

Association of Oxytocin with Glucose Intolerance and Inflammation Biomarkers in Metabolic Syndrome Patients with and without Prediabetes

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■ Abstract

OBJECTIVES: The aim of this study was to explore the differences in OXT levels in metabolic syndrome (MetS) subjects, newly diagnosed type 2 diabetes mellitus (T2DM), and prediabetes subjects vs. MetS subjects without glucose intolerance (non-diabetic MetS). It was also intended to determine the relationship between plasma OXT levels and inflammatory markers in those subjects. **METHODS:** Along with 45 lean and normoglycemic controls, a total of 190 MetS subjects (61 men, 129 women) were enrolled. Colorimetric enzymatic assays of the following components were performed: plasma OXT, high-sensitivity C-reactive protein (hs-CRP), macrophage chemoattractant protein 1 (MCP-1), plasminogen activator inhibitor 1 (PAI-1), matrix metalloproteinase 9 (MMP-9), resistin, adiponectin, leptin, macrophage migration inhibitory factor (MIF), tumor necrosis factor α (TNF- α), thrombospondin 1 (TSP-1), interleukin 10 (IL-10), interleukin 6 (IL-6), and glucagon. **RESULTS:** hsCRP, PAI-1, resistin, leptin-to-adiponectin-ratio (LAR), TNF- α , TSP-1,

and MIF were significantly higher in both MetS groups (prediabetic and T2DM) than in MetS-only subjects. Leptin and MMP-9 were significantly higher in the MetS-T2DM group (but not in MetS-prediabetics) vs. MetS-only subjects. Conversely adiponectin, OXT, MCP-1, and IL-10 were significantly lower in both MetS groups (prediabetic and T2DM) than in MetS-only subjects. There was no marked discrepancy in either glucagon or IL-6 levels among the three MetS groups. In the entire MetS study population, OXT correlated substantially and proportionally with MCP-1, IL-10, and IL-6; it correlated negatively with HbA1c, fasting plasma glucose (FPG), PAI-1, MMP-9, TNF- α , TSP-1, resistin, adiponectin, leptin, LAR, and MIF. No association could be observed between OXT and glucagon. **CONCLUSIONS:** OXT may be a substantial surrogate predictive/prognostic tool and putative pharmacotherapeutic target in metabolic anomalies and related disorders.

Keywords: diabetes · prediabetes · risk · metabolic syndrome · inflammatory marker · oxytocin

1. Introduction

xytocin (OXT) is a nine-amino acid neuropeptide produced by hypothalamic OXT neurons. It is released locally in the brain or systemically via the axonal terminals in the posterior pituitary. Systemically, OXT is known to mediate reproductive activities in women including labor and lactation. Recently, neurobiological research has led to the discovery of OXT functions

beyond its reproduction-related roles. Interestingly, recent research demonstrated that the central actions of OXT can lead to the reversal of obesity as well as glucose- and insulin-related disorders in animals [1-2] and humans [3].

Onaka *et al.* emphasized that stress, obesity, and social isolation are among the major mortality risk factors in humans [4]. In this regard, the OXT receptor system and OXT-related systems, including dopaminergic and serotonergic neurons, are

important targets for the promotion of human health, while impaired OXT release may result in dietary obesity [2, 5]. Peripheral OXT treatment via different routes may reduce food intake and visceral fat mass and ameliorate obesity, fatty liver, and glucose intolerance, providing a new avenue for the treatment of obesity and hyperphagia [1, 6-7].

OXT was recently shown to play a role in the cardiovascular system as well. In pathological conditions, the hormone exerts anti-inflammatory and cardioprotective properties, and improves vascular and metabolic functions. Also, OXT was reported to attenuate atherosclerosis and adipose tissue inflammation [8]. Therefore, OXT itself [9-10] and/or appropriate OXT analogs with practical delivery methods may offer a potentially safe and effective therapeutic strategy for obesity and diabetes [3, 11-13].

