

## ORIGINAL ARTICLE

## Oxytocin's inhibitory effect on food intake is stronger in obese than normal-weight men

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**BACKGROUND/OBJECTIVES:** Animal studies and pilot experiments in men indicate that the hypothalamic neuropeptide oxytocin limits food intake, and raise the question of its potential to improve metabolic control in obesity.

**SUBJECTS/METHODS:** We compared the effect of central nervous oxytocin administration (24 IU) via the intranasal route on ingestive behaviour and metabolic function in 18 young obese men with the results in a group of 20 normal-weight men. In double-blind, placebo-controlled experiments, *ad libitum* food intake from a test buffet was examined in fasted subjects 45 min after oxytocin administration, followed by the assessment of postprandial, reward-driven snack intake. Energy expenditure was repeatedly assessed by indirect calorimetry and blood was sampled to determine concentrations of blood glucose and hormones.

**RESULTS:** Oxytocin markedly reduced hunger-driven food intake in the fasted state in obese but not in normal-weight men, and led to a reduction in snack consumption in both groups, whereas energy expenditure remained generally unaffected.

Hypothalamic–pituitary–adrenal axis secretion and the postprandial rise in plasma glucose were blunted by oxytocin in both groups.

**CONCLUSIONS:** Oxytocin exerts an acutely inhibitory impact on food intake that is enhanced rather than decreased in obese compared with normal-weight men. This pattern puts it in contrast to other metabolically active neuropeptides and bodes well for clinical applications of oxytocin in the treatment of metabolic disorders.

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## INTRODUCTION

Oxytocin, a hypothalamic nonapeptide which is released into the circulation through the posterior pituitary and also directly acts on central nervous receptors, regulates reproductive functions like mother–infant interaction and lactation and is a potent modulator of social behaviours including attachment and sexual behaviour.<sup>1</sup> Studies in animals and pilot experiments in humans have indicated that oxytocin moreover might have a role in the regulation of eating behaviour and metabolism. In rodents<sup>2</sup> and rhesus monkeys<sup>3</sup> with normal weight, but also with diet-induced obesity, subcutaneous oxytocin administration inhibits food intake, increases energy expenditure and reduces glucose levels. Interestingly, experiments in rodents suggest that the metabolic effects of oxytocin may be even enhanced in diet-induced obese in comparison to control animals.<sup>4</sup> In studies in humans, intranasal oxytocin attenuated reward-driven snack intake and decreased postprandial glucose concentrations in normal-weight men.<sup>5</sup> Against this background, and considering that available weight loss treatments are of modest or temporary efficacy,<sup>6,7</sup> the intranasal administration of oxytocin to the human brain, which in a broad range of studies has been demonstrated to be associated with absent or minimal side effects,<sup>8</sup> might be a promising pharmacological intervention in obesity.<sup>9–11</sup>

In the present study, we investigated the impact of oxytocin on eating behaviour in obese men and compared the results with the effects obtained in normal-weight men.<sup>5</sup> As ingestive behavior is

homeostatically regulated in response to energy depletion as well as by brain circuits processing the reward-related, 'hedonic' qualities of food intake,<sup>12,13</sup> we applied a validated paradigm that includes a breakfast buffet presented to fasted participants,<sup>14,15</sup> as well as the delayed assessment of food intake from snacks.<sup>16,17</sup> In addition, energy expenditure, glucose homeostasis and hypothalamic–pituitary–adrenal (HPA) axis secretory activity were measured before and after oxytocin administration as well as after breakfast intake.

## MATERIALS AND METHODS

## Subjects

Eighteen healthy obese men were recruited and their results compared with the findings in 20 normal-weight men, which were obtained with an identical experimental set-up and are described in detail in Ott *et al* (reprinted with permission from ref. 5; copyright 2013 American Diabetes Association). Subjects were recruited via mailing lists and advertisements in local newspapers. Relevant illness was excluded by medical history and clinical examination. Habitual eating behaviour of obese subjects was assessed via a lifestyle questionnaire on dietary restraint and tendency toward disinhibition. Dietary restraint, that is, the conscious effort to restrict calorie intake to control body weight, was assessed using the restraint scale of the Dutch Eating Behaviour Questionnaire (DEBQ-R).<sup>18</sup> Tendency toward disinhibition was assessed with the disinhibition scale of the Three Factor Eating Questionnaire (TFEQ).<sup>19</sup> On average, participants achieved a DEBQ-R score ( $\pm$ s.e.m.) of  $2.55 \pm 0.13$  and a TFEQ score of

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**Table 1.** Subject characteristics

Variable	Obese men (mean $\pm$ s.e.m.)	Normal-weight men (mean $\pm$ s.e.m.)	P-value
Age (years)	27.83 $\pm$ 1.38	26.30 $\pm$ 0.89	0.35
Body weight (kg)	106.39 $\pm$ 2.25	74.81 $\pm$ 1.89	< 0.001
BMI (kg m <sup>-2</sup> )	32.10 $\pm$ 0.36	22.66 $\pm$ 0.36	< 0.001
Lean body mass (kg)	79.96 $\pm$ 1.56	61.10 $\pm$ 1.40	< 0.001
Body fat mass (kg)	26.54 $\pm$ 1.28	13.78 $\pm$ 0.84	< 0.001
Body fat mass (% of total weight)	24.80 $\pm$ 0.92	18.19 $\pm$ 0.87	< 0.001
Body cell mass (% of lean body mass)	56.37 $\pm$ 0.54	54.94 $\pm$ 0.60	0.09
Body water (liter)	58.53 $\pm$ 1.14	44.72 $\pm$ 1.02	< 0.001
Phase angle (°)	7.00 $\pm$ 0.13	6.69 $\pm$ 0.13	0.10
PFS (total scale score)	2.71 $\pm$ 0.13	2.42 $\pm$ 0.14	0.13

Mean  $\pm$  s.e.m. values are indicated. Body composition was measured by bioelectrical impedance analysis (Nutriguard-M, Data Input, Germany) in a clinical examination taking place shortly before the first experimental session. PFS, total scale score of the Power of Food Scale (PFS), a measure of propensity to consume palatable foods, assessed before substance administration and averaged across conditions. Obese men,  $n = 18$ ; normal-weight men,  $n = 20$ .

