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Oxytocin treatment prevents marrow adiposity observed in alloxan-induced diabetic rabbits using proton MR spectroscopy

Leczenie oksytocyną zapobiega stłuszczeniu szpiku kostnego obserwowanemu u królików z cukrzycą wywołaną alloksanem — badanie przy użyciu protonowej spektroskopii rezonansu magnetycznego

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Abstract

Introduction: Oxytocin might be used therapeutically as an ally to rescue osteopathy resulting from diabetes. However, the *in vivo* effects of oxytocin on marrow adipogenesis in diabetes remain unknown. In this longitudinal study, we aimed to investigate the protective effects of oxytocin on diabetes-induced marrow adiposity in rabbits using proton MR spectroscopy.

Material and methods: Forty-five female New Zealand rabbits were randomly divided into controls, diabetes, and diabetes treated with oxytocin (ip, 0.78 mg/kg) for six months. Marrow fat fraction (FF) was determined by proton MR spectroscopy at baseline, and at three and six months. Bone mineral density was measured by dual-energy X-ray absorptiometry. Serum biomarkers, glycolipid metabolism, and histological analysis of marrow adipocytes were determined.

Results: Oxytocin treatment had positive metabolic effects in diabetic rabbits, which was based on the changes in glucose metabolism, insulin sensitivity, and lipid profiles. The diabetic rabbits demonstrated dramatic marrow adiposity in a time-dependent manner; at three and six months the FF percentage changes from baseline were 10.1% and 25.8%, respectively (all $P < 0.001$). Moreover, oxytocin treatment significantly reversed FF values and quantitative parameters of marrow adipocyte in diabetic rabbits to levels of naive control rabbits. Oxytocin improved bone formation marker in diabetic rabbits compared to the saline group. Also, treatment of diabetic rabbits with oxytocin significantly mitigated bone deterioration when compared with the saline-treated diabetic group (all $P < 0.05$).

Conclusions: Oxytocin appears to alleviate harmful effects of hyperglycaemia on marrow adiposity. Proton MR spectroscopy may be a valuable tool, providing complementary information on efficacy assessments. **(Endokrynol Pol 2018; 69 (4): 416–422)**

Key words: *diabetes mellitus, oxytocin, marrow adiposity, MR spectroscopy*

Streszczenie

Wstęp: Oksytocyna może być stosowana terapeutycznie w osteopatii wynikającej z cukrzycy, jednakże jej wpływ *in vivo* na stłuszczenie szpiku kostnego w przebiegu cukrzycy pozostaje niezbadany. Niniejsze badanie przekrojowe ma na celu zbadać ochronne działanie oksytocyny na wywołane cukrzycą stłuszczenie szpiku kostnego u królików przy użyciu protonowej spektroskopii rezonansu magnetycznego.

Materiał i metody: Czterdzieści pięć samic królików nowozelandzkich podzielono losowo na grupę kontrolną, grupę z cukrzycą oraz grupę z cukrzycą leczoną oksytocyną (0.78 mg/kg, i.p.) przez sześć miesięcy. Frakcja tłuszczu *(ang. fat fraction; FF)* szpiku kostnego została określona za pomocą protonowej spektroskopii rezonansu magnetycznego na początku badania oraz po trzech i sześciu miesiącach. Gęstość mineralną kości zmierzono za pomocą absorpcjometrii promieniowania rentgenowskiego o podwójnej energii. Określono również biomarkery surowicy krwi, metabolizm glikolipidów oraz sporządzono analizę histologiczną adipocytów szpiku kostnego.

Wyniki: Leczenie oksytocyną przyniosło pozytywne efekty metaboliczne u królików z cukrzycą, co stwierdzono na podstawie zmian w metabolizmie glukozy, wrażliwości na insulinę oraz profili lipidowych. Zauważono drastyczny wzrost stłuszczenia szpiku kostnego u królików z cukrzycą w sposób zależny od czasu; po trzech i sześciu miesiącach, procentowe zmiany frakcji tłuszczu w stosunku do wartości wyjściowej wynosiły odpowiednio 10,1% i 25,8% (wszystkie $P < 0.001$). Co więcej, leczenie oksytocyną znacząco odwracało wartości frakcji tłuszczu oraz ilościowe parametry adipocytów szpiku kostnego u królików z cukrzycą do poziomu królików z grupy kontrolnej. Oksytocyna poprawiała marker tworzenia kości u królików z cukrzycą w porównaniu do grupy, której podawano sól fizjologiczną. Ponadto, leczenie oksytocyną królików z cukrzycą znacząco łagodziło niszczenie kości w porównaniu do grupy z cukrzycą, której podawano sól fizjologiczną (wszystkie $P < 0.05$).