While the effects of OXT administration in animals are well established [3, 14], evidence of the correlation between OXT, glucose intolerance, and/or inflammation in humans is scarce. Only one study evaluated the consequences of intranasal OXT administration in obese individuals without diabetes on blood glucose, insulin, and lipid profile [3]. It showed that OXT improved postprandial glucose levels and insulin sensitivity, and reduced low-density lipoprotein cholesterol (LDL-C). Accordingly, it is plausible to hypothesize that OXT can potentially ameliorate glucose intolerance and inflammation.

To date, the relationship between OXT, glucose intolerance, and/or inflammatory and metabolic markers in drug-naïve individuals with the metabolic syndrome (MetS) and prediabetes or diabetes has not been investigated. Therefore, the objective of this study was to evaluate the potential correlation between plasma OXT levels, glucose intolerance, and insulin resistance as well as their correlation with inflammatory/metabolic markers in MetS patients.

2. Patients, materials, and methods

2.1 Methods and subjects

Approval for the study was obtained from the Clinical Institutional Review Board (IRB) Committee affiliated with the Jordan University Hospital (JUH) (18/2014/IRB) and National Center for Diabetes, Endocrinology, and Genetics (NCDEG) (1151, 1152, 1153/9/SM). Informed written consent was obtained from each participant before the study.

Abbreviations:

BMI	body mass index
BP	blood pressure
ELISA	enzyme-linked immunosorbent assay
FPG	fasting plasma glucose
hs-CRP	high-sensitivity C-reactive protein
HDL-C	high-density lipoprotein cholesterol
HOMA-IR	homeostatic model assessment of insulin resistance
IL-6	interleukin 6
IL-10	interleukin 10
JUH	Jordan University Hospital
LAR	leptin-to-adiponectin-ratio
LDL-C	low-density lipoprotein cholesterol
MetS	metabolic syndrome
MCP-1	macrophage chemoattractant protein 1
MIF	macrophage migration inhibitory factor
MMP-9	matrix metalloproteinase 9
NCDEG	National Center for Diabetes, Endocrinology, and Genetics
NGT	normal glucose tolerance
OXT	oxytocin
PAI-1	plasminogen activator inhibitor 1
SBP	systolic blood pressure
T2DM	type 2 diabetes mellitus
TC	total cholesterol
TG	triglycerides
TNF- α	tumor necrosis factor α
TSP-1	thrombospondin 1
WC	waist circumference

All participants who attended the Diabetes, Endocrinology, and Nutrition outpatient clinics at JUH and NCDEG for the first time were screened for potential recruitment. Adult patients (>18 year old), either overweight (BMI > 25 kg/m²) or obese (BMI > 30 kg/m²), were included if they met at least 3 of the following 5 MetS criteria, as defined by the IDF (2006) [15]:

1. Abdominal white adipose tissue (WAT) accumulation, waist circumference >35 inches (85 cm) in women and >40 inches (100 cm) in men
2. Blood pressure (BP) >130/85 mmHg
3. Triglycerides (TG) >150 mg/dl
4. High-density lipoprotein cholesterol (HDL-C) <40 mg/dl in men or <50 mg/dl in women
5. Fasting blood glucose levels >100 mg/dl

Based on ADA (2017) guidelines [16], prediabetes was diagnosed if at least one of the following criteria was fulfilled:

1. HbA1c of ≥ 5.7 to <6.5%
2. Fasting plasma glucose (FPG) ≥ 100 to <126 mg/dl

Accordingly, diabetes was defined as: HbA1c $\geq 6.5\%$ or FPG ≥ 126 mg/dl. All patients included in this study were drug-naïve MetS patients. Fifty-three of them were diabetic, ninety-five were pre-diabetic, and forty-two had the metabolic syndrome only.