6.53  $\pm$  0.80. The propensity to consume palatable foods was measured at three levels of food proximity (food available, food present and food tasted) with the Power of Food Scale (PFS)<sup>20</sup> at each session (see Table 1 for subject characteristics). Subjects were kept unaware of hypothesized treatment effects on food intake and were informed that the experiments concerned the effect of oxytocin on mood, taste preferences and energy expenditure. Participants gave written informed consent to the study that conformed to the Declaration of Helsinki and was approved by the local ethics committee.

### Experimental procedure

Experiments were carried out in a balanced, double-blind, cross-over, within-subject comparison. Each subject participated in two experimental sessions (oxytocin and placebo) spaced at least 10 days apart. Participants were instructed to abstain from the intake of food and caffeinated and alcoholic beverages after 2000 hours on the day preceding each session.

After the subject's arrival at the laboratory at ~0800 hours, a venous cannula was inserted into the subject's non-dominant arm to enable drawing of venous blood (see Figure 1a for the experimental procedure). Thereafter, blood was sampled for baseline assessments of hormonal parameters. At 0942 hours each subject was intranasally administered 24 IU oxytocin (0.6 ml Syntocinon; Defiante Farmacêutica, Funchal/Madeira, Portugal) or placebo (0.6 ml vehicle containing all Syntocinon ingredients except for the peptide) at six individual 0.1 ml puffs (three per alternating nostril) with 30-s intervals in-between. Forty-five minutes after administration, subjects were presented with a free-choice *ad libitum* breakfast buffet from 1030 to 1100 hours. Casual snack intake was assessed 95 min later under the pretext of a snack taste test. Olfactory function was tested at 1200 hours. Feelings of hunger, mood, subjective well-being and the perceived experimenter's trustworthiness were repeatedly rated on visual analog scales (0–100) and energy expenditure was assessed by indirect calorimetry before and after substance administration and after the test breakfast. Heart rate and blood pressure were monitored. At the end of the experiments, subjects were asked to indicate their account of the study purpose and if they thought to have received oxytocin or placebo.

### Assessments of food intake

The breakfast buffet comprised a collection of bread and rolls, spreads (for example, jam, honey), sausages, cheese, fruits, puddings, fruits, milk and juice, which totaled 4562 kcal (see Table 2 for details). Subjects were left undisturbed during breakfast and were not aware that their food intake was measured by weighing buffet components before and after. This procedure enables the precise assessment of primarily hunger-driven food intake in the fasted state.<sup>5,14,15</sup> Reward-related eating in the relative absence of hunger was assessed under the pretext of a taste test<sup>5,16,17</sup> that included snacks of three different types but comparable calorie content and macronutrient composition, that is, 'TUC Cracker Classic' (salty taste; Griesson-de Beukelaer, Polch, Germany; 500 kcal per 100 g; 57.2 g carbohydrates per 100 g), 'Rice Waffles' (bland taste; Continental Bakeries B.V., Dordrecht, The Netherlands; 390 kcal per 100 g; 63 g carbohydrates per 100 g), and 'Double Chocolate Cookies' (sweet taste; EDEKA, Hamburg,

Germany; 486 kcal per 100 g; 63 g carbohydrates per 100 g). For each variety, 15 snacks broken into bite size pieces were provided on a separate plate, and labeled snack A, B and C, respectively, allowing for a considerable amount to be eaten without the plates appearing empty, to ensure that participants would not restrict snack intake based on whether the experimenter could see how much had been consumed. The participant was instructed to taste and rate each snack type on a visual analog scale anchored by 0 (not at all) and 100 mm (very palatable/sweet/salty). Subjects were informed that during and after completion of the rating they could eat as many snacks as they liked because remaining food would be discarded, and were left alone for 10 min. Intake was covertly measured by weighing the snacks before and after the test.

