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BRIEF COMMUNICATION

Clinical Research

Variable oxytocin levels in humans with different degrees of obesity and impact of gastric bypass surgery

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Abstract

Exogenous oxytocin administration in obese mice, rats, and monkeys was shown to induce sustained weight loss, mostly due to a decrease in fat mass, accompanied by an improvement of glucose metabolism. A pilot study in obese humans confirmed the weight-reducing effect of oxytocin. Knowledge about circulating oxytocin levels in human obesity might help indicating which obese subjects could potentially benefit from an oxytocin treatment. Conclusive results on this topic are missing. The aim of this study was to measure circulating oxytocin levels in lean ($n = 37$) and obese ($n = 72$) individuals across a wide range of body mass index (BMI) values (18.5–60 kg/m^2) and to determine the impact of pronounced body weight loss following gastric bypass surgery in 12 morbidly obese patients. We observed that oxytocin levels were unchanged in overweight and in class I and II obese subjects and only morbidly obese patients (obesity class III, BMI > 40 kg/m^2) exhibited significantly higher levels than lean individuals, with no modification 1 year after gastric bypass surgery, despite substantial body weight loss. In conclusion, morbidly obese subjects present elevated oxytocin levels which were unaltered following pronounced weight loss.

Introduction

While oxytocin was historically recognized for its role in parturition and lactation, we now know that it plays a role in various behaviors, as well as in the regulation of energy balance [1, 2]. Animal studies indicate that oxytocin is a potent regulator of caloric intake and metabolism. Thus, lack of oxytocin signaling in oxytocin or oxytocin receptor knockout mice results in the development of late-onset obesity and impaired glucose homeostasis. Exogenous oxytocin administration in obese mice, rats, and monkeys induces sustained weight loss, mostly due to a specific decrease in body fat mass [3–9] and improves glucose metabolism in diet-induced obese and in some diabetic or prediabetic rodents [1, 3, 4, 7]. Circulating oxytocin levels of diet-induced or genetically obese rodents are reportedly unchanged [5, 9, 10] or decreased [7, 11, 12]. In humans, dysfunctional oxytocin signaling might contribute to weight gain in some genetic obesity syndromes as the Prader–Willi syndrome (PWS) [13]. While patients with PWS clearly exhibit low circulating oxytocin levels with reduced number of oxytocin-expressing neurons, these levels were reported to be lowered [14], unchanged [15], or even increased [16–18] in other types of human obesity. In a pilot study, oxytocin treatment of human obese subjects without diabetes was shown to result in body weight loss over an 8-week period [4]. Clarification of the issue of circulating oxytocin levels in obesity would help improving our understanding of the pathophysiological importance of oxytocin in the regulation of body weight.

The aim of the present study was therefore to measure circulating oxytocin levels in different cohorts of lean and obese individuals across a wide range of body mass index (BMI) values. We also examined the impact of pronounced

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Table 1A Anthropometric characteristics of lean and obese subjects classified by their BMI: first cohort

BMI range	18.5–25	25–30	30–35	35–40	40–50	50–60
n	37	11	9	22	26	4
Female/male	18/19	3/8	4/5	9/13	19/7	4/0
Age (years)	57.41 ± 1.91	51.45 ± 4.98	52 ± 3.32	58.91 ± 2.61	**46.62 ± 2.94****	41.50 ± 3.86
Height (m)	1.69 ± 0.02	1.68 ± 0.02	1.72 ± 0.03	1.70 ± 0.02	1.65 ± 0.02	1.58 ± 0.,03
Weight (kg)	65.17 ± 1.51	**74.85 ± 2.76 ($p = 0.06$)**	**99.03 ± 4.23*****	**106.5 ± 2.65*****	**116.00 ± 2.67*****	**138.40 ± 4.13*****
BMI (kg/m^2)	22.64 ± 0.28	**26.3 ± 0.36*****	**33.52 ± 0.41*****	**37.06 ± 0.34*****	**42.73 ± 0.40*****	**55.95 ± 2.62*****
Waist (cm)	83.43 ± 1.43	**89.79 ± 1.82*****	**107.2 ± 2.85*****	**116.5 ± 2.13*****	**121.40 ± 1.87*****	**138.80 ± 3.01*****
Fasting glycemia (mM)	5.79 ± 0.36	6.54 ± 0.57	6.34 ± 0.86	6.47 ± 0.42	6.11 ± 0.36	5.92 ± 1.05
Insulinemia (mU/L)	4.26 ± 0.50	8.17 ± 2.53	**18.13 ± 6.24****	**13.19 ± 1.39*****	**17.71 ± 1.66 *****	**22.42 ± 2.91*****
HOMA-IR	1.21 ± 0.23	2.22 ± 0.65	**5.17 ± 2.24***	**3.95 ± 0.57****	**4.84 ± 0.56*****	**5.79 ± 0.98*****

