This is the peer reviewed version of the following article: Bautista CRG, Santos IVD, Moraes RM, et al. Sitagliptin's effects on bone tissue and osseointegration in diabetic rats. *Arch Oral Biol*. 2019;102:238-243, ***which has been published in final form at*** https://doi.org/10.1016/j.archoralbio.2019.04.018**.**

Sitagliptin's effects on bone tissue and osseointegration in diabetic rats

Authors:

1- Cristhian Reynaldo Gomez Bautista[1]; MSc.

2- Ingrid Valadares dos Santos[1]; undergraduate student.

3- Renata Mendonça Moraes[1]; MSc; PhD student.

4- Fernando Yamamoto Chiba[2], PhD

5- Doris Hissako Sumida[2], PhD, Full professor

6- Michele Bianchi de Moraes[3], PhD, Assistant professor.

7- Luana Marotta Reis de Vasconcellos[1]; PhD; Associate professor.

8- Ana Lia Anbinder [1*], PhD, Associated professor.

1 Authors from: Department of Bioscience and Oral Diagnosis, São Paulo State University (Unesp), Institute of Science and Technology, São José dos Campos. São José dos Campos, São Paulo, Brazil; Address: Av. Engenheiro Francisco José Longo, 777, São José dos Campos, São Paulo, 12245-000, Brazil.

2 Authors from: Department of Basic Science, São Paulo State University (Unesp), School of Dentistry, Araçatuba, São Paulo, Brazil. Address: Rua José Bonifácio, 1193, Araçatuba, São Paulo, 16015-050, Brazil.

3 Author from: Department of Diagnosis and Surgery. São Paulo State University (Unesp), Institute of Science and Technology, São José dos Campos. São José dos Campos, São Paulo, Brazil; Address: Av. Engenheiro Francisco José Longo, 777, São José dos Campos, São Paulo, 12245-000, Brazil.

* Corresponding author: PhD; Associate professor; Fax: 55-12-3947 (012) 3947-9358-9010; Tel: (012) 3947-9358; e-mail:ana.anbinder@unesp.br

Abstract

Objective: To investigate the effects of sitagliptin, a dipeptidyl peptidase 4 inhibitor used to treat type II diabetes, on bone tissue and on implant osseointegration in diabetic rats.

Design: Thirty-two male rats were divided into four groups: 1) Diabetic animals (GD); 2) Diabetic animals that received sitagliptin (GDS); 3) Normoglycemic animals (GN); and 4) Normoglycemic animals that received sitagliptin (GNS). All animals received titanium implants in the right tibia. Sitagliptin or its dilution vehicle were administered for 4 weeks. Glycemia, HOMA-IR, insulinemia, microtomographic parameters of the left tibia, implant bone area fraction occupancy (BAFO) and of the right tibia were evaluated.

Results: The model used to induce diabetes led to hyperglycemia. However, HOMA-IR results showed no insulin resistance, and insulinemia was lower in diabetic animals, demonstrating the development of type I diabetes. Sitagliptin administration did not influence glycemic control. The diabetic animals showed a lower BAFO, and bone volume fraction, as well as a lower trabecular number and thickness, revealing the deleterious effect of diabetes on bone metabolism and osseointegration.

Conclusion: In this model, sitagliptin administration did not reverse the negative effects of type I diabetes on bone, suggesting that sitagliptin has no direct action on bone tissue and has no protective bone action in decompensated diabetic animals.

Keywords: Incretins, Dipeptidyl peptidase 4, Implants

Highlights:

- Type I diabetes has a deleterious effect on bone microarchitecture.
- Type I diabetes has a deleterious effect on implant osseointegration.
- Sitagliptin had no direct action on bone osseointegration or on bone remodeling.

Introduction

Diabetes mellitus (DM) is characterized by hyperglycemia caused by a deficiency in insulin secretion (type I) and/or action (type II) (International Diabetes Federation). It is recognized as an epidemic issue, with estimates of up to 693 million people having the disease by 2045 (Cho et al., 2018). Hyperglycemia causes immune system disruption and micro- and macroangiopathy, leading to a predisposition to infections. Microangiopathy, nephropathy, neuropathy, macrovascular disease, delayed wound healing and periodontitis are the main complications of DM (Abiko & Selimovic, 2010; Luthra & Grover, 2013; Orasanu & Plutzky, 2009).