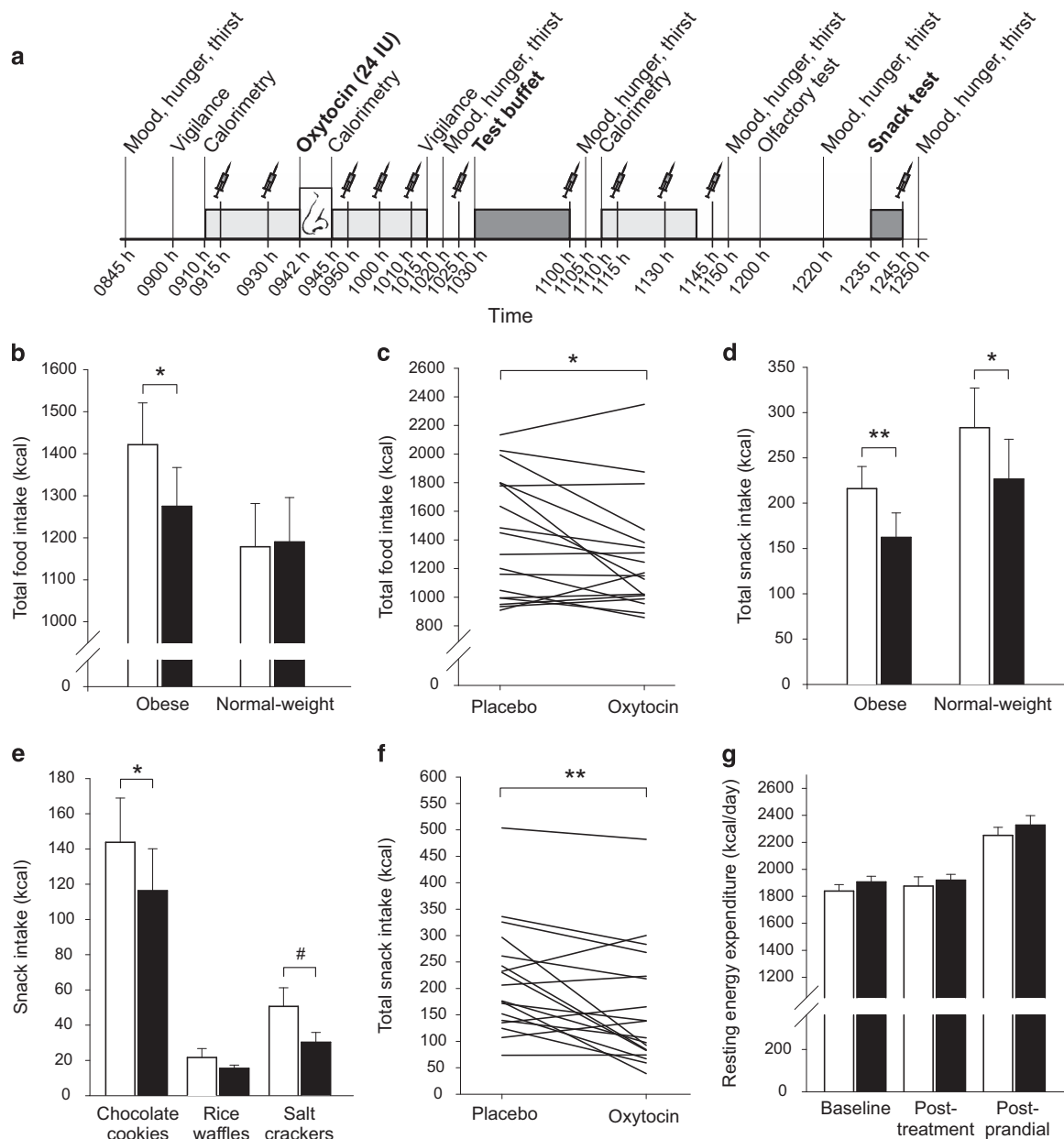
### Measurement of hunger, thirst, mood, vigilance and olfactory function

Hunger, thirst and the perceived trustworthiness of the experimenter were rated on visual analog scale (0–100 mm). Self-reported mood was assessed on 5-point scales covering the categories good/bad mood, alertness/sleepiness and calmness/agitation (MDBF).<sup>21</sup> In the 5-min personal computer-based vigilance task,<sup>22</sup> a red dot appears at random intervals either on the left or the right side of the screen, and subjects are required to press the respective key as fast as possible, receiving immediate feedback in the form of the reaction time for correct responses or an error message. Mean reaction time was registered and adjusted for mistakes by adding the square root of the product of the mean reaction time and the number of mistakes. Olfactory function was tested 60 min after the test buffet with the validated Sniffin' Sticks commercial test kit (Burghart Elektro- und Feinmechanik GmbH, Wedel, Germany) that covers the three dimensions of olfactory threshold, discrimination and identification.<sup>23</sup>

### Measurement of energy expenditure, plasma glucose and hormonal parameters

Resting energy expenditure (expressed as kcal per day) and the respiratory quotient were measured via indirect calorimetry using a DeltaTrac II ventilated-hood system (SensorMedics Vmax 29n; VIASYS Healthcare, Yorba Linda, CA, USA). Before each use, the device was calibrated with calibration gas to 5% CO<sub>2</sub> and 95% O<sub>2</sub>. Calorimetric measurements (30 min each) took place at 0910 hours (baseline), immediately after intranasal substance administration at 0945 hours to assess effects of intranasal oxytocin alone, and again at 1110 hours (that is, after the *ad libitum* test buffet) to register postprandial energy expenditure. Substrate utilization in the obese participants was calculated based on the following stoichiometric equations: carbohydrate oxidation (g per min) = 4.21  $\times$  VCO<sub>2</sub> – 2.962  $\times$  VO<sub>2</sub> – 0.4  $\times$  n; fat oxidation (g per min) = 1.695  $\times$  VO<sub>2</sub> – 1.701  $\times$  VCO<sub>2</sub> – 1.77  $\times$  n where n represents nitrogen excretion from protein oxidation (estimated at 135  $\mu$ g  $\times$  kg  $\times$  min<sup>-1</sup>).<sup>24</sup>

Blood samples obtained in the obese participants to assess serum insulin, C-peptide, cortisol and growth hormone, as well as plasma glucose, lactate, adrenocorticotrophic hormone and non-esterified fatty acids (NEFA) were centrifuged, and samples were stored at –80 °C. Concentrations of glucose and lactate in fluoride plasma were determined with the ADVIA Chemistry XPT clinical chemistry analyzer according to the hexokinase and



