Oxytocin as a novel therapeutic option for type I diabetes and diabetic osteopathy

[1]Elabd SK, [2]Sabry I, [1]Mohasseb M, [2]Algendy A

[1]Department of Physiology, Medical Research Institute, Alexandria University, Egypt
[2]Department of Zoology, Faculty of Science, Alexandria University, Egypt
E-mail: seham.elabd@alex-mri.edu.eg

Objective. The aim of the present study was to highlight the newly discovered metabolic role of oxytocin (OT) in the type I diabetic rats. Previous studies have demonstrated that OT has a beneficial role on bone physiology and therefore, the OT effect on the diabetic osteopathy will be assessed as well.

Methods. Induction of the type I diabetes was carried out by an intraperitoneal injection of 60 mg/kg body weight of streptozotocin. The metabolic role of OT on diabetic rats after OT treatment with intramuscular injection of 40 µIU/kg body weight for 6 weeks was assessed. Histological and ultrastructural studies of rat pancreas samples, before and after the OT injection, were performed and compared with the obtained physiological results.

Results. Oxytocin treatment had positive metabolic effects in diabetic rats. This is based on the change in glucose metabolism, lipid profile, and insulin sensitivity in experimental animals. In addition, OT treatment showed histological regenerative changes of pancreatic islet cells of diabetic rats. Moreover, OT administration showed that it has an anabolic effect on the bone biology.

Conclusions. The results suggest that activation of the oxytocin receptor (OTR) pathway by infusion of OT, OT analogs, or OT agonists may represent a promising approach for the treatment of diabetes and some of its complications, including diabetic osteopathy.

Key Words: diabetes mellitus, diabetic osteopathy, pancreas histology, streptozotocin, oxytocin, insulin, adiponectin, leptin

The type I diabetes mellitus is one of the most frequent chronic diseases in children and globally represents a public health challenge. Type I diabetes results from the autoimmune destruction of the insulin-producing β-cells in the pancreas. Genetic and yet undefined environmental factors act together to precipitate the disease. It eventually becomes a complex metabolic disease in which patients have insulin insufficiency, dysregulation of blood glucose control, persistent hyperglycemia, and long-term complications. The type I diabetes is associated with many complications including bone loss (Kuzuya 2002). As many as 50% of adults with the type I diabetes exhibit bone loss and are at increased risk for fractures (Motyl and McCabe 2009). The increased mortality rate associated with the complications of the type I diabetes and the increasing incidence of childhood type I diabetes emphasizes the importance of therapeutic strategies to prevent this chronic disorder.

Among the different hormonal factors affecting blood glucose level is the hormone oxytocin (OT). In addition to its well known effects during labor and lac-

Corresponding author: Elabd SK, 165, El-Horrya Avenue, El-Hadara-Alexandria, Egypt; phone: 4284318; fax: 4283719; e-mail: seham.elabd@alex-mri.edu.eg.

tation (Imamura et al. 2000; Svennersten-Sjaunja and Olsson 2005), several physiological and pathological functions have been governed directly or indirectly by OT (Lippert et al. 2003; Hashimoto et al. 2012). This makes OT and its receptor potential targets for drug therapy (Viero et al. 2010).

Oxytocin has been identified in human and rat pancreatic extracts (Gimpl and Fahrenholz 2001). It has been found that OT induces the release of glucagon and insulin from the pancreas (Gao et al. 1991; Bjorkstrand et al. 1996). In adipocytes, OT has an insulin-like activity. It stimulates glucose oxidation and lipogenesis (Gimpl and Fahrenholz 2001; Deblon et al. 2011). These insulin-like activities of OT are owing to its binding to oxytocin receptor (OTR) in adipocytes and not to the insulin receptor itself (Gimpl and Fahrenholz 2001). Furthermore, OT has been observed to antagonize the lipolytic effect induced by glucagon (Bjorkstrand et al. 1996; Fujiwara et al. 2007).

It has been recently shown that adipose tissue represents an important and highly metabolic active endocrine organ producing a large number of bioactive molecules (termed adipocytokines) regulating whole body metabolism and immune response (De-Heredia et al. 2012; Mc-Gown et al. 2014). Leptin and adiponectin are considered to belong among the most important adipocytokines. Recently, these two adipocytokines has been reported to possess various physiological activities (Kadowaki et al. 2008; Stofkova 2009; Mantzoros et al. 2011). It has been reported that both leptin and adiponectin accelerate fatty acid oxidation through adenosine monophosphate-activated protein kinases in peripheral tissues (Minokoshi et al. 2002; Yamauchi et al. 2002). It could be assumed that the balance of leptin and adiponectin in the body could influence the effect of antidiabetic drugs (Oda et al. 2008; Katsiki et al. 2011).

The present experiment aims to highlight the newly discovered metabolic role of OT in the type I diabetic rats. Previous studies have demonstrated that OT has a beneficial role on the bone physiology (Elabd et al. 2007). For that reason, the OT effect on diabetic osteopathy will be assessed as well. Histological and ultrastructural studies of rat pancreas samples, before and after injection of OT, will be performed and compared with the obtained physiological results.

Materials and Methods

Reagents. Commercial synthetic oxytocin "syntocinon" purchased from Novartis Co., Egypt and streptozotocin (STZ) purchased from SIGMA Chemicals Co., St. Louis, Mo., USA, were used in the present study. The original solution of OT (5 IU/ml) was diluted with saline (0.9% NaCl solution) to prepare a dose of 40 μIU/ml/ kg (Xie et al. 2003; Elabd et al. 2007). High dose of STZ (60 mg/kg) (Akbaradeh et al. 2007; Ragbetli and Ceylan 2010) were used in the present study; by freshly dissolved in cold citrate buffer (pH 4.5) (Arora et al. 2009).

Induction of type I diabetes. Type I diabetes was induced by intraperitoneal administration of 60 mg/kg STZ to normally fed rats (Akbaradeh et al. 2007; Ragbetli and Ceylan 2010). Rats were fasted for 12 h before induction of diabetes. The STZ-treated animals were given 5% glucose water for 24 h to prevent mortality due to the initial drug-induced hypoglycemia (Frode and Medeiros 2008). Estimation of fasting blood glucose level was carried out using a glucometer. Rats with blood glucose level over 250 mg/dl after 48-72 h of STZ administration were considered as diabetic (Gabir et al. 2000; Akbaradeh et al. 2007). Sever diabetic cases were excluded.

Experimental design. Male albino Wistar rats (n=30) kept on the normal diet were used. Animals were divided into three groups (10 per each), all kept under the same conditions and receiving the same diet. They were treated as follows:

Group I: Animals of this group received daily an intramuscular injection of vehicle (saline) for 6 weeks. This group was regarded as normal control group.

Group II: Includes 10 diabetic rats (Akbaradeh et al. 2007; Ragbetli and Ceylan 2010).

Group III: Animals of this group were daily treated with intramuscular injection of 40 μIU/ kg of OT for 6 weeks after induction of the type I diabetes. This group was described as a diabetic treated group (Xie et al. 2003; Elabd et al. 2007).

Serum measurements. At the end of experimental period, blood samples were collected by cardiac puncture from all animals and serum was separated. Fasting blood glucose was measured with the glucose oxidase method (Biosystems Co., Barcelona, Spain). Total cholesterol, triglycerides (TG), and high density lipoprotein (HDL) levels were determined with commercial kits (Biosystems Co., Barcelona, Spain) and low density lipoprotein (LDL) was calculated. Fasting blood insulin level, leptin, and adiponectin were measured (insulin, leptin: Ray Biotech, Inc.; adiponectin: B-Bridge International, Inc.), and the leptin/adiponectin (L/A) ratio was calculated. Serum levels of osteocalcin (OC) and

tartrate resistant acid phosphatase-5b (TRAP-5b), as bone related parameters, were measured with commercial kits (OC: Biomedical Technologies Inc., Stoughton, USA; TRAP-5b: Biomedical Company, Gate way Drive, Seattle, WA).

Specimens of pancreas tissue from experimental and control groups were processed for histological and ultrastructural studies.

Light microscope (LM) studies. Small specimens of pancreas tissue were fixed in 10% neutral buffered formalin for 24 h, washed in running tap water for 24 h, dehydrated in ascending series of ethyl alcohol, cleared in benzene, and embedded into paraffin wax. Sections of 5 μm thickness were cut using rotary microtome and stained with Haematoxylin and Eosin (H&E) (Bacroft 2002). Paraffin sections were brought down to distilled water, stained with Mayer's Haematoxylin for 7 min, counter stained with Eosin for 3 min, and mounted with Canadian balsam (Bacroft and Stevens 2002).

Electron microscope (EM) studies. Small pieces of pancreas tissue were fixed by immersion in formalin-glutraldehyde fixative (F_4G_1) in phosphate buffer solution (pH 7.2) at 4˚C for 3 h. Then the specimens were postfixed in 2% osmium tetroxide (OsO_4) in the same buffer at 4˚C for 2 h and washed with phosphate buffer before dehydration. Pancreatic specimens were dehydrated at 4˚C through an ascending series of ethyl alcohol. Propylene oxide was used to remove traces of alcohol. After evaporation of propylene oxide, specimens were embedded into an epon-araldite mixture labeled beam capsules. Semithin sections (1 μm thick) were stained with toluidine blue for light microscopic examination to identify the required area for ultrastructural study. Ultrathin sections (50-60 nm thick) were cut using LKB ultramicrotome, picked upon 200 mesh naked copper grids. Grids were stained using double stain of freshly prepared uranyl acetate (for 20 min) and lead citrate (for 5 min) (Glauert and Lewis 1998), and finally examined and photographed in Jeol-100CX transmission electron microscope in the Electron Microscope Unit, at the Faculty of Science, Alexandria University.

