less frequently) to +3 (much more frequently), in which 0 was 'no difference'. An example of a question is '*In the last 4 weeks, my child was…sad*'. Comparable questions were asked regarding being angry, being happy, showing food-seeking behaviour, having conflicts with others, etc.

### *Assays*

All blood samples were determined in the same laboratory according to a standardized procedure. Levels of serum creatinine, hepatic enzymes and glucose were measured with COBAS 8000 systems of Roche, and thyroid function was measured with Vitros ECIQ immunoanalyzer system of Ortho Clinical Diagnostics. Oxytocin levels in blood samples were measured in duplo with an oxytocin ELISA kit (Enzo Life Sciences). To assure the content of the vials, one puff per child per phase was measured by the same oxytocin ELISA kit. In summary, all children had one vial with and one vial without oxytocin, which confirmed perfect execution of the randomization.

### *Statistics*

Statistical analysis was performed by SPSS version 23.0. Calculation of the sample size was based on the Oxytocin Study Questionnaire. Parents answer questions about changes in eating behaviour and social behaviour, from −3 (much less frequently) to +3 (much more frequently), in which 0 was 'no difference'. A decrease of four points was considered clinically relevant. Based on an SD of four, a power of 0·9 and significance level of 0·05, a total of 24 patients had to enter the two-treatment crossover study. Data were not normally distributed; therefore, nonparametric tests were used and data are expressed as median (interquartile range (IQR)) unless otherwise stated. Statistical analysis appropriate for crossover trials was used, taking into account any carry-over or treatment-period effect, calculated by Wilcoxon signed rank test and Mann–Whitney *U*-tests, but these were not found. Depending on the data, results of the visit (i.e. questions about changes) or differences (Δ) between visit one and two, and between visit two and three (i.e. Δweight) were used. The effect of oxytocin *vs* placebo was tested by Wilcoxon tests in case of continuous data and McNemar tests in case of binary data. Correlations between effect of oxytocin or oxytocin levels and other parameters were assessed using Spearman's rho. Differences were considered significant if *P*-value was <0·05.

### *Study approval*

Written informed consent was obtained from parents and from children older than 12 years; assent was obtained in children younger than 12 years. The study protocol was approved by the Medical Ethics Committee of Erasmus University Medical Center, the Netherlands, and registered at Nederlands Trial Register NTR4950 (www.trialregister.nl).

## Results

### *Baseline characteristics*

Table 1 shows the baseline characteristics of the 25 children with PWS who were included between January and September 2015. Median age was 9·3 (range 6·0–13·7) years, and BMI was 2·4 (0·7–4·3) SDS. Thirteen (52%) patients had a deletion and 12 (48%) an mUPD. All received GH treatment with a median dose of 0·8 (0·6–1·0) mg/m$^2$/day (≈0·024 mg/kg/day), started at a median age of 1·3 (1·0–2·2) years, with a median duration of GH treatment of 8·0 (5·7–9·2) years. The median dose of oxytocin was 16 IU (range 12–24) twice daily. All 25 children completed the study.

### *Effects on social behaviour, food intake and satiety*

In the total group of 25 children with PWS between 6 and 14 years of age, no effects of oxytocin *vs* placebo treatment were found on social behaviour, food intake and satiety. In contrast to these nonsignificant effects of oxytocin in the total group, correlation analyses showed that a younger age was strongly associated with beneficial effects of oxytocin treatment on social and eating behaviour ($\rho=-0{\cdot}553$, $P = 0{\cdot}004$ and $\rho=-0{\cdot}485$, $P = 0{\cdot}014$, resp.), and therefore, subanalyses were performed. In line with the stratification, we divided the total group in 17 patients younger than 11 years and eight patients older than 11 years.

### *Subanalysis in the younger children*

*Effects on social behaviour.* Parents filled out questionnaires about their child. The items anger, sadness and conflicts improved significantly during oxytocin treatment compared to placebo ($P = 0{\cdot}001$, $P = 0{\cdot}005$ and $P = 0{\cdot}010$, resp.) (Table 2, Fig. 1). The total Oxytocin Study Questionnaire score showed a significant improvement of −4 (−7·5 to −1) points during oxytocin treatment compared to placebo ($P = 0{\cdot}001$). Ten of 17 (58·8%) parents reported an improvement in social behaviour during oxytocin treatment, while four (23·5%) parents reported improvement during placebo ($P = 0{\cdot}059$).