**Figure 1.** Experimental procedure and food intake results. **(a)** After baseline assessments of blood parameters, psychological variables, vigilance and energy expenditure, subjects were intranasally administered oxytocin (24 IU) and placebo, respectively, at 0942 hours (nose symbol). At 1030 hours, 45 min after substance administration, subjects were allowed to eat *ad libitum* from a free-choice test buffet for 30 min. About 60 min after termination of the buffet, at 1200 hours, olfactory function was assessed, and at 1235 hours, 95 min after the end of the buffet, snack intake was measured under the pretext of a taste-rating task. Throughout the sessions, mood, hunger, thirst and vigilance were assessed, and blood samples were taken (syringe symbols). Central panels indicate mean  $\pm$  s.e.m. total intake (kcal) from the test breakfast after placebo (white bars) or oxytocin (black bars) administration in obese ( $n=18$ ) and normal-weight men ( $n=20$ ) **(b)** and individual total food intake with values of both sessions connected by lines in obese men **(c)**, as well as total snack intake in obese and normal-weight men **(d)** (Data of normal-weight men reprinted with permission from ref. 5; copyright 2013 American Diabetes Association.). Lower panels depict, in the group of obese men, snack intake (kcal) according to snack categories **(e)**, individual total snack intake with individual values of both sessions connected by lines **(f)**, as well as resting energy expenditure during the baseline (0910–0940 hours), post-treatment (0945–1015 hours) and postprandial periods (1110–1140 hours) **(g)**.  $^{\#}P < 0.08$ ,  $^{*}P < 0.05$ ,  $^{**}P < 0.01$  for comparisons between conditions (paired, two-sided *t*-tests).

colorimetric lactoxidase method, respectively. Serum insulin, C-peptide and cortisol concentrations were measured with the ADVIA Centaur XPT immunology analyzer and concentrations of growth hormone and adrenocorticotrophic hormone were measured using the Immulite 2000 XPI Immunoassay-System. Plasma NEFA were determined according to the ACS-ACOD method (NEFA-HR(2), Wako Chemicals GmbH, Neuss, Germany) with the ADVIA Chemistry XPT clinical chemistry analyzer (all instruments from Siemens Healthcare Diagnostics, Eschborn, Germany).

#### Statistical analysis

Analyses were generally based on analyses of variance (ANOVA) with the between-subject factor 'group' (obese/normal weight) and the within-subject factors 'treatment' (oxytocin/placebo), 'time', 'nutrient' (carbohydrates/protein/fat), 'food type' (savory/sweet/neutral), 'snack type' (salty/sweet/bland) and 'taste' (salty/sweet) as appropriate. Note that comparisons between groups focused on the main parameters of energy intake and expenditure; for detailed results of normal-weight subjects see

**Table 2.** Composition of the test buffet

Food	Weight (g)	Energy (kcal)	Carbohydrate (g)	Fat (g)	Protein (g)
<i>Neutral</i>					
Whole wheat bread	165	360	71	2.3	12
Wheat rolls	240	275	122.4	3.4	6.3
White bread	30	72	14.6	0.4	2.2
Butter	120	928	0.7	99.8	0.8
Whole milk	750	491	36	26.3	24.8
<i>Sweet</i>					
Strawberry jam	50	147	35.8	0.1	0.1
Hazelnut spread	40	218	21.6	12.8	2.8
Honey	40	123	30	0	0.1
Sugar	24	98	24	0	0
Fruit curd	125	140	19.3	3.3	7.7
Vanilla pudding	125	134	20.8	3.8	3.5
Strawberry milk	200	167	18.2	6.8	7.4
Banana	179	168	38.3	0.4	2
Apple	195	104	22.2	1.2	0.6
Pear	140	78	17.4	0.4	0.7
Orange	180	72	15	0.4	1.8
Tangerine	80	35	8.2	0	0.5
Orange juice	400	173	36	1	4
<i>Savoury</i>					
Poultry sausage	40	74	0.1	4.3	8.3
Cervelat sausage	34	120	0.1	10.2	6.1
Sliced cheese	100	374	0	29.2	25.5
Cream cheese (natural)	33	87	0.6	7.8	3
Cream cheese (herbs)	40	124	1	11.6	3.2
Total	3330	4562	553	226	123

All values are rounded to the closest decimal.

**Table 3.** Calorie intake during the test buffet and snack test

Food type	Obese men			Normal-weight men			P-value (group × treatment)
	Placebo	Oxytocin	P-value	Placebo	Oxytocin	P-value	
Eating assessment							
Breakfast (kcal)							
Total	1421 ± 99	1274 ± 92	0.035	1180 ± 103	1190 ± 105	0.84	0.07
Carbohydrates	736 ± 51	659 ± 57	0.07	517 ± 41	540 ± 41	0.43	0.042
Fat	478 ± 54	430 ± 44	0.15	517 ± 54	509 ± 57	0.84	0.43
Protein	207 ± 15	187 ± 13	0.08	145 ± 16	142 ± 14	0.82	0.26
Neutral foods	726 ± 68	681 ± 74	0.26	519 ± 62	544 ± 68	0.42	0.16
Sweet foods	409 ± 53	375 ± 48	0.46	346 ± 40	336 ± 42	0.64	0.64
Savoury foods	287 ± 45	219 ± 31	0.040	314 ± 38	309 ± 29	0.91	0.19
Snack test (kcal)							
Total	216 ± 24	162 ± 27	0.006	283 ± 44	227 ± 44	0.03	0.95
Chocolate cookies	144 ± 25	116 ± 24	0.030	185 ± 41	138 ± 38	0.007	0.34
Rice waffles	22 ± 5	16 ± 2	0.26	18 ± 3	13 ± 2	0.15	0.78
Salt crackers	51 ± 11	30 ± 6	0.07	81 ± 19	75 ± 16	0.75	0.49

Mean ± s.e.m. values are indicated. P-values are derived from paired, two-tailed t-tests and group × treatment ANOVA interactions, respectively. Obese men, *n* = 18; normal-weight men, *n* = 20.