Due to the increased risk of periodontal disease and dental loss (Luthra & Grover, 2013), diabetic individuals have a great need for oral rehabilitation, and dental implants represent the latest therapy in dental replacement. However, osseointegration in these patients shows higher rates of failure when compared to normoglycemic ones in the long-term, and patients with uncontrolled glycemia appear to present delayed osseointegration following implantation and the highest occurrence of peri-implantitis (Naujokat, Kunzendorf, & Wiltfang, 2016). Therefore, glycemic control is crucial for bone health in DM, since chronic hyperglycemia stimulates bone resorption, decreases bone formation (Xu et al., 2014), and leads to slower repair after surgery (Younis, Al-Rawi, Mohamed, & Yaseen, 2013). Likewise, studies have shown that the bone fracture risk is twice as high in type II diabetics, and these results are mainly related to bone quality and not just to bone mineral density (BMD). In addition, computed tomography scans show profound changes in bone geometry in diabetic patients (Gorman, Chudyk, Madden, & Ashe, 2011; Russo et al., 2016).

Lifestyle modifications are recommended for patients with type II DM, and they are usually treated with a variety of hypoglycemic agents. Among these drugs are the

dipeptidyl peptidase 4 (DPP-4) inhibitors (International Diabetes Federation), a class of drugs that aims to increase the half-life of incretins. Incretins are hormones such as glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), involved in the secretion of insulin and glucagon and released by the intestine after food intake. Once released, incretins are rapidly degraded by the DPP-4 enzyme (Baggio & Drucker, 2007).

Sitagliptin (Januvia®) is a DPP-4 inhibitor approved by the Food and Drug Administration (FDA) that increases glucose-dependent insulin secretion, inhibits the secretion of postprandial glucagon, reduces hepatic glucose production and increases glucose disposition (Baggio & Drucker, 2007). Some studies have shown the pleiotropic effects of this class of drugs on bone tissue, stimulating bone turnover and calcium deposition (Aoyama, Watari, Podyma-Inoue, Yanagishita, & Ono, 2014; Bollag et al., 2000, 2001), as well as an anti-inflammatory action (Hatwal, 2012).

Receptors for GIP and GLP-1 have been identified in osteoblasts and osteoclasts (Aoyama et al., 2014; Bollag et al., 2000; Nuche-Berenguer et al., 2011; Pacheco-Pantoja, Ranganath, Gallagher, Wilson, & Fraser, 2011; Zhong et al., 2007), as well as GLP-1 receptor in osteocytes (Kim et al., 2013), suggesting a possible direct effect of incretins on bone regardless of blood glucose levels (Glorie et al., 2014). Double incretin receptor knock-out mice exhibited profound alterations of bone microarchitecture and bone strength, suggesting an important role of these hormones in regulating bone quality (Mieczkowska et al., 2015).

Because of the DPP-4 inhibitors action in increasing the half-life of incretins, studies were conducted to evaluate its effects on bone. The administration of sitagliptin increased vertebral BMD in estrogen-deficient rats (Cusick et al., 2013) and decreased trabecular bone loss in diabetic rats (Glorie et al., 2014). In humans, the data are still

controversial; although some studies have shown that DPP-4 inhibitors treatment may be associated with a reduced risk of fractures (Monami, Dicembrini, Antenore, & Mannuci 2011; Russo et al., 2016), others did not find any impact on risk of fracture associated with DPP-4 inhibition. This inconsistency may be due to variations in controls, sample size, dose and pharmacokinetics profiles (Josse et al., 2017; Yang et al., 2017).

Thus, considering the possible direct action of incretins on bone tissue, we hypothesized that sitagliptin could ameliorate the bone quality and osseointegration of implants in diabetic rats.

Materials and methods

Experimental design

This experiment was approved by the Institutional Ethics Committee (10/2014). Thirty-two male mature adult rats (*Rattus norvegi*cus Albinus, Wistar), with 120 days of age, were divided into four groups according to their glycemic status and the received therapy:

1) Normoglycemic group (GN): Eight normoglycemic animals that received water by oral gavage.