*Effects on eating behaviour.* The 17 younger children showed a significant improvement in food-related behaviour during oxytocin treatment ($P = 0{\cdot}011$). During 4 weeks of oxytocin treatment, food-seeking behaviour and satiety remained similar ($P = 0{\cdot}429$ and $P = 0{\cdot}713$, resp.); however, at baseline, food-seeking behaviour was only reported by six (35·3%) of the 17 parents, and in three of them, food-seeking behaviour was seen a few times per year. In both phases, almost all children finished their standardized breakfast meal. Only three children left pancakes (median 95, range 70–140 gram) during oxytocin

**Table 1.** Baseline characteristics of total group and per treatment schedule

| | Total group of patients of 6 to 14 years | | | |
|---|---|---|---|---|
| | PWS ($n$ = 25) | Oxytocin / Placebo ($n$ = 11) | Placebo / Oxytocin ($n$ = 14) | $P$* |
| Gender | 14 boys, 11 girls | 6 boys, 5 girls | 8 boys, 6 girls | |
| Genetic subtype (DEL/mUPD) | 13 / 12 | 6 / 5 | 7 / 7 | |
| Age (yrs) | 9·3 (6·9 to 11·9) | 9·0 (6·4 to 11·2) | 10·3 (7·0 to 12·5) | 0·403 |
| Height for age (SDS) | 0·8 (0·2 to 1·6) | 1·2 (0·4 to 1·7) | 0·5 (0·0 to 1·1) | 0·222 |
| Weight for height (SDS) | 2·0 (0·6 to 4·1) | 2·3 (0·3 to 6·1) | 1·9 (1·0 to 4·1) | 0·809 |
| BMI for age (SDS) | 2·4 (0·7 to 4·3) | 2·4 (−0·1 to 6·3) | 2·2 (1·2 to 3·9) | 1·000 |
| Age at start GH treatment (yrs) | 1·3 (1·0 to 2·2) | 1·3 (0·9 to 2·2) | 1·5 (1·0 to 2·6) | 0·609 |
| Duration of GH treatment (yrs) | 8·0 (5·7 to 9·2) | 7·3 (5·3 to 9·0) | 8·2 (5·9 to 9·3) | 0·687 |
| GH dosage (mg/m$^2$/day) | 0·8 (0·6 to 1·0) | 0·8 (0·5 to 1·0) | 0·8 (0·6 to 1·0) | 0·851 |
| Prepubertal/pubertal | 16 / 9 | 7 / 4 | 9 / 5 | |
| | Patients of 6 to 11 years | | | |
| | PWS ($n$ = 17) | Oxytocin / Placebo ($n$ = 8) | Placebo / Oxytocin ($n$ = 9) | $P$* |
| Gender | 9 boys, 8 girls | 4 boys, 4 girls | 5 boys, 4 girls | |
| Genetic subtype (DEL/mUPD) | 9 / 8 | 4 / 4 | 5 / 4 | |
| Age (yrs) | 7·3 (6·4 to 9·7) | 7·8 (6·1 to 9·3) | 7·3 (6·7 to 10·3) | 0·606 |
| Height for age (SDS) | 0·5 (0·0 to 1·3) | 1·2 (0·0 to 1·6) | 0·3 (−0·2 to 0·8) | 0·277 |
| Weight for height (SDS) | 1·7 (0·6 to 3·9) | 1·7 (0·4 to 5·3) | 1·7 (0·9 to 3·9) | 0·743 |
| BMI for age (SDS) | 1·9 (0·6 to 3·9) | 2·1 (0·0 to 5·7) | 1·8 (1·0 to 3·6) | 0·963 |
| Age at start GH treatment (yrs) | 1·3 (0·9 to 1·7) | 1·3 (0·8 to 2·0) | 1·2 (0·9 to 1·5) | 0·743 |
| Duration of GH treatment (yrs) | 6·3 (5·3 to 8·4) | 6·3 (5·2 to 8·2) | 6·3 (5·5 to 8·8) | 0·541 |
| GH dosage (mg/m$^2$/day) | 0·7 (0·5 to 1·0) | 0·7 (0·5 to 1·0) | 0·7 (0·5 to 1·0) | 0·963 |
| Prepubertal/pubertal | 14 / 3 | 6 / 2 | 8 / 1 | |
| | Patients of 11 to 14 years | | | |
| | PWS ($n$ = 8) | Oxytocin / Placebo ($n$ = 3) | Placebo / Oxytocin ($n$ = 5) | $P$* |
| Gender | 5 boys, 3 girls | 2 boys, 1 girl | 3 boys, 2 girls | |
| Genetic subtype (DEL/mUPD) | 4 / 4 | 2 / 1 | 2 / 3 | |
| Age (yrs) | 12·5 (11·6 to 13·7) | 12·3 (11·8 to 13·0) | 12·6 (11·9 to 13·7) | 0·393 |
| Height for age (SDS) | 1·2 (0·6 to 1·6) | 1·6 (1·1 to 1·8) | 1·0 (0·2 to 1·6) | 0·571 |
| Weight for height (SDS) | 2·2 (0·6 to 4·3) | 2·3 (0·4 to 4·6) | 2·0 (0·8 to 4·3) | 1·000 |
| BMI for age (SDS) | 2·8 (0·9 to 5·6) | 3·2 (1·3 to 5·5) | 2·5 (1·2 to 5·4) | 1·000 |
| Age at start GH treatment (yrs) | 2·7 (1·8 to 4·3) | 2·2 (1·6 to 2·9) | 2·9 (2·1 to 4·9) | 0·393 |
| Duration of GH treatment (yrs) | 9·5 (8·5 to 11·1) | 9·9 (9·5 to 10·6) | 8·5 (8·2 to 11·0) | 0·393 |
| GH dosage (mg/m$^2$/day) | 1·0 (0·7 to 1·0) | 0·9 (0·8 to 1·0) | 1·0 (0·7 to 1·0) | 0·786 |
| Prepubertal/pubertal | 2 / 6 | 1 / 2 | 1 / 4 | |

*$P$-value at baseline between the two-treatment schedules.
Data expressed as median with interquartile range.

treatment and one of them also left 88 grams during placebo. During the standardized breakfast meal, the rate and duration of eating was similar during oxytocin and placebo treatment (23·8 vs 22·9 gram/min, $P$ = 0·887 and 11·6 vs 10·5 min, $P$ = 0·102) (Table 2).

