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# Evaluation of Oxytocin (OXT), Endothelin-1 and Nesfatin Plasma Concentrations in Newly-diagnosed Diabetic and Non-diabetic Patients with Metabolic Syndrome

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## ABSTRACT

Oxytocin (OXT) is implicated as a novel therapy of obesity-diabetes. Nesfatin is an anorexigenic adipokine linked to improve insulin sensitivity and dysglycemia in obese/T2DM mice, while endothelin-1 (ET-1) is an endothelium vasoconstrictor that is dysregulated in metabolic insulin resistance. The aim of this study was to investigate OXT, ET-1, and nesfatin plasma levels and the correlation between these biomarkers and the various metabolic parameters in the human. In a cross-sectional study, MS-subjects attended the National Center for Diabetes Endocrinology and Genetics were enrolled based on their blood glucose levels into (82 MS-non-diabetic vs. 89 MS-pre/diabetic patients). Plasma OXT, ET-1 and nesfatin levels were measured by competitive binding and sandwich enzyme-linked immunosorbent assays (ELISA). When MS-pre/T2DM patients were compared to MS-controls, plasma OXT concentrations (pg/mL) were significantly lower ($P < 0.001$) (mean ± SD; 1206.28 ± 507.68 vs. 2224 ± 871.22); nesfatin plasma levels (ng/mL) were significantly higher ($P < 0.01$) (1.04 ± 2.20 vs. 0.31 ± 0.25); while no differences were observed in ET-1 (pg/mL) plasma levels ($P > 0.05$) (4.21 ± 4.19 vs. 4.01 ± 3.51). In conclusion, the present study is the first one which demonstrates an increase in nesfatin concentrations in MS-pre/diabetic patients vs. MS-non-diabetic. Our study reported a decrease in OXT levels in MS-pre/T2DM compared to MS-control. Besides, ET-1 concentrations had no significant difference between non-diabetic and diabetic-MS patients, serum OXT concentrations correlated with several clinical parameters; this is suggestive of OXT as a pharmacologic agent that opposes weight gain and improves insulin resistance.

**Keywords**: Metabolic syndrome (MS), Oxytocin, Endothelin-1 (ET-1), Nesfatin, Type 2 Diabetes Mellitus (T2DM), Enzyme-Linked Immunosorbent Assay (ELISA).

## 1. INTRODUCTION

Oxytocin (OXT) is produced by hypothalamic OXT neurons; it is a nine-amino acid neuropeptide that is released locally in the brain or systemically. OXT acts systemically to mediate reproductive activities of females including laboring and lactation [(6)]. Interestingly, multiple pharmacologic activities of OXT and its analogs were reported including controlling weight, lipid profile, insulin sensitization, insulin secretion and thus its potentials of being developed as a new class of small peptides for treating obesity as well as diabetes that is related or unrelated to obesity[(7)]. Furthermore, the circadian release of OXT from posterior pituitary after being synthesized in hypothalamus directs hypothalamic

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regulation of feeding circadian rhythms to maintain body weight homeostasis. Disturbance of this control system underlies obesity development. Indeed, environmental over-nutrition, such as chronic fat-enriched diet, is a major contributor to an impaired circadian release pattern of OXT, leading to altered feeding rhythms and body weight imbalance [8].

At the cellular level, balance between vasodilator and vasoconstrictor actions determines the vascular response to insulin [9]. Endothelial insulin resistance is typically accompanied by reduced nitric oxide (NO)-dependent vasodilator actions and an intact or heightened endothelin-1 (ET-1)-dependent vasoconstrictor actions [10]. The vascular ET-1 system activity is increased in insulin-resistant states such as obesity, type 2 diabetes mellitus (T2DM), and metabolic syndrome (MS). And it is enhanced secondary to abnormalities in vascular insulin signaling, as well as to changes in visceral and perivascular adipose tissue (PVAT), and may contribute to the pathogenesis of both insulin resistance and vascular dysfunction/damage. In healthy individuals, PVAT seems to have anticontractile effect and this dilator effect was lost in obese patients. PVAT hypertrophy secondary to obesity is associated with reduced partial oxygen pressure, an increase in the production of inflammatory cytokines such as TNF-α and IL-6, and elevation of reactive oxygen species. Thus, oxidative stress and hypoxia may promote imbalance in the production of vasoactive compounds and may affect vascular homeostasis by activating the ET-1 system [11].

In 2006, nesfatin was discovered and introduced as a potential novel anorexigenic modulator of food intake and body weight by its interaction with other brain transmitters to exert its food consumption inhibitory effect [12]. In nesfatin relation with T2DM/MS, functional *in vitro* studies demonstrated that nesfatin stimulates the pre-proinsulin mRNA expression and increases the glucose-induced insulin release in rat and mouse isolated islets or cultured cells. Extending these findings, NUCB2/ nesfatin released by pancreatic cell was found to be dependent on glucose concentration[13]. This stimulation was not observed when islets cells were incubated with nesfatin in low glucose concentrations. Whereas, incubation in high glucose concentrations resulted in a fourfold increase in nesfatin release [14]. Glucolipotoxic conditions, in which glucose and fatty acid are elevated, enhance the NUCB2 gene expression and this correlates with insulin gene expression, as well as with insulin secretory capacity [15]. Taken together, insulin resistance could be a possible reason for the elevation of nesfatin levels in T2DM patients, thus nesfatin acts as a potent anorexigenic factor that improves insulin resistance and opposes weight gain [16].