2) Normoglycemic treated with sitagliptin group (GNS): Eight normoglycemic animals that received 10 mg/kg/day of sitagliptin diluted in water by oral gavage.

3) Diabetic group (GD): Eight diabetic animals that received water by oral gavage.

4) Diabetic group treated with sitagliptin (GDS): Eight diabetic animals that received 10 mg/kg/day of sitagliptin diluted in water by oral gavage.

In all animals, a titanium implant was inserted in the right tibia. The administration of sitagliptin or water started the day after implant surgery and ended on the day of euthanasia, 4 weeks after surgery.

Diabetes induction

DM was induced as described by Wilson and Islam (2012). Briefly, the animals received 10% fructose solution in their drinking water for 2 weeks. After this period, they were fasted for 16 h, and 40 mg/kg of streptozocin diluted in citrate buffer (pH 4.40) (>98%, Sigma- Aldrich, Saint Louis, MO, USA) was injected intraperitoneally. One hour after injection with streptozocin, the animals received water and food *ad libitum* until the end of the experiment. Plasma glucose was measured by puncture of the caudal artery with the aid of a glucose meter (OneTouch Ultra Mini- Johnson & Johnson Medical Group, São Paulo, SP, Brazil). Animals were considered diabetic when the plasma glucose level was greater than 300 mg/dL.

Implants placement surgery

Four weeks after streptozocin injection and confirmation of DM (GDS and GD groups), all animals were anesthetized with a mixture of 10 mg/kg of xylazine hydrochloride 2% (Anasedan® Vet Brands International, Miramar, FL, USA) and 95 mg/kg of ketamine base (Dopalen® Ceva Saúde Animal Ltda., Paulínia, SP, Brazil), intramuscularly. Then, the right tibiae were surgically exposed, and the proximal third of the bone was submitted to osteotomy with implant drills to accommodate a titanium implant, using an electric surgical drill (AEU707Av2, Aseptico, Washington, DC, USA), at 1100 rpm, under constant and abundant irrigation with saline physiological solution, throughout the surgery. The implant used was made of commercially pure titanium, of a threadable type, 4.0 mm width and 2.0 mm in diameter (AS Technology,

Titanium Fix Dental Implants, São José dos Campos, SP, Brazil) and was pressed into the surgical cavity until it was fixed to the cortical bone. Following the insertion of the implant, the muscle tissue was sutured with absorbable thread #4 catgut (Cirumédica, Cotia, SP, Brazil) and the skin was sutured using #4-0 silk thread (Ethicon/Johnson & Johnson, São José dos Campos, SP, Brazil). After surgery, all animals received an intramuscular injection of antibiotic (Pentabiotico®; Fort Dodge Saúde Animal, São Paulo, SP, Brazil) and an oral solution of analgesic (15 mg/kg-paracetamol—Tylenol baby—Janssen-Cilag Farmacêutica Ltda, São José dos Campos, SP, Brazil). The experimental design is shown in Figure 1.

HOMA-IR

On the day of euthanasia, which occurred 4 weeks after implant placement, animals were submitted to cardiac puncture and blood was collected from the left heart ventricle of the animals using a needle attached to a serum separation gel vacuum tube (Tubo gel BD SST® II Advance BD, São Paulo, SP, Brazil). The glycemia (Interkit, Katal Biotecnológica, Belo Horizonte, MG, Brazil) and insulinemia (ELISA kit; EZRMI-13K, Millipore Corporation, St Charles, MO, USA) levels were evaluated according to the kit manufacturers' instructions to determine the HOMA-IR index. This index is used for the evaluation of insulin resistance and is calculated by the formula: HOMA − IR=glycemia × insulinemia/ 22.5 (Okoduwa, Umar, James, & Inuwa, 2017; Wilson & Islam, 2012).

Histomorphometric analyses

After blood collection, the animals were euthanized and their tibiae were removed. The right tibiae with the implants were fixed in paraformaldehyde for 24 h.