The difference in weight, BMI and fat percentage between start and end (Δ) of the 4 weeks of oxytocin treatment was similar as during placebo ($P$ = 0·055, $P$ = 0·149 and $P$ = 0·136, resp.).

### *Subanalyses in the older children*

*Effects on social behaviour.* We did not find beneficial effects of oxytocin in the eight children older than 11 years (Table 2). The items happiness, anger and sadness were negatively influenced by oxytocin treatment compared to placebo ($P$ = 0·039, $P$ = 0·042 and $P$ = 0·042, resp.). The Oxytocin Study Questionnaire showed an unfavourable score of +1·5 (0·3–5) points during oxytocin treatment compared to placebo

**Table 2.** Effects on social behaviour and hyperphagia of children younger and older than 11 years

| | Patients of 6 to 11 years | | | | | | | Patients of 11 to 14 years | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Items investigated | Oxytocin phase | | | Placebo phase | | | *P** | Oxytocin phase | | | Placebo phase | | | *P** |
| Improvement social behaviour (n=) | 10 of 17 | | | 4 of 17 | | | 0·059 | 0 of 8 | | | 4 of 8 | | | **0·038** |
| Questionnaires about social behaviour | Better | Same | Worse | Better | Same | Worse | | Better | Same | Worse | Better | Same | Worse | |
| Happiness | 6 | 10 | 0 | 2 | 14 | 1 | 0·344 | 0 | 7 | 1 | 5 | 3 | 0 | **0·039** |
| Anger | 11 | 6 | 0 | 0 | 10 | 7 | **0·001** | 0 | 4 | 4 | 5 | 3 | 0 | **0·042** |
| Sadness | 9 | 8 | 0 | 0 | 12 | 5 | **0·005** | 0 | 5 | 3 | 5 | 3 | 0 | **0·042** |
| Conflicts | 7 | 9 | 0 | 1 | 12 | 4 | **0·010** | 0 | 5 | 3 | 3 | 5 | 0 | 0·068 |
| Social interaction | 2 | 15 | 0 | 3 | 12 | 2 | 0·450 | 0 | 6 | 2 | 3 | 5 | 0 | 0·066 |
| Disruptive behaviour | 4 | 13 | 0 | 1 | 13 | 3 | 0·071 | 0 | 5 | 3 | 2 | 6 | 0 | 0·109 |
| Improvement eating behaviour (n=) | 6 of 17 | | | 2 of 17 | | | 0·096 | 1 of 8 | | | 3 of 8 | | | 0·157 |
| Standardized breakfast meal | | | | | | | | | | | | | | |
| Did not finished meal completely (n=) | 3 of 17 | | | 1 of 17 | | | 0·109 | 0 of 8 | | | 0 of 8 | | | 1·000 |
| Rate of eating (gram/min) | 23·8 (15·8 to 29·5) | | | 22·9 (14·6 to 29·0) | | | 0·887 | 23·8 (14·0 to 59·6) | | | 24·5 (17·4 to 52·5) | | | 0·779 |
| Duration of eating (min) | 10·5 (8·8 to 14·5) | | | 11·6 (9·3 to 18·5) | | | 0·102 | 12·0 (4·4 to 21·1) | | | 11·6 (5·3 to 15·6) | | | 0·889 |
| Measurements | | | | | | | | | | | | | | |
| Δweight (kg) | 0·3 (−0·1 to 0·6) | | | −0·3 (−0·5 to 0·0) | | | 0·055 | 0·5 (−0·2 to 1·2) | | | 0·4 (0·0 to 0·7) | | | 0·833 |
| ΔBMI (kg/m$^2$) | −0·1 (−0·3 to 0·2) | | | −0·2 (−0·3 to 0·0) | | | 0·149 | 0·0 (−0·1 to 0·0) | | | 0·1 (−0·2 to 0·3) | | | 0·327 |
| Δfat percentage by DXA scan | −0·7 (−0·9 to–0·1) | | | −0·1 (−0·6 to 1·1) | | | 0·136 | 0·5 (−0·1 to 0·9) | | | −0·1 (−0·8 to 0·3) | | | 0·092 |
| Questionnaires about food | Better | Same | Worse | Better | Same | Worse | | Better | Same | Worse | Better | Same | Worse | |
| Food-related behaviour | 7 | 10 | 0 | 1 | 9 | 7 | **0·011** | 0 | 7 | 1 | 3 | 5 | 0 | 0·066 |
| Food-seeking behaviour | 1 | 15 | 1 | 1 | 13 | 3 | 0·713 | 0 | 7 | 1 | 0 | 8 | 0 | 0·317 |
| Satiety | 4 | 11 | 1 | 2 | 14 | 1 | 0·429 | 0 | 7 | 1 | 2 | 6 | 0 | 0·102 |
| Dykens hyperphagia | 8 | 8 | 1 | 3 | 11 | 3 | 0·285 | 0 | 7 | 1 | 3 | 5 | 0 | 0·068 |

Data expressed in number or median (IQR); **P*-value between oxytocin and placebo phase, bold values are statistically significant ($p<0.05$).