There are no previous studies to evaluate the relationship between OXT and endothelin-1 and nesfatin levels in T2DM patients with MS. Thus, our study is the first clinical study to evaluate the link between MS biomarkers (endothelin-1 and nesfatin) and plasma OXT levels in the metabolic syndrome-diabetic patients.

In case of establishing the relationship between OXT levels and obesity-diabetes biomarkers dysregulation (endothelin 1 and nesfatin), it would be possible to provide a therapeutic suggestion about the neuropeptide OXT intervention as an anti-obesity and anti-diabetic.

## 2. Experimental

### 2.1 Study design

This was a cross sectional study to measure blood levels of OXT and MS biomarkers (endothelin-1 and nesfatin). The sample was obtained using convenient sampling technique. The MS-pre/T2DM group consisted of 100 newly diagnosed anti-diabetic drug-naïve patients with prediabetes or T2DM attending diabetes clinics, while the MS-control group consisted of 100 MS patients without diabetes attending nutrition and cardiology clinics at the National Center for Diabetes Endocrinology and Genetics (NCDEG). According to Adult Treatment Panel Third Report (ATP III), (2001), MS disorder is defined by the three or more of the following contributing factors: abdominal obesity defined by waist circumference > 35 inches or 88 cm in women and > 40 inches or 102 cm in men, blood pressure (BP) >130/ 85 mmHg, triglyceride (TG) > 150 mg/dL, low fasting high

density lipoprotein (HDL) < 40 mg/dL in men or < 50 mg/dL in women, and blood glucose (BG) levels of > 100 mg/dL.

Females who are pregnant or breast-feeding (lactating) were excluded from this study. Patients with prior treatment with anti-diabetic agent either for diabetes itself or for any other condition associated with hyperglycemia, patients with clinical evidence of life-threatening disease, alcohol/drug abuse or recently diagnosed untreated endocrine disorder, any individuals with known inflammatory or autoimmune conditions such as the inflammatory bowel disease, and patients with obesity secondary to endocrine derangement other than DM were as well excluded.

**2.2 clinical setting and duration**

The study started after obtaining approval from the Scientific Research Committee at the Faculty of Pharmacy and approval from the National Center for Diabetes, Endocrinology and Genetics Institutional Review Board (IRB) Committee. The informed consent was obtained from each of the participants. The study was undertaken over the course of one visit where patient's anthropometric data (height, weight, waist circumference) and blood pressure were measured. The biochemical analysis of HbA1c, fasting blood glucose, and fasting lipid profile (TG, low density lipoprotein (LDL), total cholesterol and HDL) were obtained from medical files for each consented patient. Clinical information such as risk factors of cardiovascular disease (CVD) and DM including; history of delivery of baby weighing > 4 kg, polycystic ovary syndrome (PCOS), smoking, physical activity, and family history of DM and CVD were obtained as well. Blood samples were collected in lithium heparin tubes and centrifuged at $0^0$ C, and at speed of 2000 revolutions per minutes (rpm) for 10 minutes to obtain plasma samples then it were stored at -$80^0$ C until biochemical analysis.

**2.3 Laboratory assay work principles for the MS-biomarkers**

Measurement of human OXT, and nesfatin was performed using human competitive binding ELISA kit *in vitro* (Abcam® OXT ELISA kit, USA), (RayBiotech. Inc., human nesfatin ELISA, USA), respectively. On the other hand, the measurement of ET-1 was performed using human sandwich ELISA kit (Abcam® human Endothelin-1 ELISA, USA). The lithium heparinized plasma samples of both study groups were subjected to the ELISA determination of the biomarkers levels. The ELISA plate was read at O.D. absorbance of 570 and 590 for OXT, and at 450 nm wavelengths for ET-1 and nesfatin using the ELISA plate reader (Bio-Tek® Instruments, Winooski, VT, USA). Each sample was assayed, the standard curve was obtained and the plasma concentration of the biomarkers was expressed as pg/ml.

**2.4 Statistics**

Data were coded and entered into Statistical package for the Social Science software (SPSS), release 20 (SPSS® Inc., Chicago, IL). Clinical, biochemical parameters and biomarkers were expressed as mean and standard deviation (SD) in case of continuous variables and by frequencies with percentages in case of categorical variables. Independent-sample t-test and Chi-square test were used as appropriate. All probabilities were two-tailed, and $P$ values < 0.05 were regarded as statistically significant.

## 3. Results

**3.1. Study participants**

The total number was 171 participants. The MS-control subjects were matched to MS-pre/T2DM subjects by age, gender distribution, and BMI categories. Figure 1 displays the study flow chart.