Ott *et al.*<sup>5</sup> Degrees of freedom were corrected using the Greenhouse-Geisser procedure. Areas under the curve (AUC) were calculated according to the trapezoidal rule. Significant ANOVA and AUC effects were specified by pairwise two-sided *t*-tests. For blood parameters, baseline adjustment was achieved by subtracting individual baseline values from individual post-intervention measurements. All data are presented as means ± s.e.m. A *P*-value < 0.05 was considered significant.

## RESULTS

Oxytocin reduces food intake to a greater extent in obese than normal-weight men

In the obese subjects, oxytocin in comparison to placebo-reduced overall food consumption from the breakfast buffet by ~10% ( $F(1,17) = 5.26, P < 0.04$  for treatment; Table 3 and Figures 1b and c).

In contrast, the normal-weight subjects did not alter their breakfast intake after oxytocin administration ( $P > 0.6$  for all comparisons including treatment  $\times$  macronutrients and treatment  $\times$  food types;  $F(1,36) = 3.48$ ,  $P = 0.07$  for group  $\times$  treatment). The difference between obese and normal-weight men in the hypophagic effect of oxytocin especially concerned the intake of carbohydrates ( $F(1,36) = 4.44$ ,  $P = 0.042$  for group  $\times$  treatment). In the obese subjects alone, oxytocin did not exert differential effects on the intake of macronutrients or food types (all  $P > 0.3$ ). In general, obese ate more than normal-weight participants in the respective placebo conditions ( $F(1,36) = 4.27$ ,  $P < 0.05$  for group), in particular more carbohydrates and proteins ( $F(2,72) = 9.25$ ,  $P < 0.001$  for group  $\times$  macronutrient). In both groups, hunger ratings were comparable at baseline ( $P > 0.2$ ) and generally unaltered by oxytocin ( $P > 0.9$ ), falling to comparably low values of around 12% of the maximal score after breakfast ( $P > 0.2$ ;  $F(1,36) = 350.54$ ,  $P < 0.001$  for time;  $P > 0.12$  for treatment effects). Thirst ratings were likewise unaffected by oxytocin ( $P > 0.4$  for all comparisons).

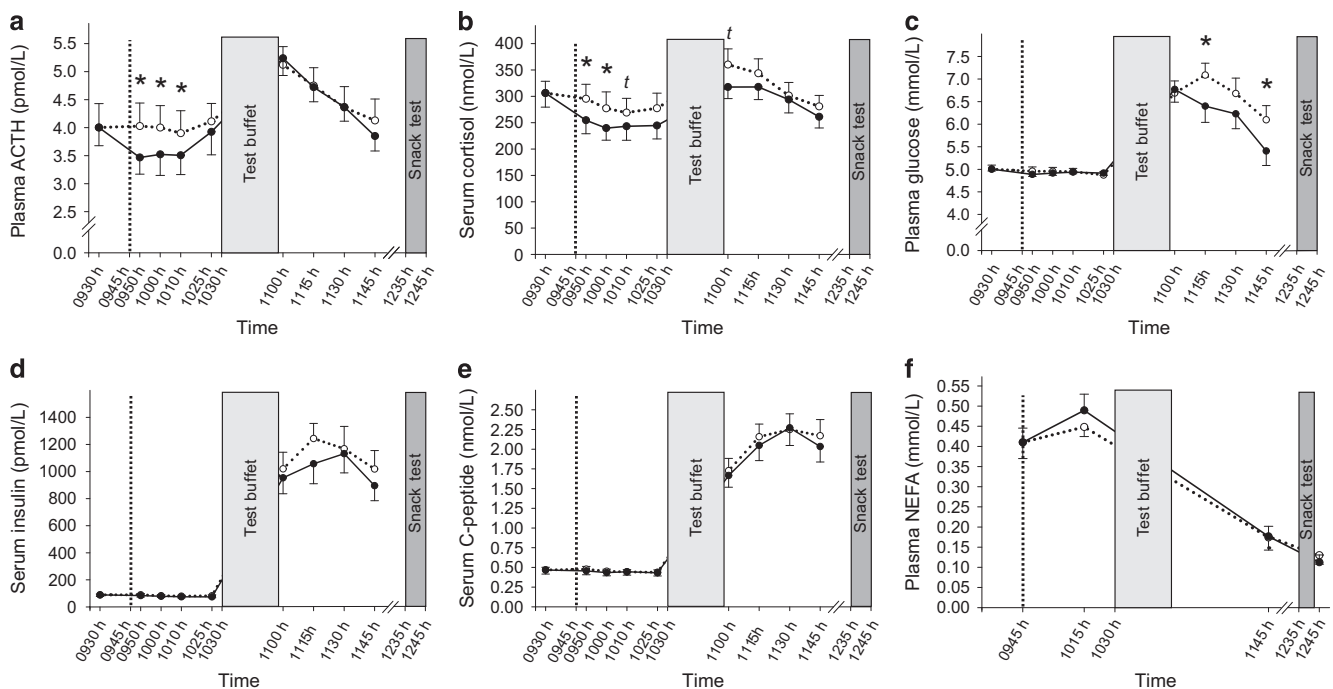
In the snack test during the postprandial period, oxytocin in comparison to placebo induced a reduction in total snack intake of 22% across all subjects ( $F(1,36) = 13.37$ ,  $P < 0.001$  for treatment; Table 3 and Figures 1d and f). This effect did not differ between the obese and the normal-weight groups ( $F(1,36) = 0.01$ ,  $P > 0.9$ ) although it was of a greater statistical effect size in the obese ( $F(1,17) = 9.89$ ,  $P = 0.006$ ;  $\omega_p^2 = 0.31$ ) compared with the normal-weight participants ( $F(1,19) = 5.5$ ,  $P = 0.03$ ;  $\omega_p^2 = 0.18$ ). In both groups, it was especially pronounced for chocolate cookie consumption ( $F(2,56) = 3.88$ ,  $P < 0.04$  for treatment  $\times$  snack type; Table 3; Figure 1e). The oxytocin-induced reductions in total and chocolate snack intake found in the obese subjects remained significant when corrected for total calorie and carbohydrate consumption during the preceding breakfast buffet (both