All patients were newly diagnosed and did not receive any treatment such as oral antidiabetic, hypolipidemic, or antihypertensive agents. Subjects with the following conditions were excluded from the study:

- Acute complications of diabetes
- Secondary obesity
- Acute and chronic inflammatory diseases
- Systemic corticosteroid treatments
- Renal dysfunction
- Hepatic dysfunction
- Pregnancy, breast feeding, or taking contraceptive pills

Forty-five apparently healthy, normoglycemic, and lean subjects were recruited as controls.

2.2 Clinical and biochemical evaluation

Height and weight were measured with standardized techniques. BMI was expressed as weight per height squared (kg/m^2). Serum creatinine was obtained from the patients' data files, and glomerular filtration rate (GFR) was calculated by Cockcroft-Gault equation: $(140 - \text{age in yr}) \times \text{mass (in kg)} / (72 \times \text{serum creatinine (in mg/dl)})$. If the patient was female, the outcome of the calculation was multiplied by 0.85 [17-18].

Participants were told to avoid stressful activities (e.g. sports and physical exercise) prior to blood sampling. Blood samples were drawn after a 10-hour overnight fast; they were put into lithium heparin for subsequent centrifugation, stored at -80°C , and thawed immediately before plasma biomarker and hormonal analyses.

Lipid profile, including total cholesterol (TC), TG, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), was measured by appropriate enzymatic assays (Beckman Coulter Inc., USA). Fasting blood glucose was determined by glucose oxidase-based assay. HbA1c was determined by turbidimetric inhibition immunoassay (COBAS C, Roche Diagnostics GmbH Mannheim, Germany). Insulin sensitivity was assessed by homeostasis model assessment of insulin resistance (HOMA-IR) using the following equation: $(\text{fasting insulin (mIU/l)} \times \text{fasting glucose (mg/dl)}) / 405$.

Commercially available human ELISA assays of plasma oxytocin (OXT), insulin, leptin, macrophage chemo-attractant protein 1 (MCP-1) (Abcam, UK), adiponectin, interleukin 6 (IL-6), interleukin 10 (IL-10), matrix metalloproteinase 9 (MMP-9), plasminogen activator inhibitor 1 (PAI-1), tumor necrosis factor α (TNF- α), (eBioscience, USA), high-sensitivity C-reactive protein (hs-CRP) (AccuBind, USA), thrombospondin 1 (TSP-1), macrophage migration inhibitory factor (MIF) (AssayBiotech, USA), glucagon and resistin (RayBiotech, USA) were performed according to manufacturers' instructions. The leptin-to-adiponectin ratio (LAR) was calculated by dividing leptin levels by adiponectin levels.

2.3 Statistical evaluation

Based on the results of Qian *et al.* [19], the sample size was calculated by the following formula:

$$N = 2 \times SD^2 (Z_{\alpha} + Z_{\beta})^2 / \Delta^2 \quad [20]$$

Where N: sample size; Z_{α} : type one error (= 1.96 if $\alpha = 5\%$); Z_{β} : type two error (= 1.28 if $\beta = 10\%$); SD: standard deviation of OXT baseline from the study by Qian *et al.* [19], equaling 1.38 ng/l; and Δ : the difference between the OXT levels of diabetic/prediabetic group vs. the control group from the study by Qian *et al.* [19] after 3 month treatment, equaling to 2.07 ng/l. Accordingly, 10 patients were required in each group to show a significant difference in OXT levels.

Anthropomorphic data, clinical data, and inflammatory biomarkers were provided as mean and standard deviation or median and interquartile range, as appropriate. Pearson or Spearman correlation was used to correlate OXT levels with HbA1c, fasting plasma glucose, lipids, insulin, and inflammatory markers. The differences in means (or medians) were assessed using the ANCOVA (or Kruskal Wallis) test among three groups. Subsequently, post-hoc Bonferroni correction was applied to detect differences between each pair of groups.