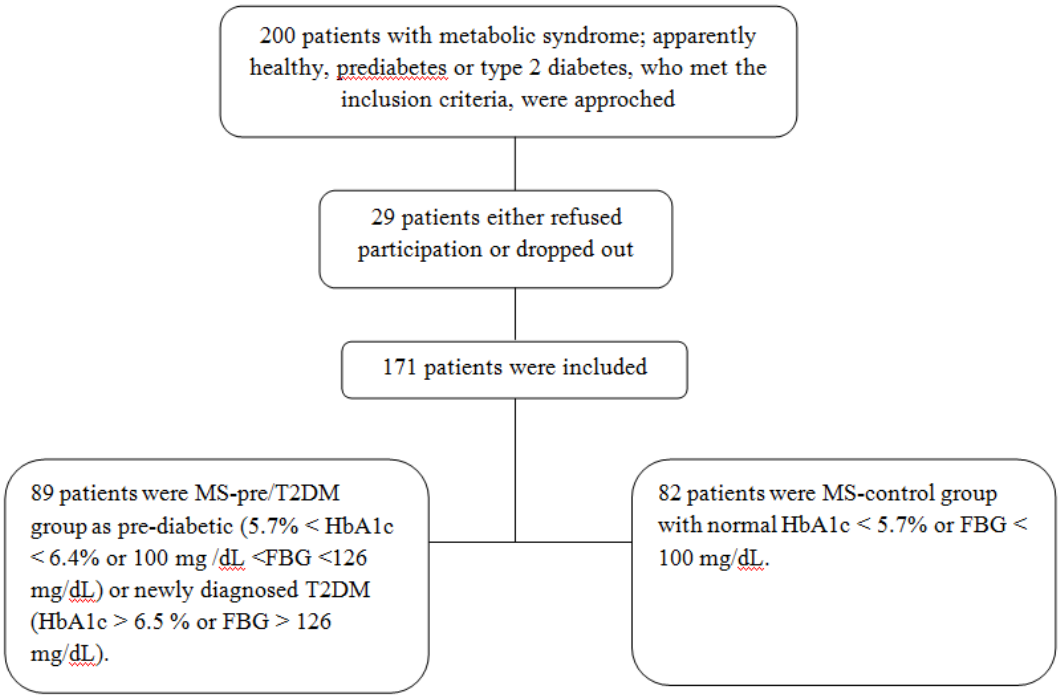


**Figure 1. The study flow chart**

### 3.2 Patients demographic data

The demographic characteristics of the study sample are summarized in Table 1. All patients were Jordanian residents, the majority of the participants were females (67.2%), and the mean age was 51.05 ± 10.72 years. The later coincides with the postmenopausal women average age in Jordan as stated elsewhere 49.6 ± 3.64 years old[17]. Breuli, *et al.* (2014) could correlate the low OXT levels with women' postmenopausal state (N=1097) [18]. However, the assessment of postmenopausal state was beyond the scope of this study. Nearly half of the patients were obese (53.3%), (29.9%) were morbidly obese and (15%) were overweight; with only (1.8%) of patients having normal body mass index (BMI); mean BMI was (33.206 ± 5.53) kg/m$^2$. There was no significant difference in the demographic characteristic of patients (mean age and BMI, BMI categories and gender) between the MS-control and MS-pre/T2DM, which ascertains the homogeneity of participants' pool.

### 3.3 Patients clinical characteristics

The clinical characteristics of the study sample are summarized in Table 2. The systolic blood pressure (SBP) and diastolic blood pressure (DBP) were significantly higher in the MS-pre/T2DM as compared to MS-controls ($P$ = 0.034 and 0.012, respectively).

The $HbA_1c$ was higher ($P$ = 0.044) in the MS-pre/T2DM group (8.58% ± 14.75) than in the MS-control group (5.31% ± 0.37). Similarly, the measured fasting plasma glucose (FPG) was significantly higher ($P$ < 0.001) in the MS-pre/T2DM group (121.06 ± 32.07) mg/dL than in the MS-control group (101.77 ± 18.96) mg/dL. The waist circumference and fasting lipid profile parameters (total cholesterol, LDL, HDL, and TG) had no significant differences between the two groups (Table 2).

**Table 1. Demographic characteristics of patients in the total sample and study groups**

| **Clinical parameter** | **Total sample [a] N= 171** | **MS-pre/T2DM n= 89** | **MS-Controls n= 82** | ***P*** **[b]** |
|---|---|---|---|---|
| Age in years (mean ± SD) | 51.05 ± 10.72 | 51.9 ± 11.345 | 50.17 ± 10.01 | 0.293 |
| Gender, N(%)[a] | | | | |
| Male | 57 (32.8) | 29 (32.6) | 28 (32.9) | 0.960^ |
| Female | 117 (67.2) | 60 (67.4) | 57 (67.1) | |
| BMI (kg/m$^2$) (mean ± SD) | 33.206 ± 5.53 | 33.7 ± 5.21 | 33.05 ± 5.07 | 0.414 |
| BMI category, N(%)[a] | | | | |
| Normal weight | 3 (1.8) | 1 (1.2) | 2 (2.4) | |
| Overweight | 25 (15) | 12 (14.3) | 13 (15.7) | 0.587^ |
| Obese | 89 (53.3) | 42 (50) | 47 (56.6) | |
| Morbidly obese | 50 (29.9) | 29 (34.5) | 21 (25.3) | |

[a] Percent within total. [b] *P*-value by independent-sample t-test for age and BMI and ^ by Pearson Chi-square test between both study groups. BMI: Body mass index, SD: Standard deviation.