Statistical evaluation. Data were expressed as means ± SEM and were statistically analyzed with two ways ANOVA followed by LSD for multiple comparisons.
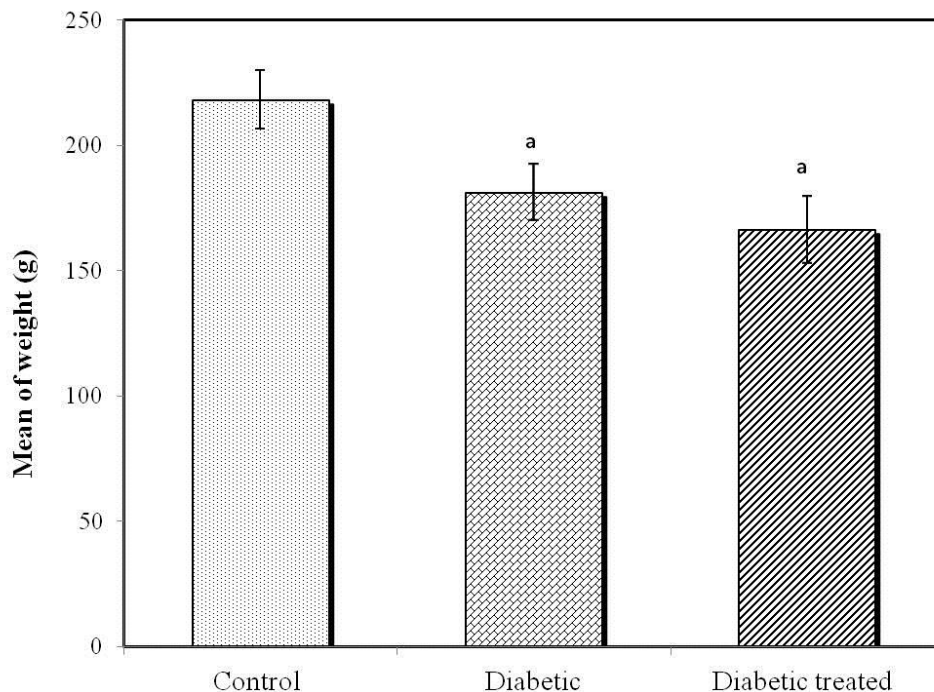

Fig. 1. Body weight (g) in untreated control, diabetic type I and diabetic rats treated with oxytocin (40 μIU/kg body weight) for six weeks. Each value represents the mean ± SD. a - statistically significant at p ≤ 0.05; p_a - p value of Post Hoc test (LSD) between control group (I) and each other group

Results

Body weight. In control male rats, body weight ranged between 200 and 240 g with a mean value 218.1 ± 11.57 g (Fig. 1). Induction of type I diabetes lead to a drop in body weight, as it ranged between 170 and 205 g with a mean value of 181.2 ± 10.91 g (p<0.001 vs. control group) (Fig. 1). In diabetic rats treated with OT daily for 6 weeks, the body weight ranged between 150 and 185 g with a mean value of 166.3 ± 13.18 g (p=0.13 vs. diabetic group) however, it was still significantly lower (p<0.001) than the control value (Fig. 1).

Fasting blood glucose concentration. In untreated control rats, fasting blood glucose concentration ranged between 89.0 and 215.0 mg/dl with a mean value 139.40 ± 4.25 mg/dl (Fig. 2). Induction of the type I diabetes lead to an increase in glucose concentration, as it ranged between 336.0 and 462 mg/dl with a mean value of 387.40 ± 42.45 mg/dl (p<0.001 vs. control group) (Fig.

2). In diabetic rats treated with OT daily for 6 weeks, the fasting blood glucose concentration ranged between 120 and 212 mg/dl with a mean value of 172.70 ± 15.79 mg/dl (p<0.001 vs. diabetic group), however, it was still significantly higher (p=0.001) than the control value (Fig. 2).

Lipid profile

Serum cholesterol concentration. In untreated control rats, serum cholesterol concentration ranged between 75.08 and 113.82 mg/dl with a mean value 91.88 ± 5.98 mg/dl (Fig. 3). Induction of the type I diabetes lead to an increase in serum cholesterol concentration, as it ranged between 124.70 and 139.48 mg/dl with a mean value of 131.83 ± 5.14 mg/dl (p<0.001 vs. control group) (Fig. 3). In diabetic rats treated with OT daily for 6 weeks, the serum cholesterol concentration ranged between 88.37 and 108.86 mg/dl with a mean value of 103 ± 3.60 mg/dl (p<0.001 vs. diabetic group)
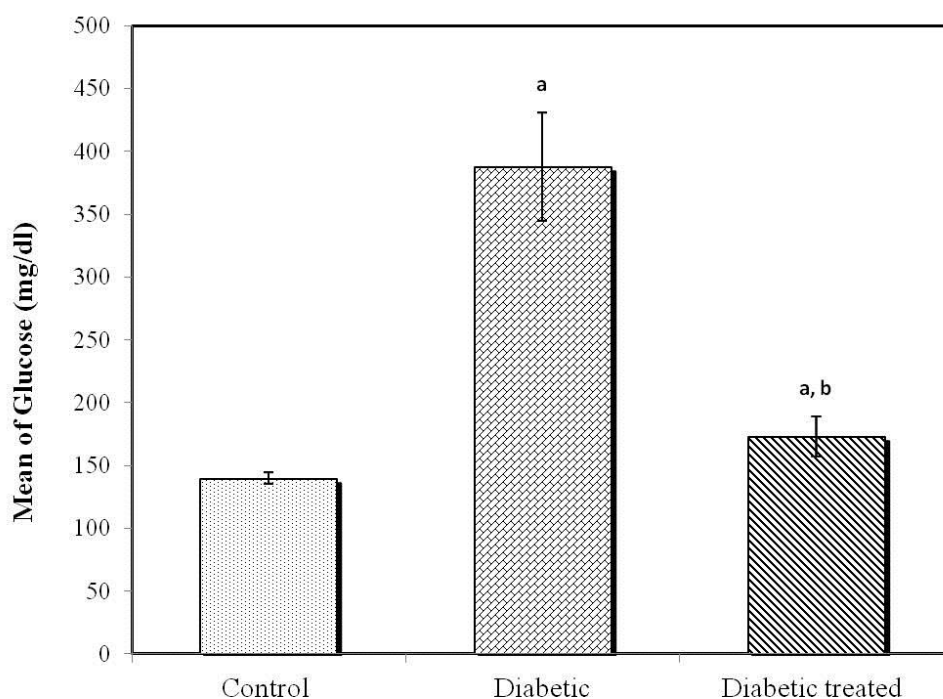

Fig. 2. Fasting blood glucose concentration (mg/dl) in untreated control, diabetic type I and diabetic rats treated with oxytocin (40 μIU/kg body weight) for six weeks. Each value represents the mean ± SD. a, b - statistically significant at p ≤ 0.05; p_a - p value of Post Hoc test (LSD) between control group (I) and each other group; p_b - p value of Post Hoc test (LSD) between group (II) and group (III)

however, it was still significantly higher (p<0.001) than the control value (Fig. 3).

Serum triglycerides (TG) concentration. In untreated control rats, serum TG concentration ranged between 53.48 and 85.08 mg/dl with a mean value 69.70 ± 9.51 mg/dl (Fig. 3). Induction of the type I diabetes lead to an increase in serum TG concentration, as it ranged between 164.0 and 196.0 mg/dl with a mean value of 180.30 ± 11.03 mg/dl (p<0.001 vs. control group) (Fig. 3). In diabetic rats treated with OT daily for 6 weeks, the serum TG concentration ranged be-

tween 81.0 and 113.0 mg/dl with a mean value of 97.80 ± 10.83 mg/dl (p<0.001 vs. diabetic group) however, it was still significantly higher (p=0.001) than the control value (Fig. 3).