$P \leq 0.003$ ). Although breakfast and snack consumption per se were unrelated in both conditions ( $P > 0.4$ ), the oxytocin-induced reduction in breakfast intake was inversely related to the respective attenuation of snack intake ( $r = -0.48$ ,  $P < 0.05$ ).

Intake and rated palatability of chocolate cookies by far exceeded those of the remaining snacks when analyzed across conditions in the obese subjects ( $F(1,17) = 70.52$ ,  $P < 0.001$  for snack type). Sweetness and saltiness ratings were highest for chocolate cookies and salt crackers, respectively ( $F(2,34) = 221.28$ ,  $P < 0.001$  for snack type  $\times$  taste). Generally comparable rating patterns were obtained in the normal-weight subjects ( $P > 0.15$  for all group comparisons). Oxytocin did not affect ratings for chocolate cookies and salt crackers (all  $P > 0.15$ ) in the obese but increased the rated palatability (placebo,  $2.89 \pm 0.50$ ; oxytocin,  $3.82 \pm 0.47$ ;  $P < 0.03$ ) and, to a lesser extent, perceived sweetness of rice waffles (placebo,  $1.74 \pm 0.40$ ; oxytocin,  $2.63 \pm 0.61$ ;  $P < 0.06$ ).

#### Energy expenditure is not acutely affected by oxytocin administration

Oxytocin did not alter resting energy expenditure assessed by indirect calorimetry during the entire experimental period in the obese ( $F(1,17) = 0.03$ ,  $P > 0.87$  for treatment  $\times$  time;  $F(1,17) = 0.32$ ,  $P > 0.58$  for treatment; Figure 1g) and the normal-weight subjects (all  $P > 0.12$ ;  $F(1,36) = 0.47$ ,  $P > 0.49$  for group  $\times$  treatment  $\times$  time). The rise in energy expenditure by  $\sim 22\%$  found in the obese subjects between the fasting state (baseline) and the postprandial state reflects diet-induced thermogenesis (that is, the energy emitted as heat during metabolizing of food;  $F(1,17) = 200.91$ ,  $P < 0.001$  for time). In the postprandial period, oxytocin compared with placebo appeared to decrease respiratory quotient (placebo,  $0.90 \pm 0.02$ ; oxytocin,  $0.83 \pm 0.02$ ) and carbohydrate utilization (placebo,  $0.26 \pm 0.03$  g min<sup>-1</sup>; oxytocin,  $0.17 \pm 0.02$  g min<sup>-1</sup>; both  $P = 0.02$ ) whereas increasing fat utilization (placebo,



**Figure 2.** Blood parameters. Mean  $\pm$  s.e.m. concentrations of plasma adrenocorticotrophic hormone (a), serum cortisol (b), plasma glucose (c), serum insulin (d), serum C-peptide (e) and plasma NEFA (f) assessed before (averaged across the 0915 hours and 0930 hours baseline values) and after intranasal administration (vertical dotted line) of oxytocin (24 IU; black circles and solid lines) and placebo (vehicle; white circles and dotted lines) in obese men ( $n = 18$ ). Subjects ate a test breakfast from 1030–1100 hours and ingested snacks under the pretext of a taste test from 1235–1245 hours. Mean baseline values of both conditions are averaged to a common baseline. \* $P < 0.05$ ,  $^tP < 0.1$  for comparisons between conditions (pairwise two-sided  $t$ -tests).



$0.05 \pm 0.01 \text{ g min}^{-1}$ ; oxytocin,  $0.10 \pm 0.01 \text{ g min}^{-1}$ ;  $P=0.01$ ) in the obese group. However, these effects were no longer significant when the analysis of respiratory quotient was corrected for total breakfast calorie intake and analyses of carbohydrate oxidation and fat utilization were corrected for carbohydrate and fat intake, respectively (all  $P>0.17$ ).