**Table 2. Clinical characteristics of participants in the total sample and the study groups**

| **Clinical parameter** | **Total sample, N= 174, (mean ± SD)** | **MS-pre/T2DM n= 89, (mean ± SD)** | **MS-Controls n= 85, (mean ± SD)** | ***P*** **[a]** |
|---|---|---|---|---|
| **SBP (mmHg)** | **135.75 ± 19** | **138.42 ± 20.54** | **132.11 ± 18.16** | **0.034** |
| **DBP (mmHg)** | **81.05 ± 11.99** | **83.31 ± 12.57** | **78.76 ± 10.89** | **0.012** |
| Waist circumference (cm) | 104.46 ± 11.8 3 | 104.03 ± 16.51 | 103.77 ± 11.38 | 0.905 |
| **Serum creatinine (mg/dL)** | **0.711 ± 0.21** | **0.83 ± 0.62** | **0.66 ± 0.19** | **0.026** |
| **HbA$_1$c (%)** | **6.28 ± 5.905** | **8.58 ± 14.75** | **5.31 ± 0.37** | **0.044** |
| **FPG (mg/dL)** | **111.26 ± 27.07** | **121.06 ± 32.07** | **101.77 ± 18.96** | **< 0.001** |
| Total cholesterol (mg/dL) | 199.12 ± 47.94 | 195.28 ± 49.08 | 202.78 ± 47.13 | 0.406 |
| LDL-C (mg/dL) | 138.08 ± 74.96 | 135.57 ± 37.53 | 139.63 ± 102.22 | 0.736 |
| HDL-C (mg/dL) | 45.47 ± 12.77 | 46.02 ± 23.80 | 47.17 ± 13.08 | 0.709 |
| TG (mg/dL) | 174 .15 ± 139.21 | 168.78 ± 85.01 | 179.95 ± 180.74 | 0.611 |
| **Oxytocin (pg/mL)** | **1709.15 ± 873.41** | **1206.28 ± 507.68** | **2224 ± 871.22** | **< 0.001** |
| **Nesfatin (ng/mL)** | **0.709 ± 1.66** | **1.04 ± 2.20** | **0.31 ± 0.25** | **0.005** |
| Endothelin-1 (pg/mL) | 4.11 ± 3.87 | 4.21 ± 4.19 | 4.01 ± 3.51 | 0.740 |

[a]*P*-value by independent-sample t-test between both study groups. DBP: diastolic blood pressure, SBP: systolic blood pressure, FPG: Fasting Plasma Glucose, HbA1c: Hemoglobin Glycosylated $A_1C$, HDL-C: High Density Lipoprotein Cholesterol, LDL-C: Low Density Lipoprotein Cholesterol, TG: Triglycerides, SD: Standard deviation.

**3.4 MS biomarkers (OXT, nesfatin, and endothelin-1) levels**

The clinical biomarkers shown in Table 2, OXT was significantly ($P$ < 0.001) lower, and nesfatin was significantly ($P$ = 0.005) higher in the MS-pre/T2DM patients as compared to MS-controls. On the other hand, endothelin-1 levels ($P$ = 0.740) did not differ between the study groups.

## 4. DISCUSSION

***Unprecedentedly, our study has the largest sample size compared to other similar studies.*** Beside, our study demonstrated for the first time the plasma nesfatin levels in MS patients. The ELISA kits were highly specific for the measurement of the selected plasma MS biomarkers. This study is the first step of three-stage study, in the other two steps further analysis will be done to find out the correlation between the biomarkers, and to do further gender-based analysis. Our study could have the novelty in case of establishing the relationship between OXT levels and obesity-diabetes biomarkers (endothelin 1 and nesfatin), and it would be possible to provide a therapeutic suggestion about the neuropeptide OXT and/or, similarly, ET-1 and nesfatin intervention as an anti-obesity and anti-diabetic. Additional possible correlations of metabolic biomarkers with clinical parameters; such as SBP, DBP, waist circumference, HbA1c, BMI, lipid profile in both MS-control and MS-pre/T2DM groups were investigated.

**4.1. OXT findings**

Recently, Qian, et al., [19] study enrolling 176 patients, including 88 subjects with newly diagnosed T2DM vs. 88 subjects with normal glucose tolerance (NGT) were allocated. All participants were divided based on BMI into four subgroups: T2DM-obese, T2DM-normal weight, NGT-obese, and NGT-normal weight [19]. With regard to our study; the emphasis was more directed into comparisons with T2DM-obese and NGT-obese subgroups (Table 3).

In the clinical trial of Qian, *et al*.,[19] 88 obese patients were divided into two subgroups based on HbA1c; T2DM-obese and NGT-obese, HbA1c was higher in T2DM than in NGT subjects (9.25 ± 1.91% and 5.34 ± 0.31%, respectively, $P$ < 0.01) [19]. Similar results were reported in our study for MS-pre/T2DM (8.58 ± 14.75%) and for MS-NGT (5.31 ± 0.37%) ($P$ = 0.044). In our study, serum OXT concentrations were decreased in MS-pre/T2DM (1206.28 ± 507.68) pg/mL compared to MS-NGT (2224 ± 871.22) pg/mL ($P$ < 0.001). These results were consistent with a similar results showing that the levels of OXT in T2DM patients (7.16 (6.45– 8.82)) pg/mL were significantly decreased ($P$ < 0.01) compared with NGT (9.23 (8.16 -10.36)) pg/mL [19]. The difference of OXT levels between our study and Qian study could be justified by using different unit. In our study, OXT levels were expressed by pg, while it were expressed by ng in Qian study. Furthermore, using different kits, in our study the measurement of human OXT was performed using human ELISA kit (Abcam® OXT ELISA kit, USA), while in Qian, OXT levels was measured using a commercially available human ELISA kit (human ELISA kit, IBL, Germany).

**4.2. ET-1findings**

Mather, *et* al.,[20] investigated the role of ET-1 in MS patients. Twenty eight subjects were divided into 3 subgroups: 8 lean subjects, 12 obese subjects, and 8 subjects with T2DM. The comparison between our results of ET-1 with those in the study by Mather, *et al.*,[20] is shown in Table 3.

The effect of ET-1 blockade produced no significant difference in obese and T2DM. ET-1 concentrations did not differ between obese and T2DM patients (13.7 ± 1.5 and 14.6 ± 2.7 pg/mL, respectively, $P$ = 0.15) [20]. Likewise, our results confirmed that ET-1 concentration was statistically indifferent between MS-control and MS-pre/T2DM groups (4.01 ± 3.51 and 4.21 ± 4.19 pg/mL, respectively, $P$ = 0.74).