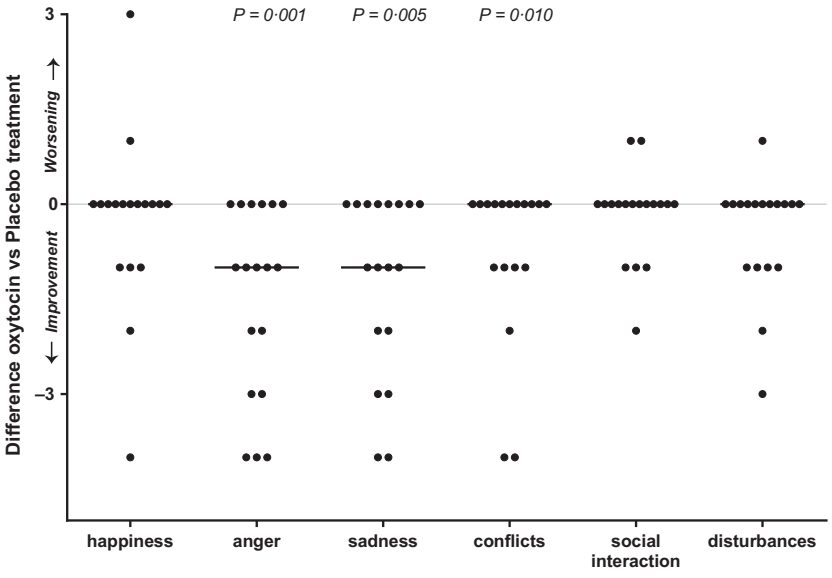


**Fig. 1** Effect of 4 weeks oxytocin *vs* placebo treatment on six items of social behaviour in children with PWS younger than 11 years of age. Individual differences between oxytocin and placebo treatment are presented as a black dot, and the black horizontal lines display the median difference. In case of a significant difference between the oxytocin and placebo phase, *P*-values are given.

($P = 0·027$). Three (37·5%) parents reported a deterioration of social behaviour during oxytocin treatment, while four (50%) parents reported an improvement during placebo treatment ($P = 0·038$).

*Effects on eating behaviour.* During oxytocin treatment, food-related behaviour, food-seeking behaviour and satiety remained similar ($P = 0·066$, $P = 0·102$ and $P = 0·317$, resp.) in the children older than 11 years. In both treatment phases, all

children finished their standardized breakfast meal, while the rate and duration of eating was similar during oxytocin and placebo treatment (23·8 vs 24·5 gram/min, $P = 0{\cdot}779$ and 12·0 vs 11·6 min, $P = 0{\cdot}889$) (Table 2). Δweight, ΔBMI and Δfat percentage were similar in the two phases (all $P > 0{\cdot}092$).

### *Associations*

There were no significant differences in oxytocin *vs* placebo effects between boys and girls, between children with a deletion or mUPD, or between children with or without serious behavioural problems. The effects of oxytocin treatment were not associated with baseline BMI or BMI SDS. In the total group, the association between age and social and eating behaviour during oxytocin treatment was stronger than the association between pubertal stage and these outcomes ($\rho = -0{\cdot}553$, $P = 0{\cdot}004$ and $\rho = -0{\cdot}485$, $P = 0{\cdot}014$ *vs* $\rho = -0{\cdot}452$, $P = 0{\cdot}023$ and $\rho = -0{\cdot}396$, $P = 0{\cdot}050$, resp.).

### *Oxytocin levels in blood*

Serum oxytocin levels before and during study were determined to further unravel the different effects in younger and older children (Fig. 2). At baseline, children younger than 11 years had a median fasting oxytocin level of 3156 (1864–5325) pg/ml, and 12–14 h after the last oxytocin dose after 4 weeks of treatment it was 4685 (2809–9490) pg/ml ($P = 0{\cdot}134$). Children older than 11 years had a baseline fasting oxytocin level of 2692 (1737–3754) pg/ml, and 4750 (1976–7831) pg/ml after oxytocin treatment ($P = 0{\cdot}327$).

In the younger children, lower oxytocin levels after 4 weeks of oxytocin treatment were associated with positive effects on social behaviour ($\rho = -0{\cdot}540$, $P = 0{\cdot}027$). This association was not found in older children. Baseline oxytocin levels or change in oxytocin levels during treatment was not associated with positive effects.