Wnioski: Oksytocyna wydaje się zmniejszać szkodliwy wpływ hiperglikemii na stłuszczenie szpiku kostnego. Protonowa spektroskopia rezonansu magnetycznego może być cennym narzędziem, dostarczającym uzupełniających informacji na temat oceny skuteczności leczenia. **(Endokrynol Pol 2018; 69 (4): 416–422)**

Słowa kluczowe: *cukrzyca, oksytocyna, stłuszczenie szpiku kostnego, spektroskopia rezonansu magnetycznego*

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Introduction

Oxytocin, a neurohypophysial peptide, may have a wide variety of physiological and pathological functions, which makes oxytocin and its receptor potential targets for drug therapy. Previous studies suggested that oxytocin has anti-oxidative, anti-apoptotic, and anti-inflammatory potential [1]. Recently, there has been accumulating evidence showing that oxytocin may promote glucose uptake and improve insulin sensitivity via direct and/or indirect effects [1–3]. Moreover, it may lead to regenerative changes in diabetic pancreatic islet cells [3]. These results strongly suggest that oxytocin might be a therapeutic target for treating diabetes.

It is well-established that bone fragility is one of the chronic complications of diabetes mellitus. Both type 1 and type 2 diabetes are associated with impairment of bone health by altering bone formation, bone resorption, bone marrow adiposity, collagen formation, inflammatory cytokine, and calcium metabolism [4, 5]. Marrow fat is a unique fat depot, which has the potential to contribute to both local and systemic metabolic processes. Although the effect of marrow adipocytes on bone integrity is complex, a growing body of evidence suggests that an inverse association exists between marrow fat content and skeletal mass in some contexts [6–9]. In humans, serum oxytocin is a marker of energy availability and may be a mediator of bone density, structure, and strength [10, 11]. In preclinical studies, several lines of evidence have demonstrated that oxytocin has a positive anabolic effect on the bone biology [12, 13]. These data suggest that oxytocin might be used therapeutically as an ally to rescue osteopathy resulting from diabetes. However, the *in vivo* effect of oxytocin on marrow adipogenesis remains unknown in animal models.

Since the interactions between bone and fat are complex and new emerging concepts regarding their relationship have the potential to transform our therapeutic targeting of the skeleton, in this longitudinal study, we aimed to investigate the protective effects of oxytocin on marrow adiposity using MR spectroscopy in an alloxan-induced diabetes rabbit model over a six-month period.

Material and methods

Animals and experimental protocol

Forty-five female New Zealand rabbits (five months old, weighing 3.50 to 4.34 kg) were used in this study. All animals were fed ad libitum and individually housed in steel cages in a temperature-controlled environment (22 ± 2°C) with 12-h light/dark cycles. After two weeks of adaptation, baseline body weight and levels of blood glucose were measured. Then, the rabbits were randomly divided into three groups (n = 15/group): a control group, an alloxan-induced diabetes mellitus group, and an oxytocin-treated diabetes mellitus group. The oxytocin-treated group received a daily intraperitoneal oxytocin injection (0.78 mg/kg, #H-2510, Bachem AG, Bubendorf, Switzerland) for six months [12], and the controls and diabetes model groups were administered saline solution.