**4.3. Nesfatin findings**

A cross-sectional study by Zhang, *et al*., [21]

investigated the plasma nesfatin levels, enrolling 74 patients with newly diagnosed T2DM and 73 subjects with NGT. The comparison between our results of nesfatin with the above study is shown in Table 3.

The plasma nesfatin levels in Zhang, *et al*., [21] study were higher in T2DM (1.91 ± 0.10) ng/mL subjects than the NGT-controls (1.40 ± 0.10) ng/mL ($P < 0.01$). Interestingly, Zhang, *et al*., [21] recruited patients who were not metabolic syndrome patients; rather diabetic patients vs. healthy controls. ***Therefore, the present study is the first one which investigates nesfatin levels in MS-pre/diabetic patients vs. MS-non-diabetic patients.*** Our results were comparable to Zhang, *et al.* (2012) where nesfatin concentrations were higher in MS-pre/T2DM than in MS-control apparently healthy subjects (1.04 ± 2.20 and 0.31 ± 0.25 ng/mL, respectively, $P = 0.005$).

As conflicting reports about plasma nesfatin dysregulation in association with MS were principally inconclusive; some animal studies reported that plasma nesfatin levels were significantly increased in diet-induced obese mice compared to controls [16, 22]. In contrary, other studies reported a decrease in nesfatin levels among T2DM patients. Differences in study design, including patient selection (obese vs. lean, diet type, glycemic level) may have contributed to these discrepancies. Li, *et al*., [23] studied patients at various stages of the disease, some of them had macrovascular and microvascular complications and had antidiabetic medications; including oral hypoglycemic drugs and insulin, which could have affected the release of nesfatin. In contrast, our study patients were newly diagnosed T2DM and were not treated with any antidiabetic drugs. In addition to its anorexigenic effects, endogenous nesfatin peptide was most recently implicated, via nesfatin neutralizing antibodies, in the regulation of food intake, thermogenesis and energy expenditure [24]. Also, nesfatin treatment proved effective in decreasing blood glucose and insulin resistance, and improving lipid disorder in diabetes mice [25]. This can clearly be linked to its elevated concentrations in T2DM and impaired glucose tolerance subjects [21] and, most importantly, in our pool of MS-participants, possibly, as a compensatory mechanism.

### 4.4 Correlation between the MS biomarkers and the clinical parameters in MS-participants

OXT correlated negatively with FPG and HbA1c (r = -0.293, $P < 0.001$ and r = -0.465, $P < 0.001$, respectively), and nesfatin correlated positively with HbA1c (r = -0.463, $P < 0.001$), while ET-1 did not correlate with HbA1c (r = -0.116, $P = 0.145$). In addition, OXT correlated negatively while ET-1 correlated positively with serum creatinine (r = -0.189, $P = 0.019$ and r = 0.174, $P = 0.036$, respectively) in the total MS-population.

HbA1c had positive correlation with FPG and serum creatinine (r = 0.508, $P < 0.001$ and r = 0.197, $P = 0.014$, respectively). Interestingly, HDL had negative correlation with both FPG and TG (r = -0.175, $P = 0.034$ and r = -0.399, $P < 0.001$, respectively). DBP correlated positively with SBP (r = 0.537, $P < 0.001$), waist circumference (r = 0.195, $P = 0.029$), BMI (r = 0.169, $P = 0.011$) and serum creatinine (r = 0.159, $P = 0.047$).

In the MS-control group, ET-1 correlated negatively with BMI (r = -0.246, $P = 0.032$), similarly to the whole MS-population DBP correlated positively with SBP and waist circumference (r = 0.582, $P < 0.001$ and r = 0.234, $P = 0.034$, respectively) (Appendix6- Table 4-a).

In the MS-pre/diabetic group, OXT correlated positively with LDL (r = 0.309, $P = 0.005$). ET-1 correlated negatively with age, BMI and HbA1c (r = -0.291, $P = 0.007$, r = -0.225, $P = 0.043$ and r = -0.256, $P = 0.020$), and positively with serum creatinine (r = 0.259, $P = 0.022$). HbA1c correlated positively with FPG (r= 0.527, $P < 0.001$), and DBP correlated positively with SBP (r = 0.484, $P < 0.001$).