Serum high density lipoprotein (HDL) concentration. In untreated control rats, serum HDL concentration ranged between 42.76 and 71.45mg/dl with a mean value 56.59 ± 9.30 mg/dl (Fig. 3). Induction of type I diabetes lead to drop in serum HDL concentration, as it ranged between 24.14 and 39.08 mg/dl with a mean value of 28.46 ± 4.19 mg/dl (p<0.001 vs. control group) (Fig. 3).
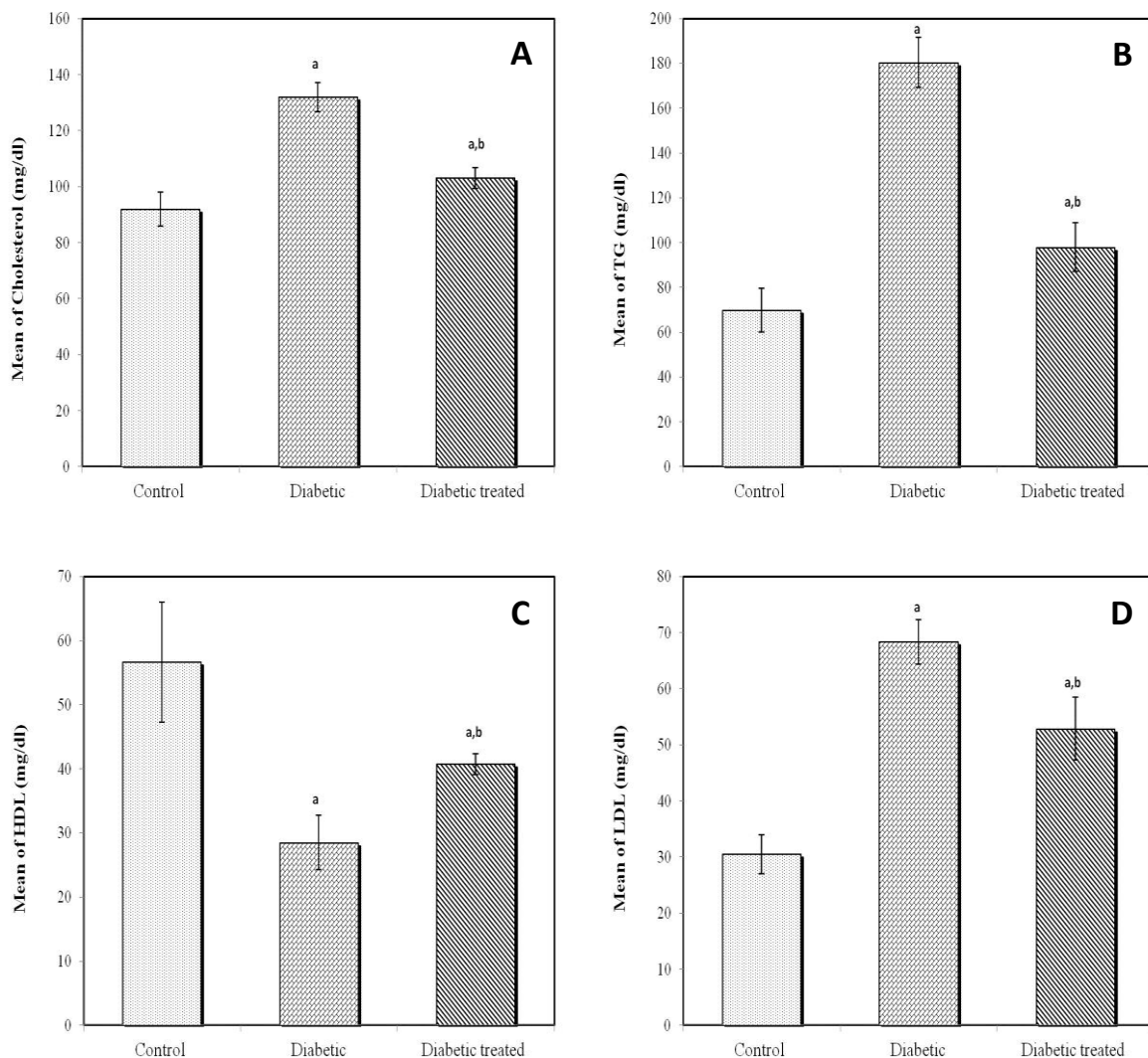

Fig. 3. Lipid profile: cholesterol (mg/dl) (A), triglyceride (TG) (mg/dl) (B), high density lipoprotein (HDL) (mg/dl) (C), low density lipoprotein (LDL) (mg/dl) (D) in untreated control, diabetic type I and diabetic rats treated with oxytocin (40 µIU/kg body weight) for six weeks. Each value represents the mean ± SD. a, b - statistically significant at p ≤ 0.05; p_a - p value of Post Hoc test (LSD) between control group (I) and each other group; p_b - p value of Post Hoc test (LSD) between group (II) and group (III)
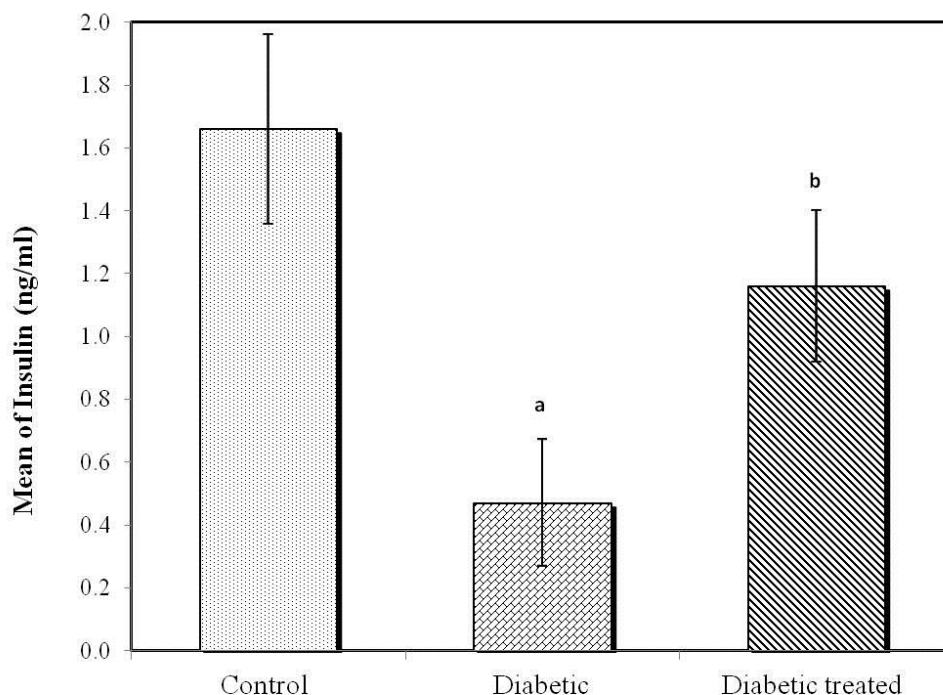
Fig. 4. Fasting blood insulin concentration (ng/ml) in untreated control, diabetic type I and diabetic rats treated with oxytocin (40 µIU/kg body weight) for six weeks. Each value represents the mean ± SD. a, b - statistically significant at $p \leq 0.05$; p_a - p value of Post Hoc test (LSD) between control group (I) and each other group; p_b - p value of Post Hoc test (LSD) between group (II) and group (III)

In diabetic rats treated with OT daily for 6 weeks, the HDL concentration ranged between 36.59 and 43.04 mg/dl with a mean value of 40.69 ± 1.59 mg/dl (p=0. 001 vs. diabetic group) however, it was still significantly lower (p<0.001) than the control value (Fig. 3).

Serum low density lipoprotein (LDL) concentration. In untreated control rats, serum LDL concentration ranged between 26.35 and 36.78 mg/dl with a mean value 30.45 ± 3.41 mg/dl (Fig. 3). Induction of type I diabetes lead to an increase in serum LDL concentration, as it ranged between 61.89 and 75.28 mg/dl with a mean value of 68.31 ± 3.92 mg/dl (p<0.001 vs. control group) (Fig. 3). In diabetic rats treated with OT daily for 6 weeks, the serum LDL concentration ranged between 42.50 and 58.33 mg/dl with a mean value of 52.78 ± 5.54 mg/dl (p<0.001 vs. diabetic group) however, it was still significantly higher (p<0.001) than the control value (Fig. 3).

Hormonal profile

Fasting blood insulin concentration. In untreated control rats, fasting blood insulin concentration ranged between 0.81 and 2.13 ng/ml with a mean value of 1.66 ± 0.3 ng/ml (Fig. 4). Induction of type I diabetes lead to drop in serum insulin concentration, as it ranged between 0.08 and 1.04 ng/ml with a mean value of 0.47 ± 0.2 ng/ml (p<0.001 vs. control group) (Fig. 4). In the diabetic rats treated with OT daily for 6 weeks, the serum insulin concentration ranged between 0.81 and 1.52 ng/ml with a mean value of 1.16 ± 0.24 ng/ml (p<0.001 vs. diabetic group) however, it was still lower (p=0.16) than the control value (Fig. 4).

Serum leptin concentration. In untreated control rats, serum leptin concentration ranged between 188.32 and 201.0 pg/ml with a mean value of 194.96 ± 4.70 pg/ml (Fig. 5). Induction of type I diabetes lead to an increase in serum leptin concentration, as it ranged between 234.57 and 252.43 pg/ml with a mean value of 242.70 ± 6.14 pg/ml (p<0.001 vs. control group) (Fig. 5). In diabetic rats treated with OT daily for 6 weeks, the serum leptin concentration ranged between 216.92 and 237.50 pg/ml with a mean value of 227.07 ± 6.69 pg/ml (p<0.001 vs. diabetic group) however, it was still significantly higher (p<0.001) than the control value (Fig. 5).

Serum adiponectin concentration. In untreated control rats, serum adiponectin concentration ranged between 95.69 and 114.56 ng/ml with a mean value of 101.61 ± 5.99 ng/ml (Fig. 5). Induction of type I diabetes lead to drop in serum adiponectin concentration, as it ranged between 14.81 and 72.21 ng/ml with a mean value of 27.99 ± 16.42 ng/ml (p=0.001 vs. control group) (Fig. 5). In diabetic rats treated with OT daily for 6 weeks, the serum adiponectin concentration ranged between 64.05 and 72.89 ng/ml with a mean value of 68.49 ± 3.18 ng/ml (p=0.002 vs. diabetic group) however, it was still significantly lower (p=0.002) than the control value (Fig. 5).