The tibiae were then washed in running water for approximately 30 min, dehydrated in increasing concentrations of alcohol, defatted in xylene, infiltrated with methyl methacrylate resin (Synth®, Diadema, SP, Brazil), and finally, embedded in the same solution using special glass molds placed in the incubator at 37°C. The blocks were then cut into slices using a diamond saw in a cutting machine for hard tissues (Labcut 1010, Extec Corp®, Enfield, CT, USA). The sections were glued to acrylic Plexiglas® slides, then ground and polished (Labpol 8-12, Extec, Enfield, CT, USA) to a final thickness of ~250 μm. The sections were stained with toluidine blue and microscopic images were obtained using an optical microscope attached to a digital camera (Axioplan 2, Carl Zeiss, Oberkochen, Baden-Württemberg, Germany).

Histometric analysis was performed using image analysis software (ImageJ 1.31p, National Institutes of Health, Bethesda, MD, USA). In the polished sections, a single-blind examiner evaluated the percentage of area between the threads occupied by bone tissue (*bone area fraction occupied*—BAFO).

Microcomputed tomography (μCt)

Three-dimensional microcomputed tomography (μCt) analyses of the left tibiae were performed using Skyscan 1176 (Aatselaar, Antwerp, Belgium). The tibiae were positioned vertically, and tomographic images were acquired at 50 kVp and 500 μA with an isotropic voxel size of 18 μm and a rotation of 0.0120. After obtaining X-ray projection, the images were reconstructed using the software NRecon (SkyScan, 2011; Version 1.6.6.0, Edinburgh, Scotland, United Kingdom), and Data Viewer (SkyScan, Version 1.4.4 64-bit, Edinburgh, Scotland, United Kingdom), according to three planes (transversal, longitudinal and sagittal). Then, using CT Analyzer software (CTAn, 2003-11SkyScan, 2012 Bruker MicroCT 1.12.4.0 version), a volume of interest (VOI)

was standardized using a transaxial cut. The analyzed VOI corresponded to 175 CT scans of the tibial metaphysis located at a distance of 80 cuts after the most caudal point of the growth plate. Bone volume fraction (BV/TV), trabecular number (Tb.N), thickness (Tb.Th) and separation (Tb.Sp) were calculated using standard methods.

Statistical analyses

The sample size was calculated using the G* Power 3.112 software (Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany), considering the Type I (α) and type II (β) errors of 5% and 20%, respectively, and a large effect size (1.6). For the statistical analysis of BAFO, microtomographic parameters, glycemia and HOMA-IR index, two-way analysis of variance (ANOVA) was performed (α=5%) considering the use of the drug and the presence or absence of DM as the two independent variables.

Results

Diabetes induction: blood glucose and HOMA-IR index

The descriptive statistical analysis of glycemia values collected on euthanasia day using the glucose meter is shown in Figure 2a. A significant difference was found regarding the main effect "Diabetes" ($F=450.6$; $p< 0.0001$) with diabetic animals showing higher glycemia values (590.7± 30.2 mg/dL) compared with normoglycemic ones (263.3± 52.4 mg/dL). No statistically significant difference was observed regarding the main effect "Drug" ($F= 0.092$; $p=0.763$) or any interaction ($F=0.759$; $p=0.391$).

The descriptive statistical analysis of the HOMA-IR index, performed with the blood collected on euthanasia day, can be observed in Figure 2b. After submitting the data to two-way ANOVA, there was not a significant difference in the "interaction" effect ($F=2.783$; $p=0.104$), main effect "Drug" ($F=2.679$; $p=0.1109$) or main effect

"Diabetes" (F=2.200; p=0.1472), demonstrating that the animals had no insulin resistance at the end of the experiment. When insulinemia was evaluated separately, serum insulin values on GD (3.836 ±0.636 µUI/mL) represented 51.13% of the plasma insulin values of GN (7.503 ±2.570 µUI/mL).

Histomorphometric analyses

The bone area fraction occupancy between the threads (BAFO) was evaluated by histomorphometry. One sample from GDS was not used due to problems during processing. It was observed that there was a significant difference only in the main effect "Diabetes", with diabetic animals presenting 14.38% significantly lower osseointegration than the normoglycemic animals (Figure 3a).