Oxytocin reduces HPA axis activity and the glucose response to food intake in obese and normal-weight subjects

During baseline, none of the blood parameters differed between conditions (all  $P>0.18$ ). Oxytocin exerted a suppressive effect on fasting HPA axis activity in the obese men, reducing plasma adrenocorticotrophic hormone and serum cortisol concentrations between administration and breakfast intake ( $F(1,17)=6.20$ ,  $P=0.02$ ;  $F(1,17)=5.81$ ,  $P<0.03$ , respectively, for treatment effects, and  $t(17)=2.65$ ,  $P<0.02$ ;  $t(17)=2.30$ ,  $P=0.03$ , respectively, for the difference in  $\text{AUC}_{0930-1025 \text{ hours}}$ ; Figures 2a and b), whereas concentrations were comparable between conditions after breakfast (all  $P\geq 0.12$  for respective comparisons). In the normal-weight individuals respective effects extended to the postprandial period<sup>5</sup> but still were largely comparable to the pattern found in obese subjects ( $P>0.14$  for respective group  $\times$  treatment interactions). Supplementary analyses in the group of obese subjects indicated that the oxytocin-induced decreases in preprandial cortisol concentrations ( $\text{AUC}_{0930-1025 \text{ hours}}$ ) were not significantly correlated with measures of breakfast intake (all  $P>0.72$ ). Likewise, decreases in postprandial cortisol levels ( $\text{AUC}_{1100-1145 \text{ hours}}$ ) were not significantly correlated with cookie intake (all  $P>0.12$ , Pearson's coefficients).

Circulating concentrations of glucose, lactate, insulin, and C-peptide showed the expected meal-related increases across conditions in the obese participants (all  $P<0.001$  for time; Figures 2c–e). Whereas levels of lactate, insulin and C-peptide were not affected by oxytocin administration (all  $P>0.19$ ), oxytocin exerted a sustained suppressive effect on glucose levels during the postprandial period ( $F(3,50)=3.77$ ,  $P<0.02$  for treatment  $\times$  time; Figure 2c). This effect was still evident when adjusted for preceding total and carbohydrate-specific breakfast intake (both  $P<0.05$ ), but statistically unrelated to the oxytocin-induced decrease in cortisol concentrations ( $P>0.43$ ). An oxytocin-triggered reduction in postprandial glucose levels was likewise found in normal-weight individuals ( $F(1,36)=0.15$ ,  $P=0.7$  for group  $\times$  treatment). In the obese subjects, total plasma NEFA concentrations were suppressed after breakfast intake ( $F(1,19)=131.42$ ,  $P<0.001$  for time) without significant treatment effects ( $P>0.67$ ; Figure 2f); circulating concentrations of growth hormone were comparable between conditions (all  $P>0.24$ ).

Olfactory performance, mood, vigilance, perceived trustworthiness and treatment awareness

In the olfactory task, no treatment effects on perceptual thresholds, olfactory discrimination and olfactory identification emerged in the obese participants (all  $P>0.17$ ). Self-rated mood, reaction times in the vigilance task and the perceived trustworthiness of the experimenter were likewise unaffected (all  $P>0.27$ ). Eight of the 18 participants (44%) correctly guessed their respective treatment conditions ( $\chi^2_{(3;N=18)}=2.68$ ,  $P>0.6$ ), indicating that the participants overall gained no treatment awareness. All of these results were well comparable with the findings in the group of normal-weight subjects.<sup>5</sup>

## DISCUSSION

We demonstrate that the acute intranasal administration of oxytocin inhibits reward- but also hunger-driven food intake in obese men and that this effect is not compensated by changes in energy expenditure. In normal-weight men, the oxytocin-induced reduction

in calorie consumption was restricted to snacking, indicating that the inhibitory effect of oxytocin on food intake is generally larger in obese than normal-weight subjects. Oxytocin moreover attenuated secretory activity of the HPA axis and curbed the postprandial excursion of glucose levels in obese and normal-weight men, suggesting an insulin-sensitizing action of the hormone.

Oxytocin has been shown in a number of experiments in rodents to inhibit feeding after intracerebroventricular injection<sup>25,26</sup> as well as after peripheral administration, which both supposedly trigger hypothalamic oxytocin release in a feed-forward fashion.<sup>2,27</sup> Recently, comparable effects have been obtained after intranasal administration.<sup>28</sup> Oxytocin's restraining effect on snacking in the postprandial period may be mediated via oxytocinergic projections to the brain reward circuit,<sup>29</sup> an assumption supported by signs of oxytocin-induced enhancements in the perceived palatability and sweetness of the moderately appealing snacks (rice waffles) offered to our obese subjects. The fact that the attenuating effect of oxytocin on snack intake focused on chocolate cookies, which were preferentially eaten and rated most palatable, further suggests a strong reward-related component of the observed oxytocin action.

In the obese in contrast to normal-weight subjects, the hypophagic effect of oxytocin clearly pertained to meal intake in the fasted state, decreasing calorie consumption from the breakfast buffet by 10%. The conclusion that oxytocin elicits stronger effects in obese than normal-weight humans is supported by findings of more pronounced weight-lowering oxytocin effects in diet-induced obese compared to control rats, which were associated with stronger oxytocin-triggered increases in c-Fos expression in the area postrema and the nucleus of the solitary tract of the hindbrain.<sup>2</sup> Moreover, prolonged anorexigenic effects of oxytocin have been reported in mice kept on a high-fat diet in comparison to control animals.<sup>27</sup> Enhanced oxytocin sensitivity in the obese state has been assumed to be mediated via improved high-affinity receptor binding of oxytocin due to elevated cholesterol levels,<sup>4</sup> and would be in line with reports that circulating oxytocin concentrations, which were not measured in the present study, are inversely related to BMI and waist circumference.<sup>30</sup> In recent experiments by Lawson and colleagues that focused on oxytocin effects in the fasted state,<sup>10</sup> *ad libitum* energy intake was reduced by oxytocin in both normal- and overweight men. The contrast to our results might stem from differences in study design including the more pronounced induction of food-anticipatory processes in the former study.<sup>10</sup> Taking into account potential rebound effects, our study indicates that the impact of oxytocin on breakfast intake in obese men is not compensated for by increases in postprandial thermogenesis and is still evident when subjects are allowed to snack. In conjunction with our observation that stronger anorexigenic effects of oxytocin during breakfast are associated with relatively smaller respective reductions in snack intake, this pattern points to a global, albeit tightly regulated enhancement of satiety signalling by oxytocin. This effect is not readily reflected in subjective hunger ratings which accords with the general treatment unawareness of our subjects. In line with our results, pilot data suggest that long-term oxytocin administration may support weight loss in obese patients.<sup>11</sup>