3. Results

3.1 Clinical parameters in the four groups

All participants were Caucasians; the majority consisted of females (66%), and the mean age was 47.2 ± 13.4 years. Mean BMI was 33.3 ± 5.3 kg/m^2 . Almost half of the MetS patients were obese

Table 1. Demographic and clinical characteristics of the whole pool of study participants

Parameter	All (n = 235)	Control (n = 45)	All MetS pa- tients (n = 190)	MetS-only (n = 42)	MetS-prediabetes (n = 95)	MetS-T2DM (n = 53)	p ^a	p ^b	p ^c
Gender (% F)	66	60	67.9	46.3 ^a	72.1 ^b	60.4 ^b	< 0.001	< 0.001	-
Age (yr)	47.2(13.4)	29.4 (9.2)	51.2 (10.5)	46.8 (10.5) ^a	52.8 (9.8) ^{ab}	51.9 (10.8) ^{ab}	< 0.001	< 0.05	-
SBP (mmHg)	130.9 (20.5)	109 (11.7)	136.2 (18.6)	134.2 (15.1) ^a	133.9 (18.4) ^a	141.6 (20.45) ^{ac}	< 0.001	-	< 0.05
DBP (mmHg)	78.5 (11.9)	70.1 (10)	80.5 (11.4)	81.3 (9.5) ^a	79 (12.42) ^a	82.3 (10.8) ^a	< 0.001	-	-
BMI (kg/m ²)	31.2 (6.6)	22.3 (2)	33.3 (5.5)	32.8 (3.9) ^a	33.1 (5.1) ^a	34 (7.1) ^a	< 0.001	-	-
WC (cm)	85 (42.7)	79 (6.8)	105.0 (12.4)	104.6 (12.2) ^a	104.5 (12.7) ^a	106.3 (12.2) ^a	< 0.001	-	-
GFR (ml/min)	NA	NA	116.1 (39.7)	129.0 (42.3)	111.3 (33.3)	114.0 (47.7)	-	-	-
HbA1c (%)*	5.6 (5.2-6)	5.1 (5-5.3)	5.9 (5.1-6.7)	5.2 (4.88-5.5)	5.9 (5.5-6.3) ^{ab}	6.50 (5.5-7.5) ^{abc}	< 0.001	< 0.0001	< 0.0001
FPG (mg/dl)	107.3 (24.9)	90.2 (7.9)	111.4 (25.9)	89.5 (7.6)	105.1 (10.3) ^{ab}	143.9 (28.5) ^{abc}	< 0.001	< 0.0001	< 0.0001
TC (mg/dl)	189.1 (44.4)	154 (27.1)	199.1 (43.4)	199.6 (45.5) ^a	201.7 (42.9) ^a	193.6 (43.1) ^a	< 0.001	-	-
LDL-C (mg/dl)*	122.5 (97.8- 148.3)	91 (75.5-109)	134 (106-153)	126 (100-160) ^a	134 (109-151) ^a	134 (101-154) ^a	< 0.001	-	-
HDL-C (mg/dl)	47.2 (18.3)	46.5(9.7)	46.3 (20.4)	53.6 (33.9)	46.6 (14.02)	41.2 (11.4) ^{ac}	< 0.05	-	< 0.05
TG (mg/dl)*	128.4 (82-180)	63 (51-85.5)	142 (105-196)	127 (94.1- 173) ^a	141 (108-187) ^a	169 (114- 226) ^a	< 0.001	-	-
FI (mIU/ml)*	7.25 (5.1-18.8)	23.4 (20.5-29.2)	5.9 (4.9-8.9)	7.9 (5.1-11) ^a	5.8 (4.9-9.3) ^a	5.6 (4.7-7.2) ^a	< 0.001	-	-
HOMA-IR*	2 (1.4-4.2)	5.3 (4.5-6.9)	1.7 (1.3-2.4)	1.7 (1.2-2.1) ^a	1.5 (1.2-2.4) ^a	1.9 (1.6-2.4) ^{ab}	< 0.001	< 0.05	-