**Table 3. Comparison between the results of OXT, ET-1, and nesfatin in our study with the results of other studies**

| Parameters, Mean±SD | OXT | | | | ET-1 | | | | Nesfatin | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Findings by Qian *et al.*, 2014 | | Our findings | | Findings by Mather *et al.*, 2002 | | Our findings | | Findings by Zhang *et al.*, 2012 | | Our findings | |
| | T2DM-obese | NGT-obese | MS-pre/T2DM | MS-Controls | T2DM-obese | NGT-obese | MS-pre/T2DM | MS-Controls | T2DM | NGT | MS pre/T2DM - | MS-Controls |
| Age (Years) | 46.19 ± 11.06 | 45.21 ± 9.24 | 51.9 ± 11.345 | 50.17 ± 10.01 | 44.5 ± 3.5 | 33.7 ± 2.6 | 51.9 ± 11.345 | 50.17 ± 10.01 | 54 ± 11 | 51 ± 7 | 51.9 ± 11.345 | 50.17 ± 10.01 |
| Gender N (%)[a] Male Female | 46 18 (39.1) 28 (60.9) | 42 13 (30.9) 29 (69.1) | 89 29 (32.6) 60 (67.4) | 85 28 (32.9) 57 (67.1) | 8 5 (62.5) 3 (37.5) | 12 8 (66.7) 4 (33.3) | 89 29 (32.6) 60 (67.4) | 85 28 (32.9) 57 (67.1) | 74 39 (52.7) 35 (47.3) | 73 36 (49.3) 37 (50.4) | 89 29 (32.6) 60 (67.4) | 85 28 (32.9) 57 (67.1) |
| BMI (kg/m$^2$) | 27.49 ± 2.03 | 27.78 ± 2.66 | 33.7 ± 5.21 | 33.05 ± 5.07 | 38.1 ± 2.7* | 34.2 ± 3.2 | 33.7 ± 5.21 | 33.05 ± 5.07 | 25.0 ± 3.7 | 24.5 ± 3.6 | 33.7 ± 5.21 | 33.05 ± 5.07 |
| SBP (mmHg) | 128.39 ± 14.48 | 128.93 ± 16.03 | 138.42 ± 20.54* | 132.11 ± 18.16 | | | 138.42 ± 20.54* | 132.11 ± 18.16 | - | - | 138.42 ± 20.54 | 132.11 ± 18.16* |
| DBP (mmHg) | 79.72 ± 6.36 | 82.64 ± 12.67 | 83.31 ± 12.57* | 78.76 ± 10.89 | | | 83.31 ± 12.57* | 78.76 ± 10.89 | - | - | 83.31 ± 12.57 | 78.76 ± 10.89* |
| Waist circumference (cm) | 94.03 ± 4.21 | 96.02 ± 9.53 | 104.03 ± 16.51 | 103.77 ± 11.38 | - | - | 104.03 ± 16.51 | 103.77 ± 11.38 | - | - | 104.03 ± 16.51 | 103.77 ± 11.38 |
| Serum creatinine (mg/dL) | - | - | 0.83 ± 0.62* | 0.66 ± 0.19 | - | - | 0.83 ± 0.62* | 0.66 ± 0.19 | - | - | 0.83 ± 0.62 | 0.66 ± 0.19* |
| HbA$_1$c (%) | 9.25 ± 1.91* | 5.34 ± 0.31 | 8.58 ± 14.75* | 5.31 ± 0.37 | 10.0 ± 1.3 | - | 8.58 ± 14.75* | 5.31 ± 0.37 | 8.8 ± 2.4 | 5.5 ± 0.4* | 8.58 ± 14.75 | 5.31 ± 0.37* |
| FPG (mg/dL) | 175.68 ± 49.68* | 90.18 ± 8.28 | 121.06 ± 32.07* | 101.77 ± 18.96 | 172.81 ± 9.8* | 97.2± 3.6 | 121.06 ± 32.07* | 101.77 ± 18.96 | 196.2 ± 68.4 | 97.2 ± 7.2* | 121.06 ± 32.07 | 101.77 ± 18.96* |
| Total cholesterol (mg/dL) | 197.21 ± 45.24 | 176.334 ± 25.52 | 195.28 ± 49.08 | 202.78 ± 47.13 | 174.01± 7.73 | 154.67±7.73 | 195.28 ± 49.08 | 202.78 ± 47.13 | 203.01 ± 54.13 | 186.77 ± 32.09 | 195.28 ± 49.08 | 202.78 ± 47.13 |
| LDL-C (mg/dL) | 128.38 ± 39.82 | 111.36 ± 23.58 | 135.57 ± 37.53 | 139.63 ± 102.22 | 108.27± 7.73 | 108.27 ± 3.86 | 135.57 ± 37.53 | 139.63 ± 102.22 | 116.39 ± 42.15 | 104.02 ± 26.29 | 135.57 ± 37.53 | 139.63 ± 102.22 |
| HDL-C (mg/dL) | 41.37 ± 7.34 | 46.79 ± 12.37 | 46.02 ± 23.80 | 47.17 ± 13.08 | 34.80 ± 3.86 | 38.66 ± 3.86 | 46.02 ± 23.80 | 47.17 ± 13.08 | 49.88 ± 26.68 | 49.11 ± 11.21 | 46.02 ± 23.80 | 47.17 ± 13.08 |
| TG (mg/dL) | 231.17± 125.77 | 157.66 ± 82.37 | 168.78 ± 85.01 | 179.95 ± 180.74 | 212.57± 26.57 | 194.86 ± 35.42 | 168.78 ± 85.01 | 179.95 ± 180.74 | 184.23 ± 138.17 | 118.68 ± 60.23* | 168.78 ± 85.01 | 179.95 ± 180.74 |
| Oxytocin (pg/mL) | 7.16 (6.45–8.82)* | 9.23 (8.16 - 10.36) | 1206.28 ± 507.68* | 2224 ± 871.22 | 14.6 ± 2.7 | 13.7 ± 1.5 | 4.21 ± 4.19 | 4.01 ± 3.51 | 1.9 ± 0.1 | 1.4 ± 0.1* | 1.04 ± 2.20 | 0.31 ± 0.25* |

[a] Percent within total. * Significant differences between study groups (P<0.05).

In conclusion, the present study is the first one which demonstrates an increase in nesfatin concentrations in MS-pre/diabetic patients vs. MS-non-diabetic, our study reported a decrease in OXT levels in MS-pre/T2DM compared to MS-control. Besides, ET-1 concentrations had no significant difference between non-diabetic and diabetic-MS patients, confirming the results of the few earlier studies, serum OXT concentrations correlated with several clinical parameters; this is suggestive of OXT as a pharmacologic agent that opposes weight gain and improves insulin resistance.

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## REFERENCES

(1) American Diabetes Association (ADA), Diagnosis and classification of diabetes mellitus. *Diabetes Care.* 2014; 37 (1), S81-S90.