The finding of reduced breakfast intake after oxytocin administration in the fasted state suggests that the peptide restrains hunger-driven, primarily homeostatically regulated eating, although under normal circumstances ingestive behaviour triggered by food deprivation also involves a strong hedonic component. Our analyses did not yield indicators that the inhibitory effect of oxytocin on breakfast intake in the obese men focused on such hedonic aspects of food consumption or could be considered a mere consequence of oxytocin acting on reward processing. Still, in obese compared with normal-weight individuals, brain reward circuits show greater activation in

response to palatable versus bland foods<sup>31</sup> whereas fronto-cortical inhibitory control of food intake appears to be reduced.<sup>32</sup> Therefore, the enhanced hypophagic effect of oxytocin in obese compared with normal-weight subjects may also be interpreted against the background of a stronger reinforcing quality of food intake and the tendency to overeat pleasurable food in obesity.<sup>33</sup> In line with this, our obese subjects consumed ~240 kcal more than the normal-weight subjects in the respective placebo conditions. In animal experiments, oxytocin receptor antagonists acutely attenuate the anorexigenic impact of hormones like corticotropin-releasing hormone,<sup>34</sup> whereas alpha-melanocyte stimulating hormone, a catabolic messenger, triggers oxytocin release from supraoptic neurons.<sup>35</sup> Oxytocin might furthermore restrain food intake by acting on downstream mediators of the leptin signal<sup>36</sup> and enhancing cholecystokinin signalling.<sup>10</sup> Tests of olfactory function in our experiments indicated that the decrease in food intake most likely was not mediated by direct effects on sensory processing. Biasing effects on ingestive behaviour related to demand characteristics and social desirability were excluded by respective interviews.

Oxytocin administration did not induce significant alterations in fasting and postprandial resting energy expenditure. Signs of decreases in the respiratory quotient and carbohydrate utilization and respective increases in fat utilization emerging in the obese subjects during the postprandial period disappeared after adjustment for preceding calorie intake, suggesting that they might rather be attributed to oxytocin-induced decreases in macronutrient consumption. In diet-induced obese rats losing weight during systemic chronic oxytocin administration, the decrease in energy expenditure normally associated with weight loss was prevented by oxytocin, probably via effects on hypothalamic thermoregulation.<sup>2</sup> In obese nonhuman primates, chronic subcutaneous administration of oxytocin increased energy expenditure in the dark cycle by 14%.<sup>3</sup> Thus, our finding suggests that rather than exerting acute effects on energy expenditure, oxytocin contributes to its regulation on a long-term basis, which might turn out to be a critical factor in future clinical applications.

Oxytocin blunted plasma glucose excursions during the postprandial period in obese as well as normal-weight subjects. Notably, this effect was still evident after correcting the data for differences in calorie and carbohydrate intake from the breakfast buffet, which suggests an oxytocin-induced improvement in insulin sensitivity. Accordingly, oxytocin enhanced insulin sensitivity and glucose tolerance in rodent models of diet-induced obesity independent of its effects on body weight<sup>2</sup> and also in rhesus monkeys with diet-induced obesity.<sup>3</sup> In the study by Lawson and coworkers,<sup>10</sup> intranasal oxytocin reduced fasting insulin secretion without affecting glucose levels, which also implies insulin-sensitizing properties of oxytocin and underlines that the role of oxytocin in glucose homeostasis in humans is in need of further investigation. The reduction in HPA axis activity by oxytocin extends previous findings of an attenuating impact of intranasal oxytocin on cortisol secretion in response to stress.<sup>37</sup> Acute and chronic activation of endocrine stress axes facilitates the intake of 'comfort food', that is, highly palatable food.<sup>38</sup> Vice versa, consuming sucrose reduces HPA axis activity in a negative feedback loop by activation of central nervous reward circuits.<sup>39</sup> With regard to the link between emotional regulation and food intake,<sup>33</sup> a mediation of oxytocin's attenuating impact on food intake via reductions in HPA axis activity might be expected to be of particular relevance in obese subjects. However, in our studies the two phenomena were statistically related only in normal-weight participants.<sup>5</sup>

In summary, our study indicates a restraining effect of oxytocin on hunger- and reward-driven eating behaviour in obese humans that goes along with a suppression of HPA axis activity and signs of enhanced peripheral insulin sensitivity. In contrast to messengers like insulin and leptin, whose anorexigenic impact is blunted

when body weight is increased,<sup>40</sup> oxytocin exerts a potent acute inhibition of food intake in obese subjects which even surpasses the effect found in normal-weight humans. These results clearly warrant further investigations on long-term oxytocin effects in metabolic disorders.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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## AUTHOR CONTRIBUTIONS

MT and MHa designed the study and wrote the manuscript. MT, AF and ME enrolled subjects and carried out experiments for the study. MT, ME, AP and MHa analyzed the data. MT, AF, MHe, HL, JB and MHa discussed the results. AF, MHe, AP, HL and JB contributed to writing the manuscript. MT and MHa are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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