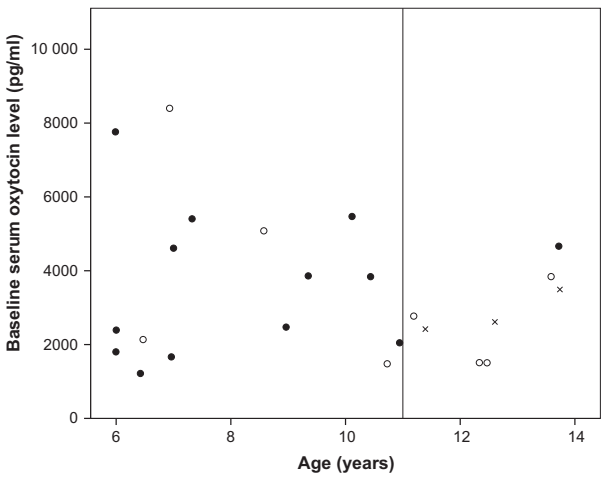


**Fig. 2** Fasting serum oxytocin levels at baseline in pg/ml per age. ● represents a patient with positive effect of oxytocin treatment. ○ represents a patient without effect of oxytocin treatment. x represents a patient with negative effect of oxytocin treatment. The grey line indicates the age of 11 years.

Only one patient older than 11 years had benefit from oxytocin treatment. Remarkably, this patient had the highest baseline oxytocin level of patients older than 11 years, which decreased considerably to the lowest level of 1126 pg/ml during oxytocin treatment (Fig. 2). The other older patients without benefit had lower baseline oxytocin levels (Fig. 2), which increased or only slightly decreased during oxytocin treatment.

### *Dosing*

The oxytocin/placebo dose was based on body surface (see Methods). In the total group, median dose was 16 IU (12–20) twice daily, which was 12·3 (12·0–13·3) IU/m$^2$ and 0·39 (0·34–0·44) per kilogram body weight (IU/kg). Given the different effects of oxytocin in the younger and older group of patients, the correlation between dosage and age was investigated. The dose in IU/m$^2$ did not correlate with age, but the given dose recalculated as IU/kg correlated inversely with age ($\rho = -0{\cdot}663$, $P < 0{\cdot}001$), meaning that older patients received a lower dose when recalculated in IU/kg (Fig. 3). The median dose was 0·42 (0·37–0·45) IU/kg in the children younger than 11 years and 0·34 (0·30–0·37) IU/kg in the older children ($P = 0{\cdot}002$). Remarkably, the only patient older than 11 years, who had benefit from oxytocin treatment, had one of the lowest recalculated doses in IU/kg of the older patients and the lowest dose in IU/kg of all patients with beneficial effects of oxytocin.

### *Safety parameters*

The intranasal administration of oxytocin was very well tolerated, and there were no side effects. Renal function, hepatic

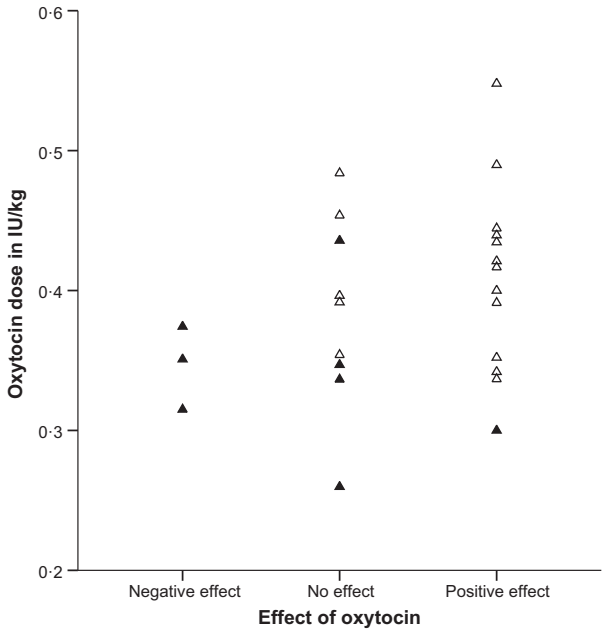


**Fig. 3** Dose of oxytocin recalculated as IU/kg *vs* effect of oxytocin on social and/or eating behaviour. Δ represents a patient younger than 11 years. ▲ represents a patient older than 11 years.

enzymes, thyroid function and glucose remained stable and normal for all patients, as did the systolic blood pressure. Diastolic blood pressure was lower during oxytocin treatment (median 64 vs 73 mmHg, $P = 0{\cdot}008$), but within normal limits. Similar results were found in patients younger or older than 11 years.

## Discussion

Our randomized, double-blind, placebo-controlled, crossover study is the first oxytocin study in children with PWS aged between 6 and 14 years. Although there were no effects in the group as a whole, subanalyses demonstrated that children with PWS between 6 and 11 years had beneficial effects of intranasal oxytocin administration on social behaviour and hyperphagia. Their parents reported significantly less anger, sadness, conflicts and food-related behaviour, and improvement of social behaviour during oxytocin treatment compared with placebo. In children with PWS, older than 11 years were the beneficial effects of oxytocin on social behaviour and hyperphagia not found. We did not find side effects or adverse events.