To create a long-term diabetic rabbit model, a single 100-mg/kg dose of alloxan monohydrate (Sigma, St Louis, MO, USA) dissolved in 10 mL of sterile saline was injected as described elsewhere [14], and non-diabetic rabbits were injected with saline. Alloxan-treated rabbits have been reported to develop severe hypoglycaemia within the first few days after injection [14, 15]. To prevent hypoglycaemic shock in the alloxan-treated rabbits, at 4, 8, and 12 h following alloxan injection, 10 ml of sterile filtered 5% pharmaceutical-grade glucose in phosphate buffer saline was administered subcutaneously. In addition, an oral solution of 20% glucose in the drinking water ad libitum was provided for 1–2 days after confirmation of hypoglycaemia (less than 70 mg/dl). Their blood glucose levels were monitored every 1–2 h until 12 h post injection. Rabbits' blood glucose levels were checked two times per day for the first four weeks and once weekly thereafter in the morning, and their weights were recorded once a week throughout the study. Rabbits with fasting plasma glucose levels ≥ 14 mmol/L during the entire experimental period were defined as diabetic and included in this study [16].

Each rabbit was anaesthetised with intravenous infusion of 3% sodium pentobarbital (1 ml/kg) via an ear vein, and they underwent MR spectroscopy scanning at baseline and at three, and six months after alloxan or saline injection. In addition, blood samples were collected for biochemical analysis. The rabbits were sacrificed with intravenous infusion of over-dose sodium pentobarbital (50 mg/kg) to obtain the bone specimens. Dual-energy X-ray absorptiometry (DXA) was performed to measure bone mineral density (BMD) at the L5 vertebral body and right femur, and the left femur was used for histopathological examination. The Guide for the Care and Use of Laboratory Animals was followed, and the study protocols were approved by the institutional Animal Experiment Ethics Committee.

MR spectroscopy

MRI exams were performed on a commercial 3 Tesla MRI system (Siemens Medical System, Erlangen, Germany). The body volume coil and quadrate knee array coil was used for the radiofrequency transmission and reception, respectively. The rabbits were anaesthetised as mentioned above, ventrally positioned with

hind limbs separated from the trunk to scan the left femur. Sagittal, coronal, and axial scout T2-weighted images of the left femur were initially acquired for positioning a 6 × 6 × 14 mm^3 voxel in the cancellous bone of the distal femur, respectively. Single voxel MR spectroscopy was performed by using point-resolved spectroscopy sequence according to the method of Li et al [7]. After local shimming and gradient adjustments, the acquisition parameters used for MR spectroscopy were repetition time (TR) 3000 ms, echo time (TE) 30 ms, signal average 64 without water suppression, and spectral bandwidth 2000 Hz and 1024 data-points. The longer TR was to minimise the T1 relaxation effect, and the shorter TE was to minimise the T2 decay of both fat and water.

A commercially available imaging workstation was used for post-processing of MR spectroscopy data. Based on previous studies [7], the bulk methylene protons and water were determined to be identifiable around 1.30 and 4.65 ppm, respectively. To quantify the amplitudes of the desired water and lipid signals, Voigt functions were used to fit the peaks in the frequency domain. The marrow fat fraction (FF) was defined as the relative fat signal amplitude in the percentage of total signal amplitude (water and fat), and the FF value was calculated according to the following equation: $FF = [I_{methylene}/(I_{methylene} + I_{water})] \times 100\%$, where I_{water} and $I_{methylene}$ are the signal amplitudes of water and bulk methylene, respectively [8].

BMD measurements

For each rabbit, BMD and bone mineral content of the L5 vertebrae and left femur were measured by a Hologic Discovery Wi DXA Scanner (Hologic Inc., Bedford, MA, USA; version 12.7); specific software for small animal-scanning mode was used as described elsewhere [7]. The BMD value was automatically calculated using the bone mineral content of the measured area.

Biochemical analysis

Serum biochemical markers of bone metabolism including bone alkaline phosphatase (BALP; Rabbit BALP ELISA Kit) and cross-linked C-telopeptide of type I collagen (CTX-I; Rabbit Cross-laps CTX-I ELISA Kit) were measured by an enzyme-linked immunosorbent assay (CUSABIO, Wuhan, China) according to the manufacturer's instructions. Serum biochemical examinations including fasting glucose, total cholesterol, triglycerides, and low- and high-density lipoprotein cholesterol were detected using a full automatic biochemical analyser. Levels of fasting insulin was assessed using a rabbit insulin ELISA Kit (Huamei Biological Engineering Co, Ltd., Wuhan, China) according to the manufacturer's instructions.