Leptin/adiponectin ratio. In untreated control rats, leptin/adiponectin ratio ranged between 1.71 and 2.07 with a mean value 1.92 ± 0.14 (Fig. 5). Induction of type I diabetes lead to an increase in leptin/adiponectin ratio, as it ranged between 3.50 and 16.11 with a mean value of 8.87 ± 2.69 (p<0.001 vs. control group) (Fig. 5). In diabetic rats treated with OT daily for 6 weeks, leptin/adiponectin ratio ranged between 3.07 and 3.74 with a mean value of 3.32 ± 0.15 (p<0.001 vs. diabetic group) however, it was still significantly higher (p=0.002) than the control value (Fig. 5).

Bone related parameters

Serum osteocalcin concentration. In untreated control rats, serum osteocalcin concentration ranged between 16.17 and 20.02 ng/ml with a mean value of 18.24 ± 1.30 ng/ml (Fig. 6). Induction of type I diabetes lead to drop in serum osteocalcin concentration, as it ranged between 6.91 and 12.31 ng/ml with a mean value of 9.26 ± 1.65 ng/ml (p<0.001 vs. control group) (Fig. 6). In diabetic rats treated with OT daily for 6 weeks, the serum osteocalcin concentration ranged between 10.25 and 16.35 ng/ml with a mean value of 13.49 ± 2.01 ng/ml (p<0.001 vs. diabetic group) however, it was still significantly lower (p<0.001) than the control value (Fig. 6).

Serum tartrate resistant acid phosphatase 5b (TRAP-5b) concentration. In untreated control rats, serum TRAP-5b concentration ranged between 7.83 and 12.19 ng/ml with a mean value of 10.0 ± 0.46 ng/ml (Fig. 6). Induction of type I diabetes lead to an increase in serum TRAP-5b concentration, as it ranged between 10.54 and 14.23 ng/ml with a mean value of 12.31 ± 1.02 ng/ml (p=0.043 vs. control group) (Fig. 6). In diabetic rats treated with OT daily for 6 weeks, the serum TRAP-5b concentration ranged between 8.56 and 13.40 ng/
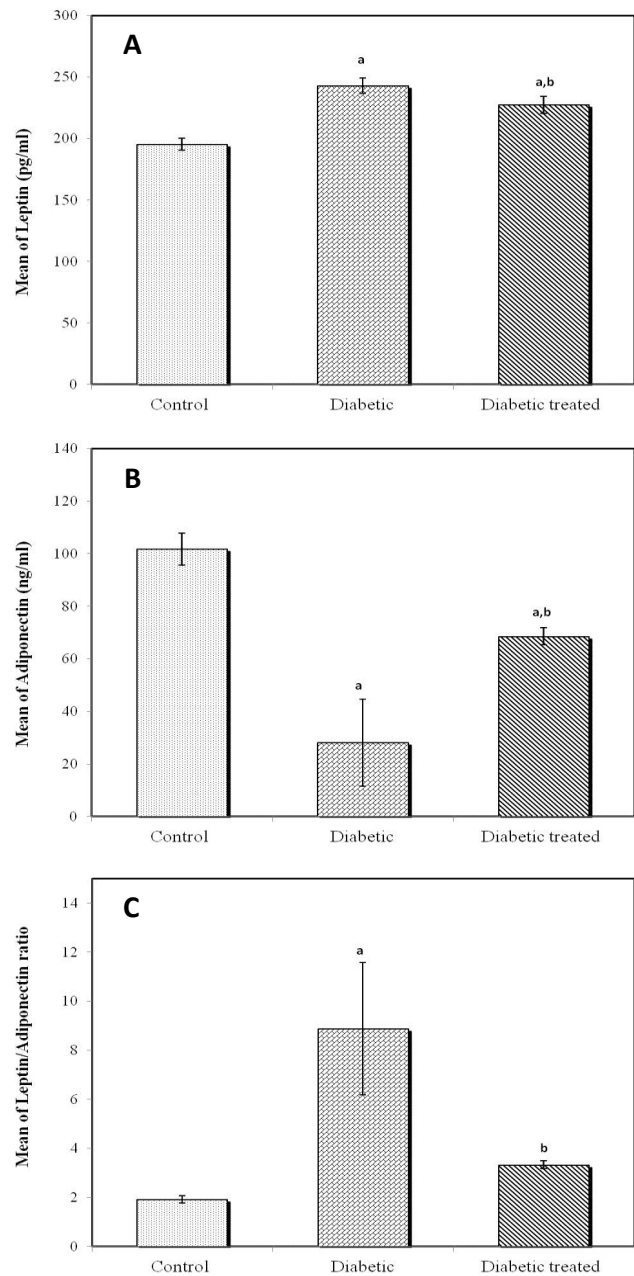

Fig. 5. Concentrations of leptin (pg/ml) (A), adiponectin (ng/ml) (B), and leptin/adiponectin ratio (L/A) (C) in untreated control, diabetic type I and diabetic rats treated with oxytocin (40 μIU/kg body weight) for six weeks. Each value represents the mean ± SD. a, b - statistically significant at p ≤ 0.05; p_a - p value of Post Hoc test (LSD) between control group (I) and each other group; p_b - p value of Post Hoc test (LSD) between group (II) and group (III)
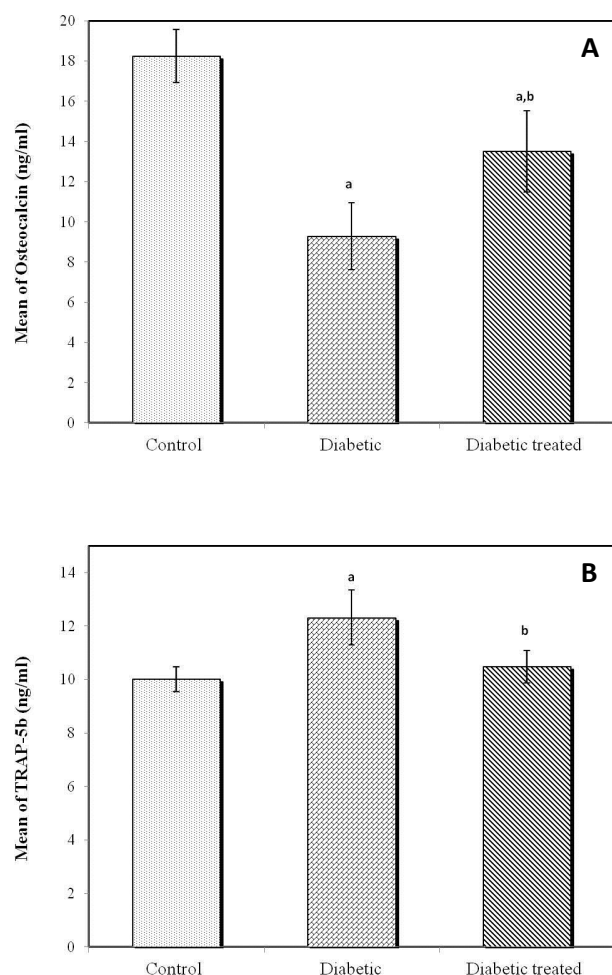
Fig. 6. Concentrations of osteocalcin (ng/ml) (A), Tartrate Resistant Acid Phosphatase-5b (TRAP-5b) (ng/ml) (B) in untreated control, diabetic type I and diabetic rats treated with oxytocin (40 µIU/kg body weight) for six weeks. Each value represents the mean ± SD. a, b - statistically significant at p ≤ 0.05; p_a - p value of Post Hoc test (LSD) between control group (I) and each other group; p_b - p value of Post Hoc test (LSD) between group (II) and group (III)

ml with a mean value of 10.47 ± 0.6 ng/ml (p=0.244 vs. control group; p=0.05 vs. diabetic group) (Fig. 6).

Histological and ultrastructural results. Histological examination of pancreas from control rats displayed normal features of the gland showing numerous exocrine (acini) and endocrine units (islets of Langerhans) (Fig. 7). Ultrastructurally, each islet consisted of 4 different cell types; β-cells (Fig. 10), α-cells, δ-cells, and PP-cells.

In the diabetic rats, the most consistent findings were the degenerative and apoptotic changes (Fig. 8). There were lymphatic infiltrations to the islet, where T-lymphocytes circulate, target, and invade the islets, a case known as "insulitis" and further proceed to destroy the islets (Fig. 8). The β-cells of diabetic rats had deformed nucleus with condensed chromatin that was sharply delineated and marginated along the nuclear membrane (Fig. 11). The ring chromatin condensation was also well observed and referred as "spheridium". In some cases, the nuclear membrane was ruptured and the nucleus underwent "karyorrhexis".

The cytoplasm contained prominent disorganized rough endoplasmic reticulum (rER), appearing with severely dilated cisternae, as well as dilated Golgi complex (Fig. 13). Mitochondria were often severely swollen with largely dispersed cristae and there was a mitochondrial vacuolization (Fig. 13). The insulin secretary granules were almost missing as compared to control (Fig. 10) and the remaining granules appeared small in size with an electron lucent or dense core. A thin or no halo between the core and the limiting membrane was evident (Fig. 11). Fibrosis also occurred, as indicated by the presence of collagen fibers both around and within several islets (Fig. 11). Occasional deposits of amyloid fibrils were detected (Fig. 11). Large areas of lysed islets with numerous infiltrated macrophages were also observed.