Legend: Data is represented as mean ± SD, unless mentioned differently. * Median (25th-75th percentile) was used to describe data that were not distributed normally. ^a Vs. controls. ^b Vs. MetS-only. ^c vs. MetS-prediabetes. *Abbreviations:* F – female, DBP – diastolic blood pressure, BMI – body mass index, GFR – glomerular filtration rate, FPG – fasting plasma glucose, FI – fasting insulin, TC – total cholesterol, LDL-C – low-density lipoprotein cholesterol, HDL-C – high-density lipoprotein cholesterol, TG – triglycerides, HOMA-IR – homeostatic model assessment insulin resistance, NA – not available, NS – not significant, SBP – systolic blood pressure, SD – standard deviation, IQR – inter-quartile range, WC – waist circumference.

(48.8%), while the rest were either morbidly obese (29.4%) or overweight (19.4%).

By definition, measures of glucose intolerance were significantly different between the three MetS groups. **Table 1** illustrates the differences between these groups in terms of their clinical parameters. HbA1c and FPG were significantly higher in the drug-naïve, newly diagnosed MetS-T2DM patients than in MetS-prediabetes and MetS-only subjects ($p < 0.0001$). HOMA-IR was significantly higher in MetS-T2DM patients than in the MetS-only group ($p < 0.05$). Except for systolic blood pressure (SBP) and HDL-C, the remaining clinical parameters (including glucagon) were not significantly different among the three MetS groups. SBP was significantly higher in MetS-T2DM than MetS-prediabetic patients ($p < 0.05$), while HDL-C was significantly lower in MetS-T2DM than in MetS-only patients ($p < 0.05$).

3.2 OXT and plasma levels of inflammatory markers

After controlling for SBP, HDL-C, sex, and age, ANCOVA was performed to compare the levels of both OXT and inflammatory markers among the four groups. The differences in inflammatory marker levels are illustrated in **Table 2**. There were significant differences in OXT and pro-inflammatory cytokine levels between all MetS patients and controls, but not in glucagon levels.

The inflammatory markers hsCRP, PAI-1, resistin, LAR, TNF- α , TSP-1, and MIF were significantly higher in both MetS groups (prediabetic and T2DM patients) vs. MetS-only patients. PAI-1 was also significantly higher in the MetS-T2DM group compared to the MetS-prediabetics. Leptin and MMP-9 were significantly higher in the MetS-T2DM group (but not in MetS-prediabetics) vs.

Table 2. Comparisons between levels of inflammatory markers among the study groups