Until now, there have been no effective treatment options for behaviour and food-related problems in PWS. Our study suggests that intranasal oxytocin administration is a novel and promising treatment for young children with PWS. Children with PWS have a specific behavioural phenotype with stubbornness, temper tantrums, manipulative and controlling behaviour, obsessive–compulsive features and difficulties in changing routines.[1–3] The social behavioural problems and hyperphagia seriously affect the quality of life of the children and their parents and caretakers.

Nowadays, most parents of children with PWS have made all kind of adjustments in everyday life to limit access to food, such as locks on the fridge.[24] This explains why, prior to the study, children had a low prevalence of food-seeking behaviour. It is therefore not surprising that we found no effects of oxytocin on food-seeking behaviour. However, the baseline food-related behaviour scores show that these children are still pre-occupied with food, characterized by talking about food, asking for food, playing that they are cooking, etc., despite all the adjustments to control hyperphagia. Oxytocin treatment decreased this food-related behaviour, which argues that oxytocin has an inhibiting effect on the hyperphagia, despite the lack of effects on food-seeking behaviour and satiety. Studies on the long-term effects of 4 weeks treatment and also long-term oxytocin treatment trials are warranted to confirm our findings on efficacy and safety.

In contrast to the beneficial effects of oxytocin in the younger children, no positive effects were found in children with PWS older than 11 years. Some parents of the older subgroup even reported negative effects of oxytocin on social behaviour, especially regarding happiness, anger and sadness, in contrast to none of the parents of younger children. These findings are in line with the results of an 8-week oxytocin trial in 22 individuals with PWS between 12 and 29 years,[20] in which no benefits in target behaviours or weight were found. The only significant difference found in that study was an increase in temper outbursts when the oxytocin dose was increased. Tauber *et al.* reported positive effects of oxytocin on social behaviour in adults with PWS, but comparison is difficult because they investigated a single dose of intranasal administration.[19]

We measured plasma oxytocin levels prior and during the oxytocin trial. At baseline, oxytocin levels in children with PWS younger than 11 years showed high interindividual variability, in line with findings in children with PWS of similar age by Johnson *et al.*,[7] and there was no relation between baseline oxytocin levels and positive effects during treatment. In contrast, a lower oxytocin level after 4 weeks of oxytocin treatment was associated with stronger positive effects on social behaviour, suggesting that it is beneficial for young children to have a lower plasma oxytocin level during oxytocin treatment. Those older than 11 years had, however, lower and less widespread baseline oxytocin levels and their levels increased during oxytocin treatment. The only older boy with beneficial effects of oxytocin treatment had a declining oxytocin level during treatment, like most of the younger children.

Why did the oxytocin treatment work in the younger, but not in the older children? One explanation could be that mistakes had been made in the preparation or delivery of the intranasal sprays in the older children. For that reason, an independent laboratory measured the content of the vials and was able to reject that explanation. Second, it could be that the sample size of the older subgroup was too small to show significant changes, but that argument is unlikely as several significant negative effects of oxytocin administration were found in the older subgroup. Third, there could have been a dosing issue. We calculated the oxytocin doses according to body surface, a common way of hormone dosing in children, which resulted in a relatively lower dose in IU/kg in children older than 11 years. However, an inappropriately low dose is also an unlikely explanation, as the only older boy with positive effects of oxytocin had the second lowest dose of all children in IU/kg. Besides, Einfeld *et al* reported adverse effects of higher oxytocin doses in older patients.[20] A fourth explanation could be that the behaviour and coping style of older patients with PWS are more embedded in their personality and are therefore not easy to change. A treatment period of 4 weeks is not short, but it could be that a longer period than four or 8 weeks of oxytocin treatment might be needed to induce beneficial effects in older children.

Another, more pathophysiological explanation might be that older children with PWS have developed an unresponsive oxytocin system over the years, with a lower number of oxytocin receptors and neurons in the hypothalamus. Adults with PWS have a 42% decrease in oxytocin neurons in the hypothalamus and relatively low plasma levels of oxytocin in relation to their obesity.[5,6] One of the nonexpressed genes in the PWS region on chromosome 15 is MAGEL2. This gene is known to be expressed in mouse hypothalamus during development and their knockout alters the number and/or function of oxytocin neurons.[25] MAGEL2–knockout pups were not hypotonic, but had an altered onset of suckling activity resulting in impaired feeding and 50% mortality,[4] while the survivors had deficits in social recognition and social interaction on the long term.[26] These suckling problems in infancy and social problems later on are similar to children with PWS, suggesting that this gene might play a role in the

suckling and behavioural problems in PWS. Adult MAGEL2–knockout mice expressed a significantly reduced number of oxytocin receptors in several regions of the brain.[26] Children with PWS, who are MAGEL2 deficient, could therefore be less or non-responsive to oxytocin treatment when they become older, because their oxytocin system deteriorated over time. In contrast, it was shown that daily administration of oxytocin in the first postnatal week prevents the deficits in social behaviour in the adult mutant mice and partly restore a normal oxytocin system in the brain. This suggests that the postnatal period is a critical period for the oxytocin system in which social behaviour is programmed. Nevertheless, our study shows that oxytocin treatment has beneficial effects in children with PWS until the age of approximately 11 years, thus also beyond the postnatal period.