(2) Expert Panel on the Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. Executive summary of the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). *JAMA.* 2001; 285, 2486-2497.

(3) Samuel V.T. and Shulman G.I. Mechanisms for insulin resistance: common threads and missing links. *Cell.* 2012; 148, 852-871.

(4) Alberti K.G., Eckel R.H., Grundy S.M., Zimmet P.Z., Cleeman J.I., Donato K.A., Fruchart J.C., James W.P., Loria C.M. and Smith S.C. Jr. Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. *Circulation.* 2009; 120, 1640–1645.

(5) McArdle M.A., Finuance O.M., Connaughton R.M., McMorrow A.M. and Roche H.M. Mechanisms of obesity induced inflammation and insulin resistance: insights into the emerging role of nutritional strategies. *Front Endocrinol*. 2013; 4(52), 1-23.

(6) Soloff M.S., Alexandrova M. and Fernstrom M.J. Oxytocin receptors: triggers for parturition and lactation**.** *Science.* 1979; 204, 1313–1315.

(7) Zhang H., Wu C., Chen Q., Chen X., Xu Z., Wu J. and Cai, D. Treatment of obesity and diabetes using oxytocin or analogs in patients and mouse models. *PLoS One.* 2013; 8 (5), e61477.

(8) Zhang G. and Cai D. Circadian intervention of obesity development via resting-stage feeding manipulation or oxytocin treatment. *Am J Physiol Endocrinol Metab.* 2011; 301, E1004–E1012.

(9) Baron A.D., Laakso M., Brechtel G. and Edelman S.V. Mechanism of insulin resistance in insulin-dependent diabetes mellitus: a major role for reduced skeletal muscle blood flow. *J - Clin Endocrinol Metab*. 1991; 73(3), 637-43.

(10) Muniyappa R. and Sowers J.R. Role of insulin resistance in endothelial dysfunction. *Rev Endocr Metab Disord.* 2013; 14, 5-12.

(11) Campia U., Tesauro M., Di Daniele N. and Cardillo C. The vascular endothelin system in obesity and type 2 diabetes: Pathophysiology and therapeutic implications. *Life Sci.* 2014; 118 (2), 149-155.

(12) Stengel A., Mori M. and Tache Y. The role of nesfatin-1 in the regulation of food intake and body weight: recent developments and future endeavors. *Obes Rev.* 2013; 14, 859-870.

(13) Gonzalez R., Perry R.L., Gao X., Gaidhu M.P., Tsushima R.G., Ceddia R.B. and Unniappan S. Nutrient responsive nesfatin-1 regulates energy balance and induces glucose-stimulated insulin secretion in rats. *Endocrinology.* 2011a ; 152, 3628-3637.

(14) Gonzalez R., Reingold B.K., Gao X., Gaidhu M.P., Tsushima R.G. and Unniappan S. Nesfatin-1 exerts a direct, glucose-dependent insulinotropic action on mouse islet beta- and MIN6 cells. *J Endocrinol*. 2011b; 208, R9–R16.

(15) Riva M**.,** Nitert M**.**D**.,** Voss U**.,** Sathanoori R., Lindqvist A**.,** Ling C. and Wierup N. Nesfatin-1 stimulates glucagon and insulin secretion and beta cell NUCB2 is reduced in human type 2 diabetic subjects. *Cell Tissue Res*. 2011; 346, 393-405**.**

(16) Ramanjaneya M., Chen J., Brown J.E., Tripathi G., Hallschmid M., Patel S., Kern W., Hillhouse E.W., Lehnert H., Tan B.K. and Randeva H.S. Identification of nesfatin-1 in human and murine adipose tissue: A novel depot-specific adipokine with increased levels in obesity. *Endocrinology.* 2010; 151, 1358-1363.

(17) Gharaibeh M., Al-Obeisat S. and Hattab J. Severity of menopausal symptoms of Jordanian women. *Climacteric*. 2010; 13 (4), 385-394.

(18) Breuil V., Panaia-Ferrari P., Fontas E., Roux C., Kolta S., Eastell R., Ben Yahia H., Faure S., Gossiel F., Benhamou C.L., Euller-Ziegler L. and Amri E.Z. Oxytocin, a new determinant of bone mineral density in post-menopausal women: analysis of the OPUS cohort. *J Clin Endocrinol Metab*. 2014; 99(4), E634-E641.

(19) Qian W., Zhu T., Tang B., Yu S., Hu, H., Sun W., Pan R., Wang J., Wang D., Yang L., Mao C., Zhou L. and Yuan G. Decreased circulating levels of oxytocin in obesity and newly diagnosed type 2 diabetic patients. *J Clin Endocrinol Metab*. 2014; 99(12), 4683-4689.

(20) Mather K.J., Mirzamohammadi B., Lteif A., Steinberg H.O. and Baron A.D. Endothelin contributes to basal vascular tone and endothelial dysfunction in human obesity and type 2 diabetes. *Diabetes*. 2002; 51, 3517-

3523.

(21) Zhang Z., Li L., Yang M., Liu H., Boden G. and Yang G. Increased plasma levels of nesfatin-1 in patients with newly diagnosed type 2 diabetes mellitus. *Exp Clin Endocrinol Diabetes*. 2012; 120, 91-95.

(22) Ogiso K., Asakawa A., Amitani H., Nakahara T., Ushikai M., Haruta I., Koyama K., Amitani M., Harada T., Yasuhara D. and Inui A. Plasma nesfatin-1 concentration in restricting-type anorexia nervosa. *Peptide.* 2011; 32, 150-153.