Histopathological evaluation

The left femurs were decalcified by 10% glycerinum-EDTA for six weeks after fixation, dehydrated with concentrated ethanol, and embedded in paraffin wax after being washed with xylene. Sagittal sections (5 μm thick) were made at the distal femoral region. Sections were deparaffinised and stained with haematoxylin-eosin (H&E). The mean diameter, density, and percentage area of marrow adipocytes were quantified for each rabbit in six fields randomly selected (400×) using Image-Pro Plus 6.0 Scion image software (Scion Corporation) as described previously [17]. The sections were examined in a blinded manner.

Statistical analysis

Statistical analyses were performed using SPSS 18.0 (SPSS Inc., Chicago, IL, USA). Data are presented as means ± standard deviations. *Shapiro-Wilk* test was performed to test the normality distribution of the data. Repeated measure two-way analysis of variance was used to compare marrow FF between the groups. Differences in bone turnover markers, serum biomarkers, and quantitative parameters of marrow adipocytes were evaluated by one-way analysis of variance (ANOVA). Post-hoc Bonferroni test was used for *post-hoc* multiple comparisons, and p values of < 0.05 were considered statistically significant.

Results

Changes in body weights

Of the initial 45 rabbits, 39 completed the whole study, whereas one naive control rabbit, three saline-treated diabetic rabbits, and two oxytocin-treated rabbits died of an adverse reaction to anaesthetics. No significant difference was found among the study groups in the initial weights. At the end of the experiment, body weights were significantly lower in the saline-treated diabetic rabbits (ranging from 3.78 to 5.30 kg) than in the naive control rabbits (ranging from 3.99–5.65 kg). In diabetic rats treated with oxytocin daily for six months, the body weight ranged between 3.60 and 5.50 kg with a mean value of 4.13 ± 0.60 kg ($P = 0.713$ vs. the diabetic group); however, it was still significantly lower ($P = 0.019$) than the control group (Table I).

Evaluation of glycolipid metabolism

Table I represents the changes in blood glucose and insulin levels as well as lipid profiles in the study groups. The fasting blood glucose concentration significantly increased, whereas fasting blood insulin levels significantly decreased in the saline-treated diabetic rabbits compared to the naive control group. Conversely, treatment of diabetic rabbits with oxytocin significantly reduced glucose levels and improved fasting blood

Table I. ***Changes in body weights, glycolipid metabolism, bone turnover biomarkers, and BMD of the rabbits***

Tabela I. ***Zmiany masy ciała, metabolizmu glikolipidów, markerów obrotu kostnego oraz*** gęstości mineralnej kości *(bone mineral density, BMD)* królików

	Naive control group (n = 14)	Saline-treated diabetes group (n = 12)	Oxytocin-treated diabetes group (n = 13)
Initial weight [kg]	3.97 ± 0.29	4.06 ± 0.20	3.98 ± 0.27
Final weight [kg]	4.93 ± 0.57	4.25 ± 0.50[a]	4.13 ± 0.60[a]
Glucose [mmol/L]	5.34 ± 0.57	15.93 ± 0.98[a]	6.18 ± 0.73[a, b]
Insulin [mmol/L]	17.15 ± 1.56	8.02 ± 0.81[a]	14.2 ± 1.03[a, b]
Plasma cholesterol [mmol/L]	1.43 ± 0.37	2.28 ± 0.49[a]	1.77 ± 0.41[a, b]
Triglyceride [mmol/L]	1.01 ± 0.28	1.60 ± 0.35[a]	1.21 ± 0.33[a, b]
LDL-c [mmol/L]	0.28 ± 0.04	0.35 ± 0.05[a]	0.30 ± 0.05[b]
HDL-c [mmol/L]	0.41 ± 0.06	0.37 ± 0.08	0.40 ± 0.09
CTX-I [ng/ml]	17.51 ± 2.97	16.83 ± 3.36	17.94 ± 3.98
BALP [mIU/ml]	4.97 ± 0.71	3.85 ± 0.63[a]	5.14 ± 0.82[b]
Lumbar spine BMD [mg/cm^2]	289 ± 33	266 ± 30[a]	285 ± 26[b]
Femur BMD [mg/cm^2]	360 ± 38	338 ± 31[a]	356 ± 35[b]