It is remarkable that the changeover in oxytocin effects occurred around the age of 11 years, the time of puberty onset. We previously demonstrated that GH-treated children with PWS have a normal age at onset of puberty, but that the majority shows a deterioration in pubertal development after Tanner stage 2–3 with a decline in gonadal function in boys.[27–29] It might be that the reactivation of the GnRH axis just before the onset of puberty together with the lack of expression of MAGEL2 and other yet unknown genes, not only result in a rapid gonadal failure after the onset of puberty but also in an enhanced deterioration of the oxytocin system in patients with PWS. We acknowledge that our supposition is very hypothetical, but we consider it noteworthy to mention that these processes seem to occur in the same timeframes. Studies are warranted to further unravel the pathophysiology and to determine whether others also find the 11-year cut-off for benefit of oxytocin treatment.

Present study was a placebo-controlled study in which we calculated the dose according to body surface, based on doses used in other trials.[12,20] We did not perform a dose-finding study, and no washout period was included to limit the number of hospital visits. We found no differences in food intake during the breakfast meal test between the oxytocin and placebo phase. This might be due to the lack of a satiety level in PWS or that their satiety level was much higher than the maximum amount of food that we offered,[30] but we considered it unethical to present an unlimited amount of food as patients with PWS feel never satiated and have an increased risk of gastric rupture.

In conclusion, administration of intranasal oxytocin appears to have beneficial effects on social behaviour and food-related behaviour in children with PWS younger than 11 years of age without side effects or adverse events. Parents reported significantly less anger, sadness, conflicts and food-related behaviour, and improvement of social behaviour during oxytocin treatment compared with placebo. In contrast to the younger children with PWS, those older than 11 years of age did not benefit from oxytocin treatment.

## Author contributions

RJK substantially contributed to conception and design of study, acquisition of data, analysis and interpretation of data and drafting the manuscript. SHD substantially contributed to acquisition of data, interpretation of data and critical revision of the manuscript. AHK substantially contributed to conception and design of study, analysis and interpretation of data and critical revisions of the manuscript.

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## Declaration of interest and Financial disclosure

The authors have nothing to disclose.

## Key points

- Prader–Willi syndrome (PWS) is known for hyperphagia with impaired satiety and a specific behavioural phenotype with stubbornness, manipulative and controlling behaviour and obsessive–compulsive features. PWS is associated with hypothalamic and oxytocinergic dysfunction.
- In humans without PWS, intranasal oxytocin administration had positive effects on social and eating behaviour, as well as weight balance.
- This randomized, double-blind, placebo-controlled, cross-over study demonstrates that intranasal oxytocin has beneficial effects on social behaviour and food-related behaviour in children with PWS younger than 11 years of age, but not in those older than 11 years of age.
- Parents of those younger than 11 years reported significantly less anger, sadness, conflicts and food-related behaviour, and improvement of social behaviour during oxytocin treatment compared with placebo.

## References

1 Goldstone, A.P., Holland, A.J., Hauffa, B.P. *et al.* (2008) Recommendations for the diagnosis and management of Prader-Willi syndrome. *Journal of Clinical Endocrinology and Metabolism*, **93**, 4183–4197.

2 Lo, S.T., Siemensma, E.P., Festen, D.A. *et al.* (2015) Behavior in children with Prader-Willi syndrome before and during growth hormone treatment: a randomized controlled trial and 8-year