Oxytocin administration showed an improvement in histological structure of pancreatic islets (Fig. 9). Pancreatic β-cells were less damaged as compared to the type I diabetic, and had some normal secretary granules which appeared to be immature (Fig. 12). However, most of the rER and Golgi complex were swollen and few mitochondria were observed (Fig. 13). A mild vacuolization in the cytoplasm was observed as compared to diabetic group (Fig. 12). Apoptosis and fibrosis were not observed.

Discussion

Oxytocin administration induced no significant change in the body weight of the diabetic treated rats as compared to the diabetic non-treated animals. The results, in accordance with Eckertova et al. (2011), showed that the adipose tissue of rats treated with OT had smaller adipocytes as compared with control. They explained this observation by activation of proliferator-activated receptor-γ (PPARγ), a key regulator of adipocyte differentiation (Tontonoz et al. 1994).
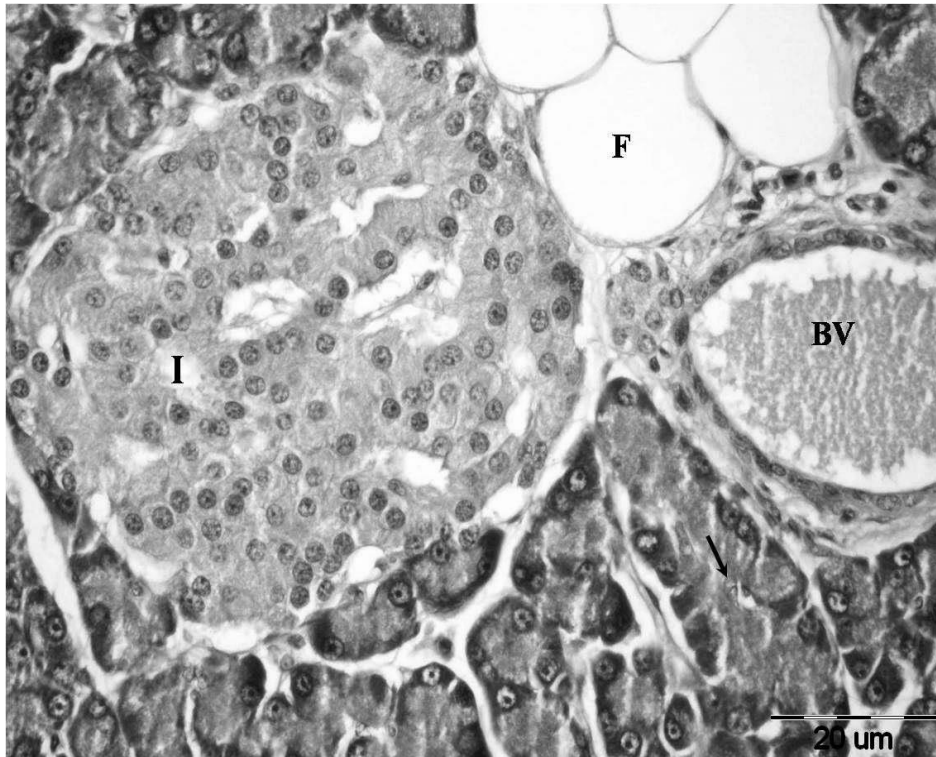
Fig. 7. Photomicrograph of the pancreas of untreated control rats. A single islet is seen as a large, pale-staining cluster of cells. It is surrounded by a dark stained exocrine acinus, which contains a faint-staining centroacinar cells (arrow). It appears in association with a blood vessels (BV) and fat droplets (F). (H&E X400).
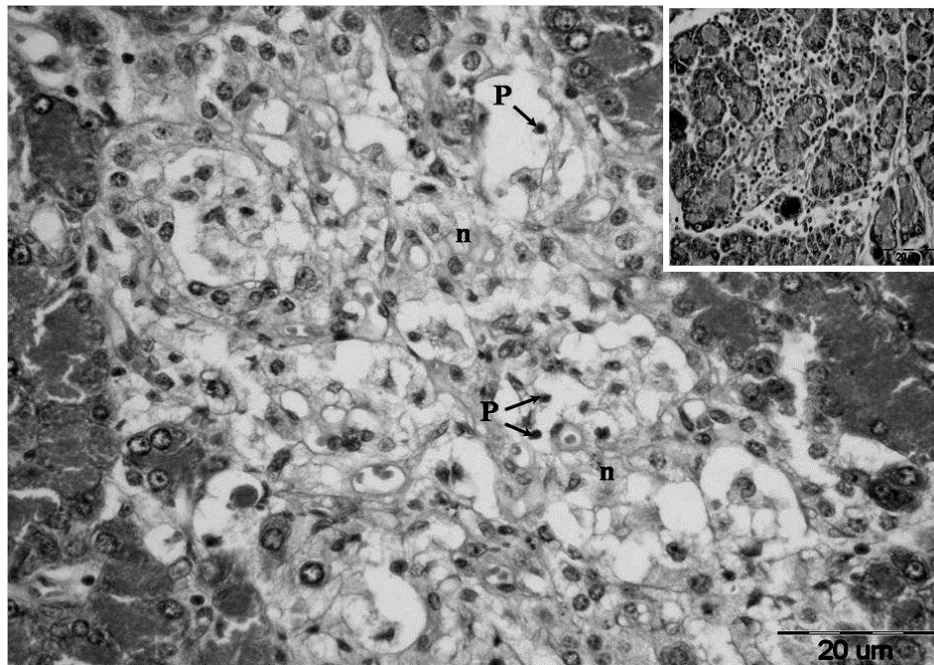

Fig. 8. Photomicrograph of the pancreas of type I diabetic rats, showing sever destroyed islet of Langerhans. Notice the presence of necrotic area (n) and apoptotic figure (P). (H&E X 400). Inset: Photomicrograph of the pancreas of type I diabetic rats (DI), showing loss of islet architecture with inflammatory cell infiltration (H&E X 400).
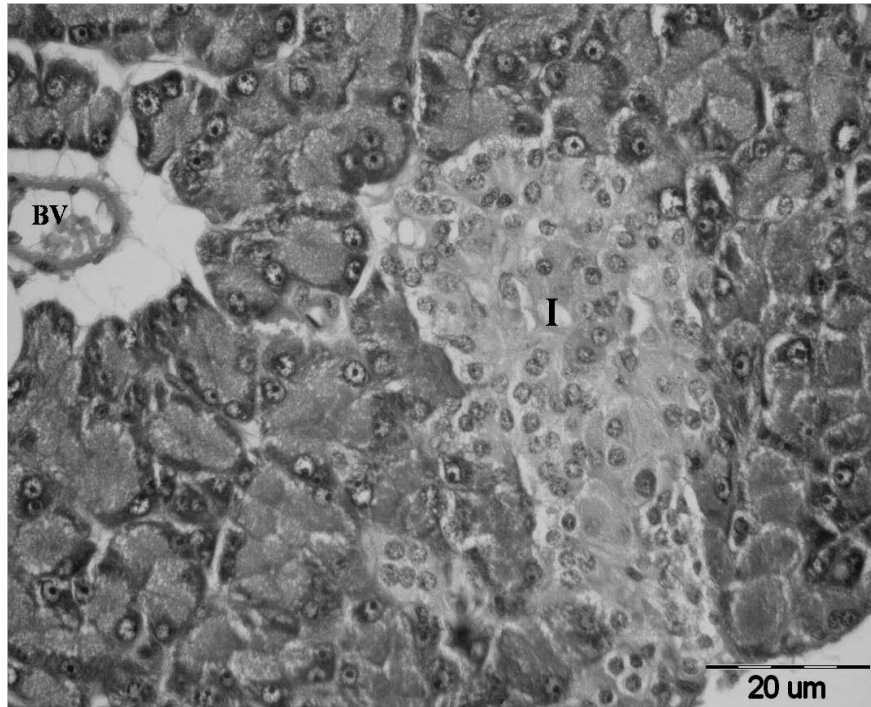
Fig. 9. Photomicrograph of the pancreas of rats treated with daily injection of 40 µIU/ml/kg body weight for six weeks showed an improvement in histological structure of the islet. Notice islet of Langerhans (I) surrounded with exocrine acinus, blood vessels are also observed (BV). (H&E X 400).
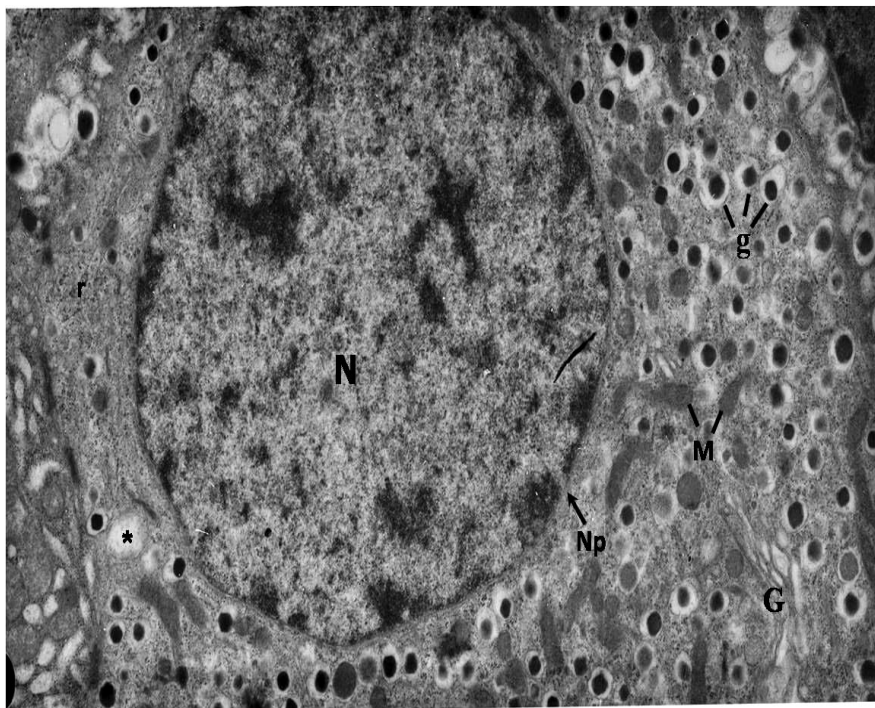