Microcomputed tomography (µCt)

After two-way ANOVA, in relation to the "Diabetes" main effect, the BV/TV was 32.50% lower in the diabetic animals, Tb.N was 29.94% lower, and Tb.Th was 5.12% lower when compared with normoglycemic controls. Tb.Sp was not significantly different among groups. There were no significant differences in "interaction" or “Drug” main effect for all of microtomographic parameters analyzed (BV/TV, Tb.Sp, Tb.N, and Tb.Th- Figure 4).

Discussion

We hypothesized that sitagliptin could have a beneficial effect on bone tissue and implant osseointegration in the tibiae of diabetic rats based on studies that demonstrated a decreased fracture rate in diabetic patients using DPP-4 inhibitors (Monami, Dicembrini, Antenore, & Mannuci, 2011, 2011; Russo et al., 2016) and others that indicated a direct action of incretins on bone metabolism (Cusick et al., 2013; Glorie et al., 2014).

To induce DM, in this study, we used the method proposed by Wilson and Islam (2012), which consists of an association between 10% fructose consumption and a low dose of streptozocin injection (40 mg/kg) in a quick and low-cost protocol that leads to the development of type II DM. Although the animals in the diabetic groups developed hyperglycemia, the HOMA-IR index analysis demonstrated that there was no insulin resistance in any of the groups, excluding the possibility of type II DM induction. Recently, Okoduwa et al. (2017) evaluated the influence of different diets (normal-diet and fortified-diet) and doses of streptozocin as type II DM induction models. Similar to us, the authors were not able to induce insulin resistance using only fructose supplementation. They only succeeded when margarine was added to the diet along with 20% fructose in the water (fortified-diet-feed groups). Furthermore, they recommended the use of residual insulin as a tool for distinguishing type I and II DM. According to the authors, the residual insulin in type II DM should not be less than 85.7% of the normal control group in a hyperglycemic condition of nonfasting blood glucose > 300 mg/dL (Okoduwa, Umar, James, & Inuwa, 2017). In our experiment, the residual insulin in the diabetic group was 51.13% of the normoglycemic group, indicating a type I DM induction.

The induction of type I DM was also confirmed by the fact that the sitagliptin dosage used did not lead to a reduction in glycemia, which would be expected in type II DM (Baggio & Drucker, 2007). Once sitagliptin increases the half-life of incretins, and thus stimulates insulin production, the pancreatic islets must be preserved to be able to produce insulin so the drug can exert an effect on blood glucose lowering. Similar to us, Glorie et al. (2014) induced type I DM with a high dose streptozocin injection and found that sitagliptin did not decrease glycemia in the animals.

Osseointegration is a prerequisite for implant stability and inflammation-free survival. The bone area fraction occupied by bone between the implant threads (BAFO) has been one of the most evaluated parameters when analyzing osseointegration (Soares et al., 2015). Statistical analysis of BAFO and microtomographic parameters in this study demonstrated that DM has a negative influence on implant osseointegration, but treatment with sitagliptin was not able to reverse this effect. Although several clinical studies found a positive influence of DPP-4 inhibition on bone fracture (Choi et al., 2016; Dombrowski, Kostev, & Jacob, 2017; Monami, Dicembrini, Antenori, & Mannucci, 2011), a recent meta-analysis concluded that alogliptin may be the only drug associated with a lower risk of bone fracture among patients with type II DM when compared with placebo (Yang et al., 2017). These results show that while all DPP-4 inhibitors improve glycemic control, there may be some differences between the various drugs regarding their effects on other tissues (Evans & Bain, 2016; Kania, Gonzalvo, & Weber, 2011; Yang et al., 2017).

We used the same dose (10 mg/kg) and administration period of sitagliptin from a previous study conducted by our group, in which the drug was used over a 28-day period in a periodontitis induction model in rats (Moraes et al., 2015). Applying a conversion formula, this is the recommended manufacturer's dosage for humans (100 mg/day) and it is widely used in clinical studies (Reagan-Shaw, Nihal, & Ahmad, 2008; Wang, Gou, Wang, Ma, & Zhai, 2014). In the previous study, we proved that under this condition, sitagliptin significantly decreased DPP-4 serum levels (Moraes et al., 2015). Several studies have obtained improvement of bone parameters using higher sitagliptin doses than that recommended for humans (Cusick et al., 2013; Glorie et al., 2014). Dosages between 50 and 100 mg/day of DPP-4 inhibitor did not influence the risk of

fractures in patients after 1 year (Driessen et al., 2014). Therefore, the dosage appears to be essential for the onset of this medication's effects.