body weight loss following Roux en-Y gastric bypass (RYGB) surgery on circulating oxytocin levels.

Materials and methods

Participants

Seventy-two obese and 37 lean subjects were recruited at the Service of Therapeutic Education for Chronic Diseases (Geneva University Hospitals) or randomly selected—based on the BMI—from the Bus Santé (cross-sectional population-based study [19]). Table 1A provides the basic characteristics of all the individuals who were classified according to the different BMI ranges defined by the World Health Organization (normal range (BMI 18.50–24.99 kg/m^2), overweight (25.00–29.99 kg/m^2), class I (30.00–34.99 kg/m^2), class II (35.00–39.99 kg/m^2), and class III obesity (≥40 kg/m^2), which, respectively, correspond to an increased, moderate, severe, or very severe risk of comorbidities [20]. A second cohort included 12 morbidly obese patients before and 1 year after RYGB surgery (basic characteristics, Table 1B). The study was conducted according to the ethical principles for medical research involving human subjects released by the Declaration of Helsinki. The study protocol was approved by the local ethics committee. All participants signed the informed consent form.

Biochemical measurements

Oxytocin was measured using a commercial ELISA kit (Cayman Chemical, Michigan, IL, USA) with 9.6% intra-assay and 6.3% inter-assay coefficients of variations reported at a concentration of 46.9 pg/mL. A subset of samples was also measured using an alternative ELISA kit (Enzo Life Sciences Chemical, Farmingdale, NY, USA) with 10.2% intra-assay and 16.5% inter-assay coefficients of variations reported at concentrations of 121.4 and 145.1 pg/mL, respectively. Serum and plasma (EDTA as the anticoagulant) samples were extracted using solid-phase extraction (C18-E 200 mg/3 mL columns, Phenomenex, Torrance, CA, USA) to eliminate interfering molecules and to concentrate the sample for analysis following the provider instructions. Insulin and glycemia were measured by commercial kits (Crystal Chem, Elk Grove Village, IL, USA and Roche, Basel, Switzerland).

Table 1B Anthropometric characteristics and oxytocin levels of obese subjects who underwent a Roux en-Y gastric bypass surgery, before (pre bypass) and 1 year after surgery (post bypass): second cohort

BMI range	Pre bypass	Post bypass
n	12	12
Female/male	9/3	9/3
Age (years)	37.25 ± 2.32	38.25 ± 2.32
Height (m)	1.65 ± 0.03	1.65 ± 0.03
Weight (kg)	119.07 ± 5.75	**78.66 ± 4.80*****
BMI (kg/m^2)	43.82 ± 2.06	**28.96 ± 1.78*****
Oxytocin (pg/mL)	34.04 ± 2.25	33.06 ± 3.35

Characteristics of the subjects recruited, classified by their BMI. Anthropometric characteristics of (A) Lean and obese subjects classified by their BMI. **$p < 0.01$, ***$p < 0.001$ (in bold) vs. the 18.5–25 BMI group. (B) Obese subjects who underwent a Roux en-Y gastric bypass surgery, before (pre bypass) and 1 year after surgery (post bypass). ***$p < 0.001$ (in bold) vs. pre bypass group

Statistical analysis

Quantitative data are expressed as mean ± SEM. The GraphPad (Prism, La Jolla, CA, USA), SPSS (IBM, New York, USA) or R [21] statistical software was used to analyze the data. Outlier analysis was performed by the ROUT test. Gaussian distribution of the data was determined using the Kolmogorov–Smirnov test and presence of different variances was assessed by the *F* test. Depending