Marker	All (n = 235)	Control (n = 45)	MetS-only (n = 42)	MetS-prediabetes (n = 95)	MetS-T2DM (n = 53)	p ^a	p ^b	p ^c
hs-CRP (mcg/ml)	605.5 (456.9)	1565.1 (1478.1)	249.9 (140.5) ^a	344.5 (149.6) ^{ab}	409.8 (129.4) ^{ab}	< 0.001	<0.01	-
MCP-1 (pg/ml)	173.2 (117.9)	150.2 (45.4)	267.8 (136.3) ^a	162.1 (128.3) ^b	142.9 (96.3) ^b	< 0.001	< 0.0001	-
PAI-1 (pg/ml)	6973.1 (6830.8)	12524.4 (10445.1)	1301.6 (306.6) ^{a1}	5756.8 (4077.5) ^{a1,b}	7830.9 (4209.7) ^{a2,b,c}	< 0.001 ^{a1} < 0.05 ^{a2}	< 0.0001	< 0.0001
MMP-9 (ng/ml)	616.9 (326.4)	51.1 (24.3)	511.8 (333.8) ^a	756.0 (587.1) ^a	993.6 (758.7) ^{ab}	< 0.001	<0.05	-
Resistin (pg/ml)	38914.2 (43221.1)	6333.3 (2047.5)	5634.7 (4217.6)	57744.9 (44710.8) ^{a1,b}	65461.2 (41814.4) ^{a2,b}	< 0.05 ^{a1} < 0.001 ^{a2}	< 0.0001	-
Adiponectin (ng/ml)	5071.95 (2816.41)	6567.5 (3061.9)	6932.3 (723.6)	3910.9 (2533.1) ^{ab}	2892.9 (2112.1) ^{ab,c}	< 0.001	<0.0001	< 0.05
Leptin (ng/ml)	27.1 (23.1)	2.8 (1.6)	27.6 (13.6) ^a	32.4 (26.2) ^a	40.1 (31.8) ^{ab}	< 0.001	<0.05	-
LAR*	0.005 (0.001- 0.010)	0.0005 (0.0003- 0.0006)	0.004 (0.002-0.005) ^{a1}	0.019 (0.002-0.026) ^{a1,b}	0.021 (0.006-0.034) ^{a2,b}	< 0.01 ^{a1} < 0.001 ^{a2}	<0.001	-
TNF- α (pg/ml)	5.21 (1.8)	2.1 (1.7)	3.2 (1.9)	6.6 (2.03) ^{ab}	7.3 (2.6) ^{ab}	< 0.001	< 0.001	-
TSP-1 (ng/ml)	613.7 (435.6)	470 (100.9)	324.8 (136) ^a	700.4 (419.49) ^{ab}	819.1 (622.01) ^{ab}	< 0.001	< 0.001	-
MIF (ng/ml) ^{*y}	99.8 (24.6- 177.3)	1.47 (1.14- 2.15)	74.0 (46.5-132) ^a	130 (42.5-224) ^{ab}	143 (99.5-217) ^{ab}	< 0.001	< 0.05	-
IL-10 (pg/ml) ^{*y}	6.03 (4.50- 7.83)	2.85 (2.15- 5.15)	8.14 (7.1-11.1) ^a	6.20 (4.70-7.99) ^{ab}	5.6 (5.1-7.1) ^{ab}	< 0.001	< 0.05	-
IL-6 (pg/ml)	1.6 (0.81)	1.89 (1.03)	1.81 (0.9)	1.38 (0.94)	1.5 (1.2)	NS	NS	NS
Glucagon (pg/ml) [*]	27.9 (14.6- 38.9)	19 (6.6- 27.8)	27.2 (12.8-37)	32.43 (19.6-41.5) ^{a1}	34.7 (22.5-48.1) ^{a2}	< 0.05 ^{a1} < 0.001 ^{a2}	-	-
OXT (pg/ml)	2.3 (0.9)	3.9 (0.38)	2.3 (0.9) ^a	1.4 (0.8) ^{ab}	1.4 (0.8) ^{ab}	< 0.001	< 0.0001	-

Legend: The data is shown as mean \pm SD, unless mentioned differently. * Median (25th-75th percentile) was used to describe data not distributed normally. ^y ANCOVA test was performed using log values. ^a Vs. control. ^b Vs. MetS-only. ^c Vs. MetS-prediabetes. **Abbreviations:** hsCRP – high-sensitivity C-reactive protein, IL-6 – interleukin 6, IL-10 – interleukin 10, LAR – leptin-to-adiponectin-ratio, MCP-1 – macrophage chemo-attractant protein 1, MIF – macrophage migration inhibitory factor, MMP-9 – matrix metalloproteinase 9, OXT – oxytocin PAI-1 – plasminogen activator inhibitor 1, TNF- α – tumor necrosis factor α , TSP-1 – thrombospondin 1.

MetS-only subjects. Conversely, adiponectin, OXT, MCP-1, and IL-10 were significantly lower in MetS-T2DM patients and MetS-prediabetics vs. MetS-only subjects (**Table 2**).