Fig. 10. Electron micrograph of β-cell of the endocrine pancreas of untreated control rats. The nucleus (N), Golgi complex (G), pleomorphic mitochondria (M), free ribosomes (r) and secretory granules (g) are evident. The membrane bound structures contain lipid "ceroid bodies" (*) are also observed. (X7500).
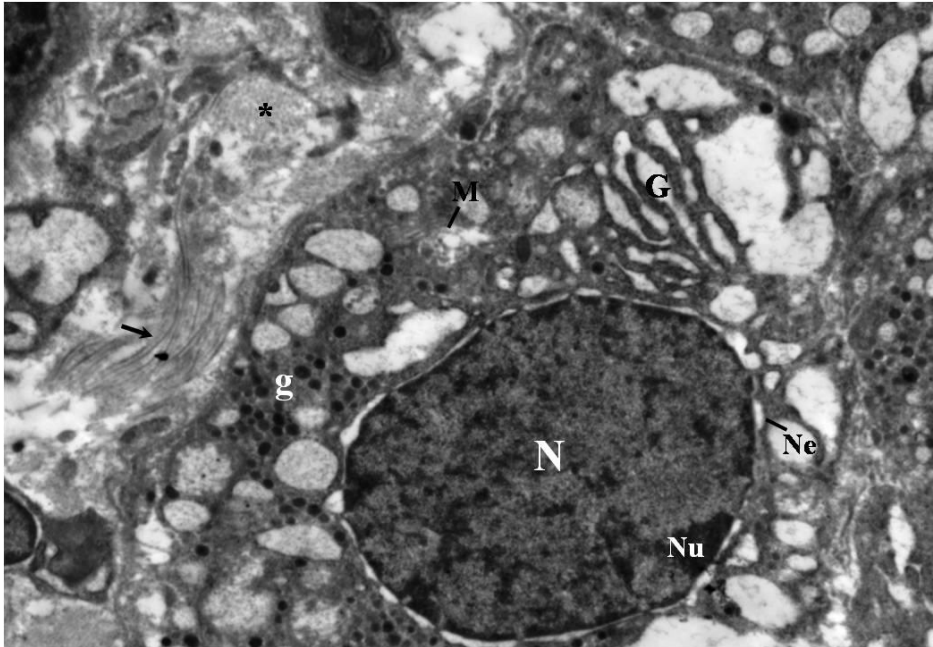
Fig. 11. Electron micrograph of the endocrine pancreas of type I diabetic rats, showed intact β-cells with nucleus have margin-ated condensed chromatin and surrounded by swollen nuclear envelope (Ne) and marginated nucleus (Nu). Their cytoplasm is highly vaculated and has swollen mitochondria (M) with largely dispersed cristae, and dilated Golgi complex (G). Small secretory granules (g) having dense cores with a thin or no halo were evident. Collagen (*) and amyloid (arrow) fibers were also observed. (X4000).
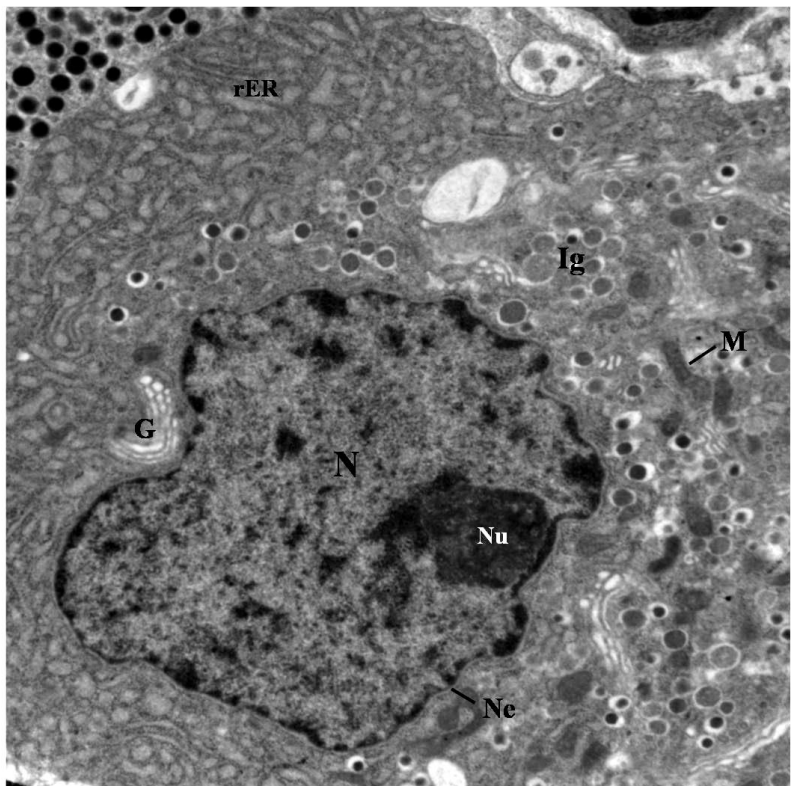

Fig. 12. Electron micrograph of β-cell of the endocrine pancreas of rats treated with daily injection of 40 μIU/ml/kg body weight for six weeks. Nucleus (N) and its nucleolus (Nu) surrounded by irregular nuclear envelope (Ne) and marginated dense heterochromatin. Swollen rough endoplasmic reticulum (rER) and Golgi complex (G) and mitochondria (M) and slight vacuated cytoplasm were observed. Several immature secretary granules (Ig) also observed. (X4000).
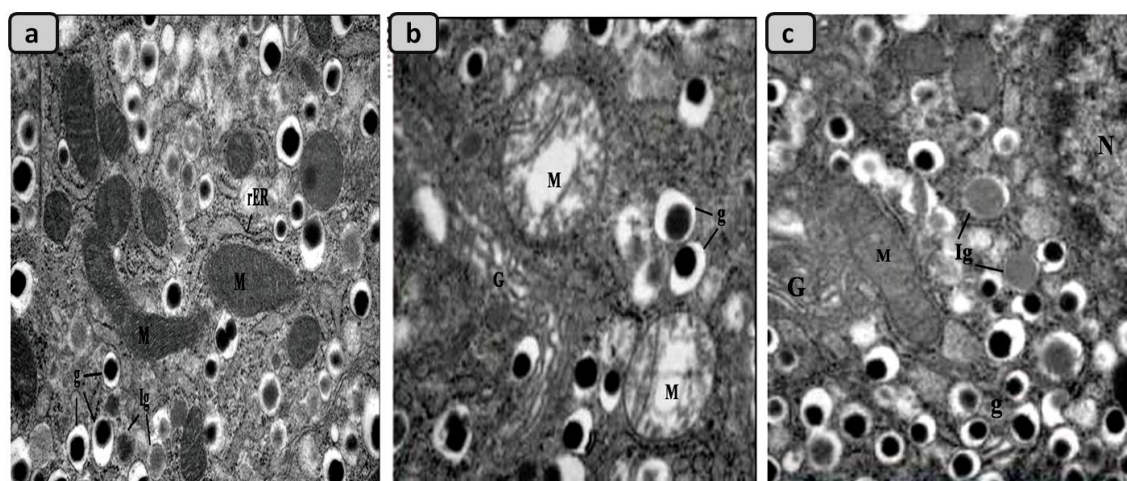
Fig. 13. Electron micrograph showing higher magnification of β-cell of all groups: control group (a), diabetic group (b) and diabetic treated with oxytocin (c). Mitochondria (M), rough endoplasmic reticulum (rER), Golgi complex (G), mature (g) and immature (Ig) secretary granules are observed. (X25000).

Oxytocin treatment induces a significant decrease in total cholesterol, TG, LDL, and an increase in HDL. In fact, OTRs are expressed in adipocytes (Gimpl and Fahrenholz 2001). It has recently been reported that OT induced lipolysis and β-oxidation of fatty acids via its direct effect on adipocytes (Deblon et al. 2011; Maejima et al. 2011).

The fasting blood glucose level was significantly decreased and insulin level was increased by OT administration as compared to diabetic rats. Indeed, it can increase glucose uptake via insulin-like signaling pathway (Lee et al. 2008; Florian et al. 2010). Florian et al. (2010) have reported that the glucose uptake in cardiomyocytes (CMs) is increased by OT through stimulation of intracellular release of calcium, and also activation of phosphoinositide-3-kinase (PI3K), calcium-calmodulin kinase kinase (Ca-CAMKK) and AMP-activated protein kinase (AMPK) pathways.