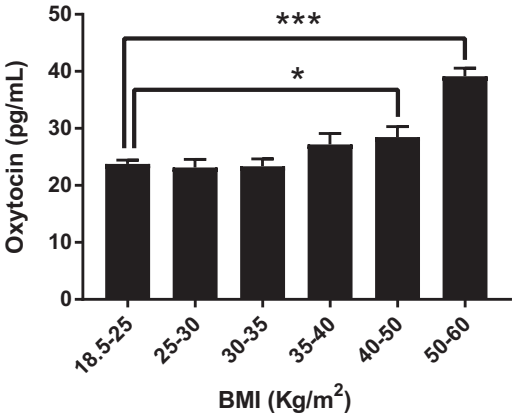

Fig. 1 Oxytocin levels in humans classified by their BMI. *$p < 0.05$, ***$p < 0.001$ for the indicated comparisons. Subjects were classified according to the different body mass index (BMI) ranges defined by the WHO (normal range (BMI 18.50–24.99 kg/m^2), overweight (25.00–29.99 kg/m^2), class I (30.00–34.99 kg/m^2), class II (35.00–39.99 kg/m^2), and class III obesity (≥40 kg/m^2)

on the data distribution and the number of groups, statistical significance was determined by the Student's t test, the Mann–Whitney test (two-group analysis, Table 1B), an analysis of variance (ANOVA) analysis, the Kruskal–Wallis test or generalized linear models (analysis of covariance (ANCOVA)) with a Dunnet's post hoc test (more than two-group analysis, Table 1A, Fig. 1, and Sup. Figure 1). Correlation was analyzed using the Pearson's test. Biochemical and statistical studies were not blinded. Sample size was not a priori calculated, but post hoc data analysis showed an achieved power higher than 95% (G*Power [22]).

Results

A first study was performed in a cohort of 72 obese and 37 lean subjects. All obese subjects had a normal fasting glycemia, but higher fasting insulin levels and Homeostasis Model Assessment (HOMA) index than controls. With regard to the circulating oxytocin levels measured in this cohort of obese patients, they were normal compared to lean individuals in overweight (BMI 25–30 kg/m^2) and in class I obesity (BMI 30–35 kg/m^2) conditions, to gradually increase with higher BMIs. Only class III obese subjects (BMI >40 kg/m^2) exhibited significantly higher circulating oxytocin levels than lean individuals (Fig. 1, ANOVA analysis). Further analysis showed that oxytocin levels significantly correlated with obesity indexes (weight, waist, and BMI, $p < 0.0001$, $R^2 = 0.15$–0.16), as well as with some biochemical defects accompanying obesity (insulinemia, $p < 0.001$, $R^2 = 0.12$ and HOMA-IR, $p < 0.01$, $R^2 = 0.10$). There was no significant correlation between oxytocin levels and age, height, sex and fasting glycemia. After data adjustment for HOMA index, age and sex (ANCOVA analysis), the statistical difference between lean and obese individuals with a BMI higher than 50 kg/m^2 remained, while these covariables had no statistically significant effect.

We next examined oxytocin levels in a cohort of 12 morbidly obese patients before and 1 year after RYGB surgery, which resulted in a mean weight loss of 40.4 ± 2.0 kg over the 1-year period. Interestingly, their circulating oxytocin levels were similar when measured before and 1 year after the bypass surgery (Table 1B).

Finally, we performed a third study involving eight lean subjects (female/male = 4/4, BMI = 22.6 ± 1.5 kg/m^2) to compare plasma and serum oxytocin levels after an overnight fast. The values obtained were similar for both sets of values, being 26.8 ± 0.3 and 25.5 ± 0.5 pg/mL for serum and plasma levels, respectively.