3.3 Correlations between OXT, clinical parameters, glucose intolerance, and inflammatory markers

The correlations between OXT levels, inflammatory markers, and indices of glucose intolerance were analyzed in the whole pool of study participants. Plasma OXT levels in the MetS pool of participants correlated inversely with both HbA1c and FPG ($r = -0.439$ and -0.178 , $p < 0.001$, respectively, **Table 3**). No relationship of OXT with glucagon or any other clinical parameters in the study pool of

MetS subjects could be detected. OXT also correlated substantially with each of the following inflammatory markers in the entire MetS pool of participants (as shown in **Table 3**):

- Positively: MCP-1, IL-10, and IL-6
- Negatively: PAI-1, MMP-9, TNF- α , TSP-1, and MIF

Resistin, adiponectin, leptin, and LAR correlated inversely with OXT in the entire pool of MetS patients (**Table 3**).

4. Discussion and conclusions

The objective of this study was to evaluate the potential association of OXT and inflammatory

markers in diabetic, prediabetic, and non-diabetic MetS patients. We demonstrated that OXT is significantly higher in MetS patients with T2DM and prediabetes compared to those with MetS alone (i.e., normal glucose tolerance).

Previous studies showed that OXT increases insulin release from a perfused rat pancreas via vasopressin V1 or OXT receptors [21]. This finding indicates a potential stimulatory effect of OXT on insulin release from beta-cells, and suggests a therapeutic role in diabetes mellitus, which was confirmed later by studies in animals [1-3, 11] and humans [3, 19]. Zhang *et al.* evaluated the effect of OXT nasal spray on weight loss and metabolic improvement in human patients with obesity [3]. While OXT treatment did not affect fasting blood glucose or insulin levels, it tended to reduce post-prandial glucose and insulin levels towards healthier profiles within the normal ranges. In addition, OXT treatment significantly reduced serum LDL-C and cholesterol levels, and tended to increase serum HDL-C levels. In a recent study by Qian *et al.*, a total of 176 patients were enrolled, including 88 subjects with newly diagnosed T2DM and 88 subjects with normal glucose tolerance (NGT) [19]. The subjects were divided into four groups, namely NGT-normal weight vs. NGT-obese and T2DM-normal weight vs. T2DM-obese. Similar to our findings, the T2DM group had lower OXT concentrations (7.2 pg/ml) than the NGT group (9.2 pg/ml) ($p < 0.001$). Plasma OXT levels (pg/ml) were decreased in T2DM (1.4 ± 0.8) compared to prediabetics (1.4 ± 0.8) vs. MetS-only patients (2.3 ± 0.8 , $p < 0.0001$), and OXT levels correlated inversely ($p < 0.001$) with BMI, waist circumference, HbA1c, FPG, SBP, TG, LDL, and TC. In contrast, in our study, OXT correlated negatively only with HbA1c and FPG in the entire MetS population ($r = -0.439$, $n = 182$, and -0.178 , $n = 176$, $p < 0.001$, respectively). These findings confirm the positive effects of OXT in glycemic control.

While the effect of OXT in animal models is common, there is limited evidence in humans. Only one study on obesity, in which blood glucose, insulin, and lipid levels were evaluated, assessed the effect of intranasal OXT in healthy individuals [3]. However, this study excluded patients with diabetes. No study to date has evaluated the relationship between OXT and measures of either insulin sensitivity or glucose tolerance in diabetic individuals. Therefore, our study is the first clinical trial to evaluate the relationship between OXT levels and insulin sensitivity in drug-naïve diabetic patients with MetS. Also, it is the first study to evaluate the relationship between a wide array

Table 3. Correlates of OXT with clinical parameters and inflammatory markers in the entire pool of participants