Understanding of the islet inflammation is an important factor in the pathogenesis of diabetes. The protection of β-cells from death presents itself as a new therapeutic target (Yamaoka 2003). This inflammatory process is probably a combined consequence of dyslipidemia, hyperglycemia, and increased circulating proinflammatory adipocytokines (leptin, interleukin-1 (IL-1), interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α)) (Cnop et al. 2005). As well, toxic molecules such as reactive oxygen species (ROS) play a significant

role in islet cell death by inducing DNA damage (Cnop et al. 2005).

Histological and ultrastructural observations demonstrated that OT treatment clearly show a definite improvement in the β-cells of the islets of Langerhans and induced a restoration of the normal architecture of the islet cells. The question remains, whether OT directly promotes β-cell regeneration or it acts indirectly. Still, the exact mechanism is unknown. Oxytocin receptors were identified in several cell types including pancreatic β-cells (Jeng et al. 1996). The direct effect may be caused via activation of PPARγ in pancreatic islet. It has been reported that PPAR-γ agonists can protect the β-cells from apoptosis, maintaining its neogenesis, reduction of islet amyloid, and restore β-cell responses to glucose in subjects with impaired glucose tolerance (Kim and Ahn 2004). The indirect effect(s) may be related to its action as insulin sensitizers, with a reduction in gluco- and lipo-toxicity. Oxytocin has antioxidant and anti-inflammatory effects (Clark et al. 2009; Gutkowska and Jankowski 2009). It has been suggested that OT may have antidiabetic properties by reducing oxidative stress and inflammatory processes in β-cells. However, many antidiabetic drugs act on peripheral tissues and have no direct effect on pancreatic islets (Bhonde et al. 2007).

One would expect that smaller adipocytes, which are more sensitive to insulin, produce substantially lower amounts of leptin and higher amounts of adiponectin

(Skurk et al. 2007), however, in the present study, OT decreased leptin and enhanced adiponectin secretion. This is in agreement with a previously reported correlation between leptin and adiponectin secretion and adipose tissue volume (Skurk et al. 2007).

It could be reasoned that the balance of leptin and adiponectin in the body could influence the effect of antidiabetic drugs (Oda et al. 2008). Therefore, the effect of OT at a dose 40 µIU/kg using leptin/adiponectin ratio (L/A) as an index of metabolic diseases (Yoon et al. 2011), was evaluated. The L/A ratio is more sensitive and reliable marker for insulin resistance than the homeostasis model assessment (HOMA) index (Rashid 2013), due to some limitations that have been reported, its clinical use was restricted (Ferrara and Goldberg 2001). Furthermore, L/A explained the variation in insulin sensitivity independent on gender, age and obesity itself (Koebnick et al. 2007). The L/A ratio in diabetic patients are considered for a predictor of insulin resistance and a useful indicator for the choice of drug to treat diabetes mellitus.

The present study confirms earlier reports demonstrating that OT administration has an anabolic effect on bone (Elabd et al. 2007; Tamma et al. 2009; Colli et al. 2012). Oxytocin administration significantly increases osteocalcin and decreases TRAP-5b in diabetic rats. The positive bone balance and the decreased bone resorption observed during the treatment with OT suggest that it could be used therapeutically in the recovery of osteopathy resulting from diabetes.

The significant increase in osteocalcin indicates that OT stimulates osteoblast activity and new bone formation, since osteocalcin is an excellent biochemical marker of bone formation activity (Seibel 2005). The effect of OT on bone could be resulted from the interaction of the hormone with its receptors in bone cells (Zingg and Laporte 2003), leading to intracellular events, such as those described by Copland et al. (1999). Thus, OT could increase the release of intracellular calcium and increase prostaglandin E2 synthesis, with consequent positive bone balance (Soloff et al. 2000; Aguirre et al. 2007). The interaction between OT and OTR in osteoblasts may also involve other intracellular events such as MAP kinase phosphorylation and induce c-Fos expression (Copland et al. 1999; Soloff et al. 2000).

Despite the fact that OT may favor osteoclastogenesis, it can inhibit the resorptive function of mature osteoclasts through a calcium signaling mechanism activated by OTR, since OT treatment induces an increase in intracellular calcium (Tamma et al. 2009). This could explain the significant decrease in the levels of TRAP-5b, a bone resorption marker (Seibel 2005). The significant decrease in TRAP-5b levels after OT treatment suggests that OT acts by inhibiting the bone resorption process, these in agreement with Colli et al. (2012).

The potential therapeutic uses for OT and more long-acting and specific analogues of OT are huge. As a short nine amino acid peptide, OT is considered to be ideal for the design of agonists and antagonists of its receptor. A lot of studies will strengthen the development of new drugs including the use of OT, OT agonists and antagonists for various human disorders including diabetes.

Acknowledgements

References

Aguirre JI, Leal ME, Rivera MF, Vanegas SM, Jorgensen M, Wronski TJ: Effects of basic fibroblast growth factor and a prostaglandin E2 receptor subtype 4 agonist on osteoblastogenesis and adipogenesis in aged ovariectomized rats. J Bone Miner Res 22, 877-888, 2007. http://dx.doi.org/10.1359/jbmr.070313

Akbaradeh A, Norouzian D, Mehrabi MR, Jamshidi Sh, Farhangi A, Verdi AA, Mofidian SM, Rad BL: Induction of diabetes by Streptozotocin in rats. Indian J Clin Biochem 22, 60-64, 2007. http://dx.doi.org/10.1007/BF02913315

Arora S, Ojha SK, Vohora D: Characterisation of streptozotocin induced diabetes mellitus in swiss albino mice. Global J Pharmacol 3, 81-84, 2009.

Bancroft JD: Theory and practice of histological techniques, ed. 5. London, Churchill Livingstone, 2002.

Bhonde R, Shukla RC, Kanitkar M, Shukla R, Banerjee M, Datar S: Isolated islets in diabetes research. Indian J Med Res 125, 425-440, 2007.

Bjorkstrand E, Eriksson M, Uvnas-Moberg K: Evidence of a peripheral and a central effect of oxytocin on pancreatic hormone release in rats. Neuroendocrinol 63, 377-383, 1996. http://dx.doi.org/10.1159/000126978

Clark SL, Simpson KR, Knox GE, Garite TJ: Oxytocin: new perspectives on an old drug. Am J Obstet Gynecol 200, 35 e1-6, 2009.

Cnop M, Welsh N, Jonas JC, Jorns A, Lenzen S, Eizirik DL: Mechanisms of pancreatic beta-cell death in type 1 and type 2 diabetes: many differences, few similarities. Diabetes 54, S97-107, 2005. http://dx.doi.org/10.2337/diabetes.54.suppl_2.S97

Colli VC, Okamoto R, Spritzer PM, Dornelles RC: Oxytocin promotes bone formation during the alveolar healing process in old acyclic female rats. Arch Oral Biol 57, 1290-1297, 2012. http://dx.doi.org/10.1016/j.archoralbio.2012.03.011

Copland JA, Ives KL, Simmons DJ, Soloff MS: Functional oxytocin receptors discovered in human osteoblasts. Endocrinol 140, 4371-4374, 1999. http://dx.doi.org/10.1210/endo.140.9.7130

Deblon N, Veyrat-Durebex C, Bourgoin L, Caillon A, Bussier AL, Petrosino S, Piscitelli F, Legros JJ, Geenen V, Foti M, Wahli W, Di -Marzo V, Rohner-Jeanrenaud F: Mechanisms of the anti-obesity effects of oxytocin in diet-induced obese rats. PLoS One 6, e25565 (1-13), 2011.

De-Heredia FP, Gomez-Martinez S, Marcos A: Obesity, inflammation and the immune system. Proc Nutr Soc 71, 332-338, 2012. http://dx.doi.org/10.1017/S0029665112000092

Eckertova M, Ondrejcakova M, Krskova K, Zorad S, Jezova D: Subchronic treatment of rats with oxytocin results in improved adipocyte differentiation and increased gene expression of factors involved in adipogenesis. Br J Pharmacol 162, 452-463, 2011. http://dx.doi.org/10.1111/j.1476-5381.2010.01037.x

Elabd SK, Sabry I, Hassan WB, Nour H, Zaky K: Possible neuroendocrine role for oxytocin in bone remodeling. Endocr Regul 41,131-141, 2007.

Ferrara CM, Goldberg AP: Limited value of the homeostasis model assessment to predict insulin resistance in older men with impaired glucose tolerance. Diabetes Care 24, 245-249, 2001. http://dx.doi.org/10.2337/diacare.24.2.245

Florian M, Jankowski M, Gutkowska J: Oxytocin increases glucose uptake in neonatal rat cardiomyocytes. Endocrinol 151, 482-491, 2010. http://dx.doi.org/10.1210/en.2009-0624

Frode TS, Medeiros YS: Animal models to test drugs with potential antidiabetic activity. J Ethnopharmacol 115, 173-183, 2008. http://dx.doi.org/10.1016/j.jep.2007.10.038

Fujiwara Y, Hiroyama M, Sanbe A, Yamauchi J, Tsujimoto G, Tanoue A: Mutual regulation of vasopressin- and oxytocin-induced glucagon secretion in V1b vasopressin receptor knockout mice. J Endocrinol 192, 361-369, 2007. http://dx.doi.org/10.1677/joe.1.06864

Gabir MM, Hanson RL, Dabelea D, Imperatore G, Roumain J, Bennett PH, Knowler WC: The 1997 American Diabetes Association and 1999 World Health Organization criteria for hyperglycemia in the diagnosis and prediction of diabetes. Diabetes Care 23, 1108-1112, 2000. http://dx.doi.org/10.2337/diacare.23.8.1108

Gao ZY, Drews G, Henquin JC: Mechanisms of the stimulation of insulin release by oxytocin in normal mouse islets. Biochem J 276, 169-174, 1991.