Discussion

Contradictory results on circulating oxytocin levels in human obesity have been reported in the literature. This may partly be related to the fact that oxytocin measurements in blood samples are lacking reliability when performed using some of the commercially available assays, underlying recurrent discussion and controversy [23–26]. One factor that varies among the different reports is the type of blood sample analyzed (i.e., serum vs. plasma). However, as shown in the present study, plasma and serum samples provide similar oxytocin values. The type of sample conservation was also discussed in some studies as a possible confounding parameter that could influence the circulating oxytocin levels. That this is not the case was concluded by Szeto et al. [24] using different conservation temperatures and incubation times for their assays. With regard to our own samples, they were constantly kept at −80 °C. Finally, sample preparation, in particular treatment with an extraction procedure, seems to be crucial for the oxytocin measurement. Thus, similar oxytocin levels were reported using bioassays and immunoassays after sample extraction, whereas immunoassays with unextracted samples led to higher and inaccurate values [24, 26]. A recent study pointed to a reduction/alkylation step as a way to measure free and protein-bound oxytocin, obtaining higher values than traditional approximations [25]. Our data were obtained with a commercial ELISA kit from Cayman after a solid-phase extraction, which removed some of the interfering substances and likely measured the free-oxytocin fraction (the biological differences and dynamics between protein-bound vs. free oxytocin are unknown as yet [27]). Of note, qualitatively similar results were obtained in a subset of samples reanalyzed using an alternative ELISA kit

from Enzo, although the absolute values were much higher (three times) (Sup. Figure 1). This allowed us to perform relative comparisons among the groups and to analyze the oxytocin levels of obese individuals across a wide range of BMI values [27].

In our first cohort, obese subjects were distributed into different categories according to the BMI. This revealed that circulating oxytocin levels are normal in overweight and in class I and II obese subjects, and elevated in class III obese patients (BMI > 40 kg/m^2) only. Our results are coherent with most of the existing literature showing normal or increased oxytocin levels in human obesity and/or positive correlation with body weight, BMI, and body fat mass [16–18]. In contrast, they are inconsistent with the results of Qian et al. [14] describing a negative correlation between serum oxytocin levels and BMI. However, a close examination of this study shows that lean and obese normal glucose-tolerant subjects exhibited similar circulating oxytocin values, whereas lean and T2D patients showed lower values than non-diabetic individuals. Therefore, the mean value of the obese group was lowered due to the fact that it comprised samples from both the diabetic and the non-diabetic subgroups, leading to the bias conclusion that obesity is characterized by low oxytocin levels. Accordingly, decreased circulating oxytocin levels in T2D were also reported by others [28, 29].

With regard to the issue of the underlying mechanisms responsible for the increased oxytocin levels observed in severely obese subjects, they could be numerous and may include elevated estrogen levels or an attempt to reduce the increased food intake present in severely obese subjects [2, 30]. An alternative hypothesis is based on the observations of a circadian rhythm of hypothalamic oxytocin release in mice fed a standard diet and suppression of this rhythm in animals chronically fed a high fat diet [7]. Lack of rhythmicity of oxytocin secretion in obese patients could occur, as it was previously demonstrated for other hormones, such as ghrelin and cortisol [31].

An additional observation of the present study is that elevated oxytocin levels of morbidly obese subjects were unaltered 1 year following RYGB surgery that induced a substantial weight loss. Lack of impact of body weight loss on basal circulating oxytocin levels is in keeping with previously reported results [15], but in contrast to other data obtained in extremely obese subjects following gastric banding [16]. Together, these results suggest that body weight loss in itself is not the main determinant of circulating oxytocin levels in humans. They also suggest that the type of bariatric surgery used (i.e., gastric banding vs. RYGB) may differently influence circulating oxytocin levels. This could be related to several parameters, such as different changes in the gut microbiota or in the levels of some gastrointestinal hormone after bariatric surgery. As insulin resistance is reportedly not normalized in obese subjects 16 months after RYGB [32], it may be hypothesized that oxytocin levels remain elevated in an attempt to improve insulin sensitivity.

It can be concluded that the oxytocin levels are unchanged in most forms of overweight and obesity in humans (BMI 25–40 kg/m^2), which could therefore benefit from an oxytocin/oxytocin analog treatment. Appearance of elevated oxytocin levels in morbidly obese subjects (BMI > 40 kg/m^2) may be an attempt to alleviate the marked insulin resistance known to prevail in these patients.

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Compliance with ethical standards

Conflict of interest FRJ has a patent application (PCT/IB2011/052156) covering therapeutic uses of oxytocin. The other authors declare that they have no conflict of interest.

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