Values are expressed as mean ± SD.
[a]$P < 0.05$ vs. naive control rabbits; [b]$P < 0.05$ vs. saline-treated diabetic rabbits
BALP, bone alkaline phosphatase; BMD, bone mineral density; CTX-I, cross-linked C-telopeptide of type I collagen; HDL-c, high-density lipoprotein cholesterol; LDL-c, low-density lipoprotein cholesterol
*P < 0.001 vs. the control group; †P < 0.001 vs. the saline-treated diabetes group (one-way ANOVA followed by Bonferroni post-hoc test)

insulin concentration when compared with those in the saline-treated diabetic group.

As shown in Table I, levels of plasma triglyceride, cholesterol, and low-density lipoprotein increased in all diabetic rabbits. In diabetic rabbits treated with oxytocin, serum triglyceride, cholesterol, and low-density lipoprotein concentration were lower than the saline-treated diabetic group; however, the serum triglyceride and cholesterol concentration was still significantly higher than the untreated control group. No significant difference was found in high-density lipoprotein concentration among the three groups.

Changes in biomarkers of bone turnover and BMD

Examinations of bone formation marker in the diabetic rabbits showed a decrease in serum BALP levels compared with the naive controls, and daily oxytocin injections could increase levels of BALP. However, bone resorption marker CTX-I levels were unaltered in the diabetic rabbits, and osteoclast activity was not affected by the oxytocin treatment (Table I).

As shown in Figure 1, decreases in the L5 vertebral body and femur BMD ($P < 0.05$ for all) were observed in the diabetes group compared with the naive control group, which were reversed by oxytocin treatment ($P < 0.05$).

Changes in marrow fat content and quantitative parameters of marrow adipocytes

The *in vivo* MR spectroscopy measurements taken at each time point allowed the monitoring of the FF changes in each rabbit. Figure 1 is a representation of the MR spectroscopy scans of a rabbit from each of the three groups, done at the different time points. The FF values for the saline-treated diabetic rabbits markedly increased over the six-month period (Figure 2). The FF values from baseline, and months 3 and 6 were significantly different when tested by adjusted repeated-measures ANOVA ($P < 0.001$ for all). At month 3 of the FF percentage changes from baseline were 2.1%, 10.1%, and 4.1%, for the naive control, saline-, and oxytocin-treated diabetic rabbits, respectively, and at month 6 the percentage changes from baseline were 4.2%, 25.8%, and 5.4%, respectively. There was a significant difference in FF value between the saline-treated diabetic rabbits and naive controls or oxytocin-treated diabetic rabbits. No differences in FF measures over time were noted within the same time-point between the naive controls and oxytocin-treated diabetic rabbits.

Histology images stained with H&E validated the MR spectroscopy results. Figure 3 summarises the changes in quantitative parameters of marrow adipocytes in the studied groups. Quantitative parameters of marrow adipocytes including adipocyte density, adipocyte volume, and area percentage of fat cells significantly increased in the saline-treated diabetic rabbits compared to the naive control animals. Conversely, treatment of diabetic rabbits with oxytocin significantly reduced quantitative parameters of marrow adipocytes when compared with those in the saline-treated diabetic group.