Another factor that could contribute to the lack of sitagliptin action is the period of treatment used in this experiment. Glorie et al. (2014) found significant differences in trabecular bone between the diabetic rats with and without sitagliptin treatment from the ninth week of treatment. Increases in the mechanical properties of the cortical bone were found only after the twelfth week. Thus, a longer treatment time could enable the onset of sitagliptin's effect on bone tissue.

Other authors administered exenatide, a GLP-1 agonist, in order to evaluate implant osseointegration in type II diabetic rats (Zhou et al., 2015). Although they demonstrated that exenatide enhanced the alkaline phosphatase serum levels and implant survival in diabetic animals, the results cannot be attributed with certainty to the direct effect of GLP-1 on bone cells, because the blood glucose of the animals was controlled by the treatment. When DM is well controlled, implant procedures are safer and predictable with a complication rate similar to that of healthy patients (Naujokat, Kunzendorf, & Witfang, 2016).

The end result of DPP-4 inhibition is difficult to predict (Glorie, D'Haese, & Verhulst, 2016). The DPP-4 enzyme has a wide distribution of substrates and targets proteins in bone cells beyond its indirect action in the hypothalamus stimulating the release of leptin and adiponectin, which are related to bone resorption. Considering the action of DDP-4 on incretins, insulin-like growth factor (IGF)-2 and vasoactive intestinal polypeptide (VIP), a protective effect on bone with its inhibition could be considered. However, in relation to other substrates, such as stromal cell-derived factor α (SDF1α), neuropeptide Y (NPY), peptide YY and substance P, it would not be

expected. This may explain the difference in results involving the inhibition of DPP-4 and bone homeostasis.

In summary, because studies have demonstrated the presence of incretin receptors in the bone, as well as the influence of these hormones on bone turnover, the idea that DPP-4 enzyme blockade could increase the half-life of these molecules, thus influencing bone metabolism, seems promising. However, in this experiment, treatment with sitagliptin did not reverse the deleterious effects of DM on bone tissue or the osseointegration of implants. We also demonstrated that DM impairs osseointegration and bone microarchitecture. DPP-4 presents with a wide distribution and acts on diverse substrates, which makes it a difficult target for treatments. Future studies should consider this characteristic of DPP-4, as well as the possible dose and time-dependent action of its inhibitors.

Declaration of interest

The authors report no conflict of interest related to this study.

Funding: This work was supported by São Paulo Research Foundation (FAPESP) [grant number 2014/20973-6 and 2017/26461-5] (IVS and RMM) and by Coordination for the Improvement of Higher Level Education Personnel (CAPES) (CRGB and RMM).

Acknowledgments: We would like to thank AS Technology Company for the donation of the implants used in this research. The authors report no conflicts of interest related to this study.

References

Abiko, Y. and Selimovic, D., The mechanism of protracted wound healing on oral mucosa in diabetes. Review. *Bosnian Journal of Basic Medical Sciences***10**(3), 2010, 186–91. https://doi.org/10.17305/bjbms.2010.2683

Aoyama, E., Watari, I., Podyma-Inoue, K.A., Yanagishita, M., and Ono, T. Expression of glucagon-like peptide-1 receptor and glucose-dependent insulinotropic polypeptide receptor is regulated by the glucose concentration in mouse osteoblastic MC3T3-E1 cells. *International Journal of Molecular Medicine* **34**(2), 2014, 475–82. https://doi.org/10.3892/ijmm.2014.1787

Baggio, L.L. and Drucker, D.J. (2007). Biology of Incretins: GLP-1 and GIP. *Gastroenterology* **132**(6), 2007, 2131–2157. https://doi.org/10.1053/j.gastro.2007.03.054

Bollag, R.J., Zhong, Q., Phillips, P., Min, L., Zhong, L., Cameron, R., & Isales, C.M. Osteoblast-derived cells express functional glucose-dependent insulinotropic peptide receptors. *Endocrinology* **141**(3), 2000., 1228–35. https://doi.org/10.1210/endo.141.3.7366