Gimpl G, Fahrenholz F: The oxytocin receptor system: structure, function, and regulation. Physiol Rev 81, 629-683, 2001.

Glauert AM, Lewis PR: Biological specimen preparation for transmission electron microscope. Portland Press, London, 1998.

Gutkowska J, Jankowski M: Oxytocin: Old Hormone, New Drug. Pharmaceuticals 2, 168-183, 2009. http://dx.doi.org/10.3390/ph203168

Hashimoto H, Uezono Y, Ueta Y: Pathophysiological function of oxytocin secreted by neuropeptides: A mini review. Pathophysiol 19, 283-298, 2012. http://dx.doi.org/10.1016/j.pathophys.2012.07.005

Imamura T, Luedke CE, Vogt SK, Muglia LJ: Oxytocin modulates the onset of murine parturition by competing ovarian and uterine effects. Am J Physiol Regul Integr Comp Physiol 279, R1061-1067, 2000.

Jeng YJ, Lolait SJ, Strakova Z, Chen C, Copland JA, Mellman D, Hellmich MR, Soloff MS: Molecular cloning and functional characterization of the oxytocin receptor from a rat pancreatic cell line (RINm5F). Neuropeptides 30, 557-565, 1996. http://dx.doi.org/10.1016/S0143-4179(96)90039-6

Kadowaki T, Yamauchi T, Kubota N: The physiological and pathophysiological role of adiponectin and adiponectin receptors in the peripheral tissues and CNS. FEBS Lett 582, 74-80, 2008. http://dx.doi.org/10.1016/j.febslet.2007.11.070

Katsiki N, Mikhailidis DP, Gotzamani-Psarrakou A, Yovos JG, Karamitsos D: Effect of various treatments on leptin, adiponectin, ghrelin and neuropeptide Y in patients with type 2 diabetes mellitus. Expert Opin Ther Targets 15, 401-420, 2011.

Kim HI, Ahn YH: Role of peroxisome proliferator-activated receptor-gamma in the glucose-sensing apparatus of liver and beta-cells. Diabetes 53, S60-65, 2004. http://dx.doi.org/10.2337/diabetes.53.2007.S60

Koebnick C, Shaibi GQ, Kelly LA, Roberts CK, Lane CJ, Toledo-Corral C, Davis JN, Byrd-Williams C, Weigensberg MJ, Goran MI: Leptin-to-adiponectin ratio as independent predictor of insulin sensitivity during growth in overweight Hispanic youth. J Endocrinol Invest. 30, RC13-16, 2007. http://dx.doi.org/10.1007/BF03346344

Kuzuya T: Diabetic complications: definition, classification and epidemiology. Nihon Rinsho 60, 19-27, 2002.

Lee ES, Uhm KO, Lee YM, Kwon J, Park SH, Soo KH: Oxytocin stimulates glucose uptake in skeletal muscle cells through the calcium-CaMKK-AMPK pathway. Regul Pept 151, 71-74, 2008. http://dx.doi.org/10.1016/j.regpep.2008.05.001

Lippert TH, Mueck AO, Seeger H, Pfaff A: Effects of oxytocin outside pregnancy. Horm Res 60, 262-271, 2003. http://dx.doi.org/10.1159/000074243

Maejima Y, Iwasaki Y, Yamahara Y, Kodaira M, Sedbazar U, Yada T: Peripheral oxytocin treatment ameliorates obesity by reducing food intake and visceral fat mass. Aging 3, 1169-1177, 2011.

Mantzoros CS, Magkos F, Brinkoetter M, Sienkiewicz E, Dardeno TA, Kim SY, Hamnvik OR, Koniaris A: Leptin in human physiology and pathophysiology. Endocrinol Metabolism 301, E567-E584, 2011.

Mc-Gown C, Birerdinc A, Younossi ZM: Adipose tissue as an endocrine organ. Clin Liver Dis 18, 41-58, 2014. http://dx.doi.org/10.1016/j.cld.2013.09.012

Minokoshi Y, Kim YB, Peroni OD, Fryer LG, Muller C, Carling D, Kahn BB: Leptin stimulates fatty-acid oxidation by activating AMP-activated protein kinase. Nature 415, 339-343, 2002. http://dx.doi.org/10.1038/415339a

Motyl K, McCabe LR: Streptozotocin, Type I Diabetes Severity and Bone. Biol Proced Online 11, 296-315, 2009. http://dx.doi.org/10.1007/s12575-009-9000-5

Oda N, Imamura S, Fujita T, Uchida Y, Inagaki K, Kakizawa H, Hayakawa N, Suzuki A, Takeda J, Horikawa Y, Itoh M: The ratio of leptin to adiponectin can be used as an index of insulin resistance. Metabolism 57, 268-273, 2008. http://dx.doi.org/10.1016/j.metabol.2007.09.011

Ragbetli C, Ceylan E: Effect of Streptozotocin on Biochemical Parameters in Rats. Asian J Chem 22, 2375-2378, 2010.

Rashid NF: Adiponectin to Leptin ratio as marker for Insulin Resistance in Diabetic Males in relation to Glycemic Control. Mustansiriya Med J 12, 14-21, 2013.

Seibel MJ: Biochemical markers of bone turnover: part I: biochemistry and variability. Clin Biochem Rev 26, 97-122, 2005.

Skurk T, Alberti-Huber C, Herder C, Hauner H: Relationship between adipocyte size and adipokine expression and secretion. J Clin Endocrinol Metab 92, 1023-1033, 2007. http://dx.doi.org/10.1210/jc.2006-1055

Soloff MS, Jeng YJ, Copland JA, Strakova Z, Hoare S: Signal pathways mediating oxytocin stimulation of prostaglandin synthesis in select target cells. Exp Physiol 85, 51S-58S, 2000. http://dx.doi.org/10.1111/j.1469-445X.2000.tb00007.x

Stofkova A: Leptin and adiponectin: from energy and metabolic dysbalance to inflammation and autoimmunity. Endocr Regul 43,157-168, 2009.

Svennersten-Sjaunja K, Olsson K: Endocrinology of milk production. Domest Anim Endocrinol 29, 241-258, 2005. http://dx.doi.org/10.1016/j.domaniend.2005.03.006

Tamma R, Colaianni G, Zhu LL, Di-Benedetto A, Greco G, Montemurro G, Patano N, Strippoli M, Vergari R, Mancini L, Colucci S, Grano M, Faccio R, Liu X, Li J, Usmani S, Bachar M, Bab I, Nishimori K, Young LJ, Buettner C, Iqbal J, Sun L, Zaidi M, Zallone A: Oxytocin is an anabolic bone hormone. Proc Natl Acad Sci USA 106, 7149-7154, 2009. http://dx.doi.org/10.1073/pnas.0901890106

Tontonoz P, Hu E, Spiegelman BM: Stimulation of adipogenesis in fibroblasts by PPAR gamma 2, a lipid-activated transcription factor. Cell 79, 1147-1156, 1994. http://dx.doi.org/10.1016/0092-8674(94)90006-X

Viero C, Shibuya I, Kitamura N, Verkhratsky A, Fujihara H, Katoh A, Ueta Y, Zingg HH, Chvatal A, Sykova E, Dayanithi G: Oxytocin: Crossing the bridge between basic science and pharmacotherapy. CNS Neurosci Ther 16, 138-156, 2010. http://dx.doi.org/10.1111/j.1755-5949.2010.00185.x

Xie DP, Chen LB, Liu CY, Liu JZ, Liu KJ: Effect of oxytocin on contraction of rabbit proximal colon in vitro. World J Gastroenterol 9, 165-168, 2003.

Yamaoka T: Regeneration therapy for diabetes mellitus. Expert Opin Biol Ther 3, 425-433, 2003.

Yamauchi T, Kamon J, Minokoshi Y, Ito Y, Waki H, Uchida S, Yamashita S, Noda M, Kita S, Ueki K, Eto K, Akanuma Y, Froguel P, Foufelle F, Ferre P, Carling D, Kimura S, Nagai R, Kahn BB, Kadowaki T: Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. Nat Med 8, 1288-1295, 2002. http://dx.doi.org/10.1038/nm788

Yoon JH, Park JK, Oh SS, Lee KH, Kim SK, Cho IJ, Kim JK, Kang HT, Ahn SG, Lee JW, Lee SH, Eom A, Kim JY, Ahn SV, Koh SB: The ratio of serum leptin to adiponectin provides adjunctive information to the risk of metabolic syndrome beyond the homeostasis model assessment insulin resistance: the Korean Genomic Rural Cohort Study. Clin Chim Acta 412, 2199-2205, 2011. http://dx.doi.org/10.1016/j.cca.2011.08.003

Zingg HH, Laporte SA: The oxytocin receptor. Trends Endocrinol Metab 14, 222-227, 2003. http://dx.doi.org/10.1016/S1043-2760(03)00080-8