longitudinal study. *European Child and Adolescent Psychiatry*, **24**, 1091–1101.
3 Holm, V.A., Cassidy, S.B., Butler, M.G. *et al.* (1993) Prader-Willi syndrome: consensus diagnostic criteria. *Pediatrics*, **91**, 398–402.
4 Schaller, F., Watrin, F., Sturny, R. *et al.* (2010) A single postnatal injection of oxytocin rescues the lethal feeding behaviour in mouse newborns deficient for the imprinted Magel2 gene. *Human Molecular Genetics*, **19**, 4895–4905.
5 Swaab, D.F. (1997) Prader-Willi syndrome and the hypothalamus. *Acta Paediatrica. Supplement*, **423**, 50–54.
6 Hoybye, C., Barkeling, B., Espelund, U. *et al.* (2003) Peptides associated with hyperphagia in adults with Prader-Willi syndrome before and during GH treatment. *Growth Hormone & IGF Research*, **13**, 322–327.
7 Johnson, L., Manzardo, A.M., Miller, J.L. *et al.* (2015) Elevated plasma oxytocin levels in children with Prader-Willi syndrome compared with healthy unrelated siblings. *American Journal of Medical Genetics. Part A*, **170**, 2097–2102.
8 Ott, V., Finlayson, G., Lehnert, H. *et al.* (2013) Oxytocin reduces reward-driven food intake in humans. *Diabetes*, **62**, 3418–3425.
9 Lawson, E.A., Marengi, D.A., DeSanti, R.L. *et al.* (2015) Oxytocin reduces caloric intake in men. *Obesity*, **23**, 950–956.
10 Zhang, H., Wu, C., Chen, Q. *et al.* (2013) Treatment of obesity and diabetes using oxytocin or analogs in patients and mouse models. *PLoS One*, **8**, e61477.
11 Zhang, G. & Cai, D. (2011) Circadian intervention of obesity development via resting-stage feeding manipulation or oxytocin treatment. *American Journal of Physiology. Endocrinology and Metabolism*, **301**, E1004–E1012.
12 Macdonald, K. & Macdonald, T.M. (2010) The peptide that binds: a systematic review of oxytocin and its prosocial effects in humans. *Harvard Review of Psychiatry*, **18**, 1–21.
13 Lo, S.T., Siemensma, E., Collin, P. *et al.* (2013) Impaired theory of mind and symptoms of Autism Spectrum Disorder in children with Prader-Willi syndrome. *Research in Developmental Disabilities*, **34**, 2764–2773.
14 Festen, D.A., Wevers, M., de Weerd, A.W. *et al.* (2008) Cognition and behavior in pre-pubertal children with Prader-Willi syndrome and associations with sleep-related breathing disorders. *American Journal of Medical Genetics. Part A*, **146A**, 3018–3025.
15 Siemensma, E.P., Tummers-de Lind van Wijngaarden, R.F., Festen, D.A. *et al.* (2012) Beneficial effects of growth hormone treatment on cognition in children with Prader-Willi syndrome: a randomized controlled trial and longitudinal study. *Journal of Clinical Endocrinology and Metabolism*, **97**, 2307–2314.
16 Domes, G., Heinrichs, M., Michel, A. *et al.* (2007) Oxytocin improves "mind-reading" in humans. *Biological Psychiatry*, **61**, 731–733.
17 Hollander, E., Bartz, J., Chaplin, W. *et al.* (2007) Oxytocin increases retention of social cognition in autism. *Biological Psychiatry*, **61**, 498–503.
18 Hollander, E., Novotny, S., Hanratty, M. *et al.* (2003) Oxytocin infusion reduces repetitive behaviors in adults with autistic and Asperger's disorders. *Neuropsychopharmacology*, **28**, 193–198.
19 Tauber, M., Mantoulan, C., Copet, P. *et al.* (2011) Oxytocin may be useful to increase trust in others and decrease disruptive behaviours in patients with Prader-Willi syndrome: a randomised placebo-controlled trial in 24 patients. *Orphanet Journal of Rare Diseases*, **6**, 47.
20 Einfeld, S.L., Smith, E., McGregor, I.S. *et al.* (2014) A double-blind randomized controlled trial of oxytocin nasal spray in Prader Willi syndrome. *American Journal of Medical Genetics. Part A*, **164A**, 2232–2239.
21 Schonbeck, Y., Talma, H., van Dommelen, P. *et al.* (2013) The world's tallest nation has stopped growing taller: the height of Dutch children from 1955 to 2009. *Pediatric Research*, **73**, 371–377.
22 Schonbeck, Y., Talma, H., van Dommelen, P. *et al.* (2011) Increase in prevalence of overweight in Dutch children and adolescents: a comparison of nationwide growth studies in 1980, 1997 and 2009. *PLoS One*, **6**, e27608.
23 Dykens, E.M., Maxwell, M.A., Pantino, E. *et al.* (2007) Assessment of hyperphagia in Prader-Willi syndrome. *Obesity*, **15**, 1816–1826.
24 Ho, A.Y. & Dimitropoulos, A. (2010) Clinical management of behavioral characteristics of Prader-Willi syndrome. *Neuropsychiatric Disease and Treatment*, **6**, 107–118.
25 Grinevich, V., Desarmenien, M.G., Chini, B. *et al.* (2014) Ontogenesis of oxytocin pathways in the mammalian brain: late maturation and psychosocial disorders. *Frontiers in Neuroanatomy*, **8**, 164.
26 Meziane, H., Schaller, F., Bauer, S. *et al.* (2015) An Early postnatal oxytocin treatment prevents social and learning deficits in adult mice deficient for Magel2, a gene involved in Prader-Willi syndrome and autism. *Biological Psychiatry*, **78**, 85–94.
27 Siemensma, E.P., de Lind van Wijngaarden, R.F., Otten, B.J. *et al.* (2012) Testicular failure in boys with Prader-Willi syndrome: longitudinal studies of reproductive hormones. *Journal of Clinical Endocrinology and Metabolism* **97**, E452–E459.
28 Siemensma, E.P., de Lind van Wijngaarden, R.F., Otten, B.J. *et al.* (2011) Pubarche and Serum Dehydroepiandrosterone Sulfate Levels in Children with Prader-Willi Syndrome. *Clinical Endocrinology*, **75**, 83–89.
29 Bakker, N.E., Wolffenbuttel, K.P., Looijenga, L.H. *et al.* (2015) Testes in infants with Prader-Willi syndrome: human chorionic gonadotropin treatment, surgery and histology. *Journal of Urology*, **193**, 291–298.
30 Lindgren, A.C., Barkeling, B., Hagg, A. *et al.* (2000) Eating behavior in Prader-Willi syndrome, normal weight, and obese control groups. *Journal of Pediatrics*, **137**, 50–55.