Bollag, R.J., Zhong, Q., Ding, K.H., Phillips, P., Zhong, L., Qin, F., et al. Glucose-dependent insulinotropic peptide is an integrative hormone with osteotropic effects. *Molecular and Cellular Endocrinology* **177**(1–2), 2001 35–41. http://www.ncbi.nlm.nih.gov/pubmed/11377818

Cho, N.H., Shaw, J.E., Karuranga, S., Huang, Y., da Rocha Fernandes, J. D., Ohlrogge, A. W., et al. IDF Diabetes Atlas: Global estimates of diabetes prevalence for 2017 and projections for 2045. *Diabetes Research and Clinical Practice* **138**, 2018, 271–281. https://doi.org/10.1016/j.diabres.2018.02.023

Choi, H.J., Park, C., Lee, Y.-K., Ha, Y.-C., Jang, S., and Shin, C.S. Risk of fractures and diabetes medications: A nationwide cohort study. *Osteoporosis International* **27**(9), 2016, 2709–2715. https://doi.org/10.1007/s00198-016-3595-6

Cusick, T., Mu, J., Pennypacker, B. L., Li, Z., Scott, K. R., Shen, X., et al. Bone loss in the oestrogen-depleted rat is not exacerbated by sitagliptin, either alone or in combination with a thiazolidinedione. *Diabetes, Obesity & Metabolism* **15**(10), 2013, 954–957. https://doi.org/10.1111/dom.12109

Dombrowski, S., Kostev, K. and Jacob, L. (2017). Use of dipeptidyl peptidase-4 inhibitors and risk of bone fracture in patients with type 2 diabetes in Germany-A retrospective analysis of real-world data. *Osteoporosis International* **28**(8), 2421–2428. https://doi.org/10.1007/s00198-017-4051-y

Driessen, J.H.M., van Onzenoort, H.A.W., Henry, R.M.A., Lalmohamed, A., van den Bergh, J.P., Neef, C., et al. Use of dipeptidyl peptidase-4 inhibitors for type 2 diabetes mellitus and risk of fracture. *Bone* **68**, 2014, 124–130. https://doi.org/10.1016/j.bone.2014.07.030

Evans, P.M.S. and Bain, S.C. Omarigliptin for the treatment of type 2 diabetes mellitus. *Expert Opinion on Pharmacotherapy* **17**(14), 2016, 1947–1952. https://doi.org/10.1080/14656566.2016.1218472

Glorie, L., Behets, G.J., Baerts, L., De Meester, I., D'Haese, P.C. and Verhulst, A. DPP IV inhibitor treatment attenuates bone loss and improves mechanical bone strength in male diabetic rats. American Journal of Physiology. *Endocrinology and Metabolism* **307**(5), 2014, E447-55. https://doi.org/10.1152/ajpendo.00217.2014

Glorie, L., D'Haese, P.C. and Verhulst, A. Boning up on DPP4, DPP4 substrates, and DPP4-adipokine interactions: Logical reasoning and known facts about bone related

effects of DPP4 inhibitors. *Bone* **92**, 2016, 37–49. https://doi.org/10.1016/j.bone.2016.08.009

Gorman, E., Chudyk, A.M., Madden, K.M., and Ashe, M.C. Bone health and type 2 diabetes mellitus: A systematic review. *Physiotherapy Canada*, **63**(1), 2011, 8–20. https://doi.org/10.3138/ptc.2010-23bh

Hatwal, A. Inflammation and incretins. *Indian Journal of Endocrinology and Metabolism* **16**(Suppl 2), 2012, S239-41. https://doi.org/10.4103/2230-8210.104049

International Diabetes Federation. (2018). https://www.idf.org/about-diabetes/what-is-diabetes.html. [Accessed 28 November 2018].