(23) Li Q.C., Wang H.Y., Chen X., Guan H.Z. and Jiang Z.Y. Fasting plasma levels of nesfatin-1 in patients with type 1 and type 2 diabetes mellitus and the nutrient-related fluctuation of nesfatin-1 level in normal humans. *Regul Pept.* 2010; 159, 72-77.

(24) Wilz A.M., Wernecke K., Burmeister L., Lehnert H. and Schulz C. Endogenous nesfatin-1 is involved in the regulation of food intake and energy expenditure. *Exp Clin Endocrinol Diabetes.* 2015; 123-OP2_3, DOI:10.1055/s-0035-1547609

(25) Dong J., Xu H., Xu H., Wang P.F., Cai G.J., Song H.F., Wang C.C., Dong Z.T., J. and Jiang Z.Y. Nesfatin-1 stimulates fatty-acid oxidation by activating AMP-activated protein kinase in STZ-induced type 2 diabetic mice. *PLoS One*. 2013; 8 (12), e83397.

# تقييم تركيز الاوكسيتوسين، الإندوثيلين-1، والنيسفاتين في بلازما الدم لمرضى للاضطراب الأيضي المشخصين حديثا بمرض السكري والغير مصابين بالسكري

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## ملخص

**التمهيد والأهداف:** إن الأوكسيتوسين يعد كعامل علاجي جديد ضد مرض السكري والسمنة. النيسفاتين هو أديبوكاين جديد مفقد للشهية اللذي يرتبط بتحسين حساسية الأنسولين ومستوى السكر في الفئران المصابة بالسكري الذين يعانون من السمنة. أيضا، الإندوثيلين-1 هو مضيق للأوعية واختلاله يكون في حالات مقاومة الأنسولين الأيض. مع ذلك، لم يتم التحقق ما إذا كان هناك علاقة تربط بين الأوكسيتوسين مع كل من النيسفاتين والإندوثيلين-1 في مرضى الاضطراب الأيضي والسكري من النوع الثاني أو المرضى في مرحلة ما قبل السكري.

**الطرق:** في دراسة مسحية، التحق من مرضى الاضطراب الأيضي (82 شخص غير مصابين بالسكري و89 شخص من المصابين بالسكري أو ما قبل السكري). تم قياس مستويات الأوكسيتوسين، الإندوثيلين-1، والنيسفاتين عن طريق الفحص المناعي للربط الإنزيمي التنافسي. لقد تم تقييم العلاقة بين تلك المؤشرات الحيوية والخضائص السريرية مثل نسبة السكر التراكمي، سكر صيام الدم، ضغط الدم، مستوى الدهون في الدم، محيط الخصر، ومؤشر كتلة الجسم.

**النتائج**: حين تم مقارنة مرضى الاضطراب الأيضي المصابين بالسكري بالمرضى غير مصابين بالسكري، قل مستوى الأوكسيتوسين في البلازما ($P<0.001$) (المتوسط ± الانحراف المعياري؛ 1206.28 ±507.68 مقارنة مع 2224 ± 871.22 بيكوغرام /مل). مستويات النيسفاتين في البلازما كانت مرتفعة ($P<0.01$) (1.04 ± 2.20 مقارنة مع 0.31 ± 0.25 نانوغرام /مل). مع ذلك، لم توجد اختلافات في مستويات الأندوثيلين-1 في البلازما ($P> 0.05$) (4.21± 4.19 مقارنة مع 4.01 ± 3.51 بيكوغرام/مل). في جميع مرضى الاضطراب الأيضي؛ مستوى الأوكسيتوسين ارتبط عكسيا بالنيسفاتين (معامل الإرتباط سبيرمان = 0.297-، $P<0.001$). ولكن لم يرتبط مع الأندوثيلين-1 (معامل الإرتباط سبيرمان = 0.008-، $P= 0.922$). على نحو مماثل، عندما قمنا بدراسة كل من الجنسين على حدا، مستوى الأوكسيتوسين ارتبط عكسيا بالنيسفاتين (معامل الإرتباط سبيرمان =0.346-، $P <0.001$). ولكن لم يتبط مع الأندوثيلين-1 (معامل الإرتباط سبيرمان =0.030 , $P= 0.744$) في مرضى الاضطراب الأيضي الإناث. في إجمالي العينة، تراكيز الأوكسيتوسين كانت مرتبطة عكسيا بسكر صيام الدم (معامل الإرتباط بيرسون = 0.293- , $P < 0.001$) لم تلاحظ أي فروقات في المؤشرات الحيوية بين الذكور والإناث سواء في مرضى الاضطراب الأيضي الغير مصابين بالسكري ($P > 0.05$) أو في مرضى مرضى الاضطراب الأيضي المصابين بالسكري ($P > 0.05$).

الخلاصة: الأوكسيتوسين ارتبط عكسيا مع النيسفاتين، ولكن لم يرتبط مع الأندوثيلين-1 في جميع مرضى الاضطراب الأيضي وفي إناث مرضى الاضطراب الأيضي. مستوسات الأوكسيتوسين قلّت والنيسفاتين ارتفعت في مرضى الاضطراب الأيضي المصابين بالسكري مقارنة بالمرضى الغير مصابين بالسكري، في حين لم تختلف مستويات الأندوثيلين-1 بين المجموعتين. هنالك حاجة إلى المزيد من الأبحاث لتوضيح دور هذه المؤشرات الحيوية في مرضى الاضطراب الأيضي.

**الكلمات الدالة:** الاضطراب الأيضي، الأندوثيلين، النيسفاتين، المصابين بمرضى السكري.

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