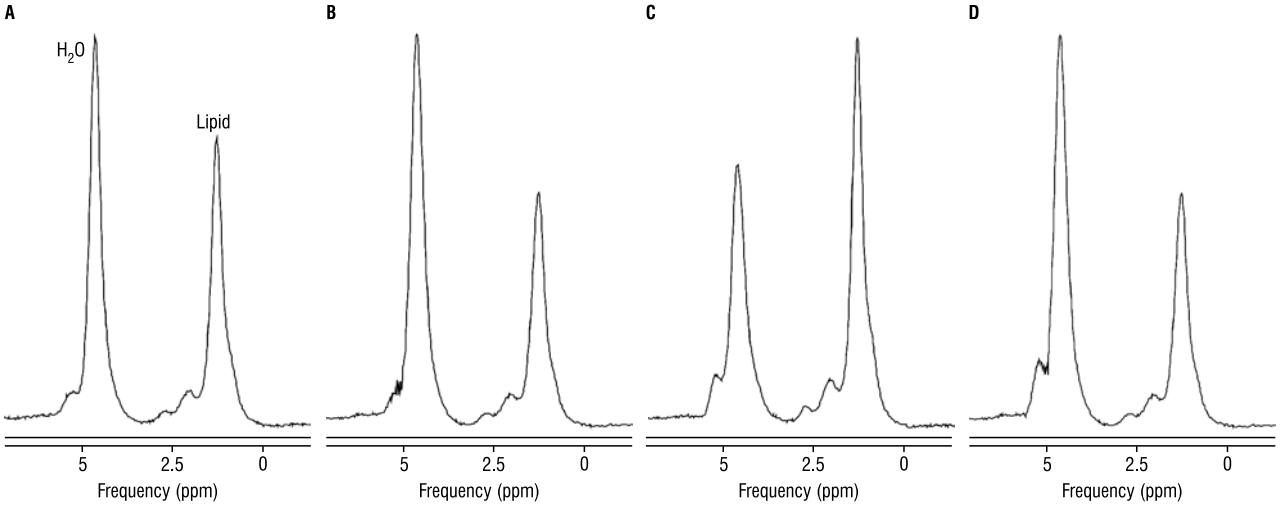

Figure 1. *Representative MR spectroscopy. FF = 38.9% in the naive control at month 6 (**A**); FF = 37.0% and 58.7% in the saline-treated diabetic rabbit at baseline (**B**) and month 6 (**C**), respectively; FF = 36.1% in the oxytocin-treated rabbit at month 6 (**D**)*

Rycina 1. Reprezentatywna *spektroskopia rezonansu magnetycznego. FF = 38,9% u królików z grupy kontrolnej w szóstym miesiącu badania (**A**); FF = 37,0% i 58,7% u królika z grupy z cukrzycą, której podawano sól fizjologiczną, odpowiednio na początku badania (**B**) i w szóstym miesiącu badania (**C**); FF = 36,1% u królika z grupy leczonej oksytocyną w szóstym miesiącu badania (**D**)*

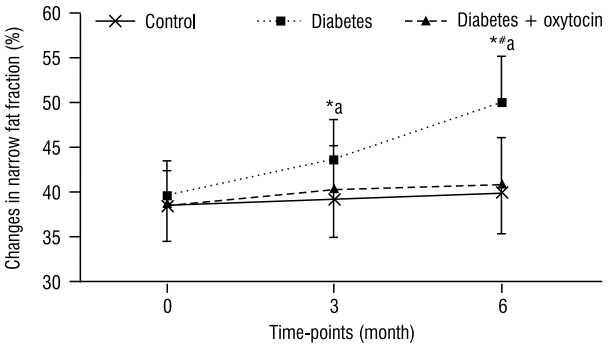

Figure 2. *Plots of the marrow fat fraction monitored over time for the three groups of rabbits. Data are expressed as mean ± SD (n =14, 12, and 13 for the naive controls, saline-, and oxytocin-treated diabetic rabbits, respectively)*

Rycina 2. *Wykresy frakcji tłuszczu szpiku kostnego, monitorowane w czasie dla trzech grup królików. Dane wyrażono jako średnią ± odchylenie standardowe (ang. standard deviation, SD) (n = 14, 12 i 13 odpowiednio dla grupy kontrolnej, grupy z cukrzycą, której podawano sól fizjologiczną oraz grupy z cukrzycą leczonej oksytocyną)*

Discussion

The list of diabetes-associated complications is extensive and involves nearly every organ system, including the skeleton, and the area of research on the deleterious effects of diabetes on bone homeostasis is still in its infancy. Our results are consistent with the existing literature, which shows impaired bone formation rather than increased bone resorption in T1-diabetic animals [18, 19].