Parameter/marker	R _{all} (n = 235)	R _{allMetS} (n = 190)
Age	0.181 [†]	-0.084
SBP	0.153*	-0.065
DBP	0.130	-0.019
Waist circumference	0.309 [†]	-0.016
GFR	NA	0.137
FPG	0.019	-0.178 [†]
HbA1c	-0.125	-0.439 [†]
Total cholesterol	0.157*	-0.036
LDL-C	0.232 [†]	0.106
HDL-C	-0.008	-0.040
Triglycerides	0.196 [†]	0.058
Fasting insulin	-0.168*	0.069
HOMA-IR	-0.189 [†]	-0.019
Glucagon	-0.001	-0.164
MCP-1	0.237 [†]	0.2375 [‡]
PAI-1	-0.402 [†]	-0.483 [†]
MMP-9	-0.003	-0.239 [‡]
Resistin	-0.258 [†]	-0.551 [†]
Adiponectin	-0.268 [†]	-0.506 [†]
Leptin	-0.015	-0.249 [‡]
LAR	-0.118	-0.383 [†]
TNF- α	-0.163*	-0.415 [†]
TSP-1	-0.205 [‡]	-0.320 [†]
MIF	0.105	-0.386 [†]
IL-10	0.428 [†]	0.445 [†]
IL-6	0.104	0.279 [‡]

Legend: * $p < 0.05$. [‡] $p < 0.01$. [†] $p < 0.0001$. **Abbreviations:** DBP – diastolic blood pressure, FPG – fasting plasma glucose, GFR – glomerular filtration rate, HbA1c – glycated hemoglobin, HDL-C – high-density lipoprotein cholesterol, HOMA-IR – homeostatic model assessment insulin resistance, IL-6 – interleukin 6, IL-10 – interleukin 10, LAR – leptin-to-adiponectin-ratio, LDL-C – low-density lipoprotein cholesterol, MIF – macrophage migration inhibitory factor, MCP-1 – macrophage chemo-attractant protein 1, MIF – macrophage migration inhibitory factor, MMP-9 – matrix metalloproteinase 9, OXT – oxytocin, PAI-1 – plasminogen activator inhibitor 1, SBP – systolic blood pressure, TNF- α – tumor necrosis factor α , TSP-1 – thrombospondin 1.

of inflammatory markers of MetS. Our study showed a negative correlation between pro-inflammatory cytokines (PAI-1, MMP-9, resistin, leptin, TNF- α , TSP-1, and MIF) and adiponectin. A positive correlation with the anti-inflammatory cytokines, namely IL-10, IL-6, and MCP-1, was detected. Our study confirms previous studies which showed that OXT can attenuate atherosclerotic inflammatory processes in male rats [22], and reduce inflammation in ApoE^{-/-} mice [8].

The study by Ahmad *et al.* showed that exogenous OXT administration decreased plasma levels of IL-6, MCP-1, and CRP, and decreased oxidative stress, as indicated by increased plasma levels of nitric oxide and glutathione and decreased plasma levels of malondialdehyde in the blood [8]. Moreover, adipose tissues of OXT-treated mice had lower IL-6 levels than vehicle-treated ones [22]. Impressively, hyperglucagonemia and glucose production were linked with prediabetes and diabetes pathogenesis [23-24]. The same applied to the stimulatory effect of OXT on glucagon secretion [25]. However, no OXT-glucagon relationship could be found in the present study.

In our study, patients were recruited from two large medical centers in Jordan. This ensured that enough patients were included to comprise a fairly representative sample of the Jordanian population. However, the results may not be generalizable because of the lack of statistics regarding the

prevalence of MetS in the Jordanian population. Nevertheless, this study included MetS patients who were apparently healthy. However, due to its cross-sectional nature, a causative relationship between OXT levels and glycemic control or inflammatory markers cannot be drawn. Therefore, more studies need to be performed to evaluate the effect of OXT or OXT analogues on glycemic control indices and inflammatory markers. The results imply that OXT and its analogues could be a novel potential therapeutic option to alleviate diabetes and MetS-related inflammation.

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