Oxytocin-oxytocin receptor signalling network can prove to be of great importance in therapeutics and drug targeting due to its diverse biological functions. In preclinical studies, oxytocin has recently been implicated in bone homeostasis, favouring osteoblastic over osteoclastic activity and promoting osteogenesis over adipogenesis [12, 20]. However, these data mainly resulted from osteoporotic animal models but not diabetic animals. Work by Elabd and Altirriba showed that oxytocin may represent a promising approach for the treatment of diabetes and some of its complications, including diabetic neuropathy [2, 21]. Our results are consistent with the existing literature that shows the lack of a visible suppression of osteoclast activity in the oxytocin-treated rabbits, suggesting that oxytocin treatment increases osteoblast activity but does not alter osteoclast activity [3, 12, 22]. Our data confirm these results and provide some additional interesting information. We found that oxytocin treatment could rescue diabetes-related bone deterioration.

In addition to vascular disruption, enhanced marrow adiposity has become a hallmark of the diabetic bone phenotype. In the present study, in accordance with previous experimental studies [23–25], MR spectroscopy findings showed a significant increase in marrow fat content in diabetic rabbits when compared with the naive control animals. A body of growing evidence implicates enhanced marrow adipogenesis and cellular changes in the bone marrow in a novel mechanistic link between various diabetic secondary complications, as observed in both type 1 and 2

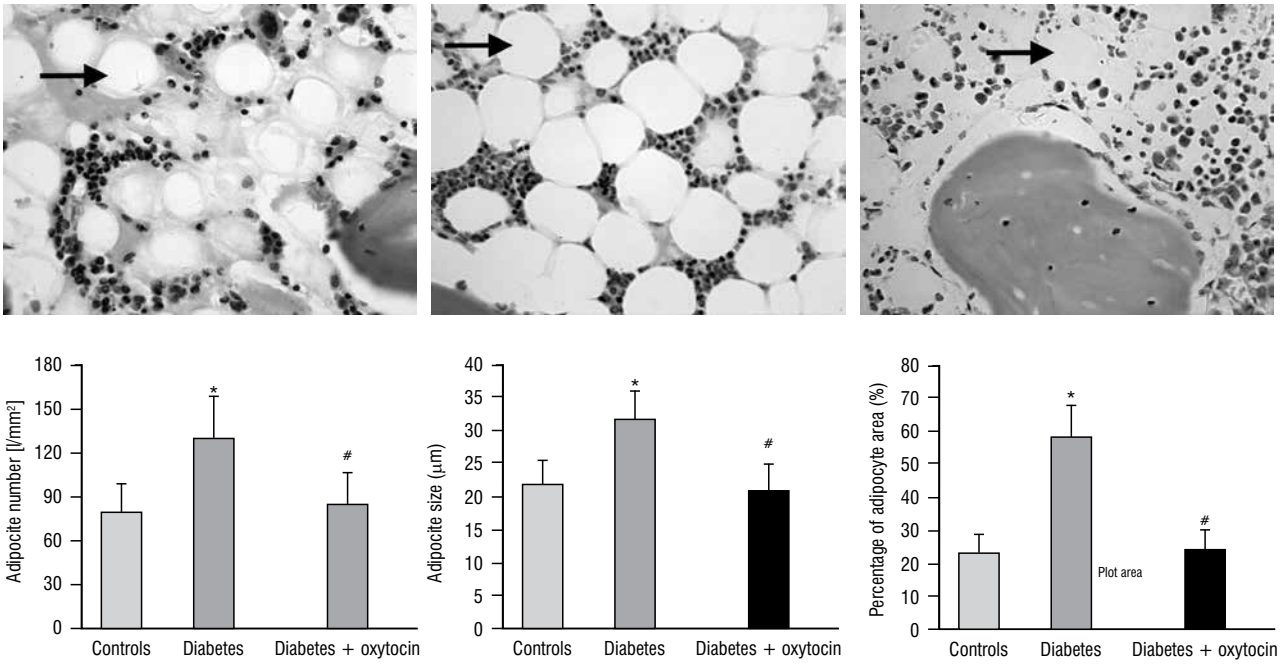

Figure 3. *Changes in marrow fat cells in diabetic rabbits treated with oxytocin for 6 months. Representative serial sections of marrow fat cells (arrow) stained with haematoxylin-eosin stain (400×) are presented in A1 to A3. For the naive control (**A1**), few marrow fat cells were embedded in the marrow nuclear cells. Diabetic rabbit (**A2**) exhibited markedly increased marrow fat infiltration and decreased trabecular bone. This pathological expansion of marrow fat was restored by oxytocin treatment (**A3**). The quantitative parameters of fat cells are presented in B1 to B3. Values in columns are presented as mean ± SD (n =14, 12, and 13 for the naive controls, saline-, and oxytocin-treated diabetic rabbits, respectively)*

Rycina 3. *Zmiany w komórkach tłuszczowych szpiku kostnego u królików z cukrzycą leczonych oksytocyną przez sześć miesięcy. Ryciny A1 do A3 przedstawiają reprezentatywne serie preparatów komórek tłuszczowych szpiku kostnego (zaznaczenie strzałką), wybarwionych hematoksyliną i eozyną* (400x). *W przypadku grupy kontrolnej* (**A1**), *kilka komórek tłuszczowych szpiku kostnego zostało umieszczonych w jądrzastych komórkach szpiku. Królik z cukrzycą* (**A2**) *wykazywał wyraźnie zwiększoną obecność tłuszczu w szpiku kotnym oraz zmniejszoną istoty gąbczastej kości. Ten patologiczny rozwój tłuszczu w szpiku kostnym został odwrócony dzięki leczeniu oksytocyną* (**A3**). *Ilościowe parametry komórek tłuszczowych przedstawiono na rycinach B1 do B3. Wartości w kolumnach zaprezentowano jako średnią ± SD (n = 14, 12 i 13 odpowiednio dla grupy kontrolnej, grupy z cukrzycą, której podawano sól fizjologiczną oraz grupy z cukrzycą leczonej oksytocyną)*

models of diabetes [6, 26], including three possible mechanisms: 1. diabetes results in depletion of stem/ /progenitor cells in the bone marrow; 2. diabetes may reduce mobilisation of marrow stem cells; and 3. high levels of glucose may reduce endothelial and mesenchymal progenitor cell numbers that have mobilised. Unfortunately, assessment of marrow fat content in T1 diabetic patients has yielded less impressive results. For example, Slade et al. [27] reported marrow adiposity from any site tested (e.g. distal femur, proximal tibia, and lumbar spine) was not altered by T1-diabetes status. This indicates that further studies are warranted to fully understand whether marrow adipose tissue is altered during T1 diabetes in larger clinical trials.

One of the key findings in the present study is that daily injection of oxytocin could completely reverse marrow fat expansion seen in alloxan-induced diabetic rabbits. One explanation could be that oxytocin might suppress the expression of peroxisome proliferator-activated receptors-γ2, a key regulator of adipocyte differentiation. Bone metabolism is the combination of bone formation by osteoblasts and bone resorption by osteoclasts. Adipocytes and osteoblasts share the same precursor cells. An inverse association existing between osteogenesis and adipogenesis is well documented, and thus controlling the fine balance between the two lineages is of great therapeutic significance [28]. Another explanation could be that oxytocin can be a good choice to increase the insulin levels and improve insulin sensitivity by reducing gluco- and lipotoxicity [2]. This hypoglycaemic effect of oxytocin can potentiate the differentiation and migration of bone mesenchymal stem cells by inhibiting glycogen synthase kinase-3β to osteoblasts [29], thereby inhibiting marrow adipogenesis. Furthermore, the presence of marrow adiposity in diabetic bone is linked to the lipotoxic effect on bone cells through the secretion

of fatty acids by adipocytes within the bone marrow, and this lipotoxic milieu affects osteoblast function and survival [30]. Interestingly, oxytocin has antioxidant and anti-inflammatory effects [3], which in turn stimulate mesenchymal stem cells osteogenesis at the expense of adipogenesis. This indicates that the benefits of preventing lipotoxicity could have a substantial influence in the treatment of metabolic diseases.

In conclusion, we have demonstrated that oxytocin appears to alleviate harmful effects of hyperglycaemia on marrow adiposity in diabetic rabbits. This anti-adipogenic potential of oxytocin may attribute to reduced adipocytes density and volume in bone tissue. Proton MR spectroscopy may be a valuable tool, providing complementary information on efficacy assessments for bone metabolic diseases.

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Conflict of interest

The authors declare that they have no conflict of interest.

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