Josse, R.G., Majumdar, S.R., Zheng, Y., Adler, A., Bethel, M.A. and Buse, J.B. TECOS Study Group, Sitagliptin and risk of fractures in type 2 diabetes: Results from the TECOS trial. *Diabetes, Obesity & Metabolism* **19**(1), 2017, 78–86. https://doi.org/10.1111/dom.12786

Kania, D.S., Gonzalvo, J.D. and Weber, Z.A. Saxagliptin: A Clinical review in the treatment of type 2 diabetes mellitus. *Clinical Therapeutics* **33**(8), 2011, 1005–1022. https://doi.org/10.1016/j.clinthera.2011.06.016

Kim, J.-Y., Lee, S.-K., Jo, K.-J., Song, D.-Y., Lim, D.-M., Park, K.-Y et al. Exendin-4 increases bone mineral density in type 2 diabetic OLETF rats potentially through the down-regulation of SOST/sclerostin in osteocytes. *Life Sciences* **92**(10), 2013, 533–40. https://doi.org/10.1016/j.lfs.2013.01.001

Luthra, S. and Grover, H. Molecular mechanisms involved in the bidirectional relationship between diabetes mellitus and periodontal disease. *Journal of Indian Society of Periodontology* **17**(3), 2013, 292. https://doi.org/10.4103/0972-124X.115642

Mieczkowska, A., Mansur, S., Bouvard, B., Flatt, P.R., Thorens, B., Irwin, N. et al. Double incretin receptor knock-out (DIRKO) mice present with alterations of trabecular

and cortical micromorphology and bone strength. *Osteoporosis International* **26**(1), 2015, 209–18. https://doi.org/10.1007/s00198-014-2845-8

Monami, M., Dicembrini, I., Antenore, A. and Mannucci, E. Dipeptidyl Peptidase-4 Inhibitors and Bone Fractures: A meta-analysis of randomized clinical trials. *Diabetes Care* **34**(11), 2011, 2474–2476. https://doi.org/10.2337/dc11-1099

Moraes, R.M., Lima, G.M.G., Oliveira, F.E., Brito, A.C.V., Pereira, R.C., Oliveira, L.D., et al. Exenatide and sitagliptin decrease interleukin 1β, matrix metalloproteinase 9, and nitric oxide synthase 2 gene expression but does not reduce alveolar bone loss in rats with periodontitis. *Journal of Periodontology* **86**(11), 2015 https://doi.org/10.1902/jop.2015.150278

Naujokat, H., Kunzendorf, B. and Wiltfang, J. Dental implants and diabetes mellitus—a systematic review. *International Journal of Implant Dentistry* **2**(1), 2016, 5. https://doi.org/10.1186/s40729-016-0038-2

Nuche-Berenguer, B., Lozano, D., Gutiérrez-Rojas, I., Moreno, P., Mariñoso, M. L., Esbrit, P., et al. GLP-1 and exendin-4 can reverse hyperlipidic-related osteopenia. *The Journal of Endocrinology* **209**(2), 2011, 203–10. https://doi.org/10.1530/JOE-11-0015

Okoduwa, S.I.R., Umar, I.A., James, D.B. and Inuwa, H.M. Appropriate insulin Level in Selecting Fortified Diet-Fed, Streptozotocin-Treated Rat Model of Type 2 Diabetes for Anti-Diabetic Studies. *PloS One* **12**(1), 2017, e0170971. https://doi.org/10.1371/journal.pone.0170971

Orasanu, G. and Plutzky, J. The pathologic continuum of diabetic vascular disease. *Journal of the American College of Cardiology* **53**(5 Suppl), 2009, S35-42. https://doi.org/10.1016/j.jacc.2008.09.055

Pacheco-Pantoja, E. L., Ranganath, L. R., Gallagher, J. A., Wilson, P. J. M., & Fraser, W. D. (2011). Receptors and effects of gut hormones in three osteoblastic cell lines. BMC Physiology, 11(1), 12. https://doi.org/10.1186/1472-6793-11-12

Reagan-Shaw, S., Nihal, M. and Ahmad, N. Dose translation from animal to human studies revisited. *The FASEB Journal* **22**(3), 2008, 659–61. https://doi.org/10.1096/fj.07-9574LSF

Russo, G.T., Giandalia, A., Romeo, E.L., Nunziata, M., Muscianisi, M., Ruffo, M.C., et al. Fracture risk in type 2 diabetes: Current perspectives and gender differences. *International Journal of Endocrinology*, 2016, 1–11. https://doi.org/10.1155/2016/1615735

Soares, P.B.F., Moura, C.C.G., Claudino, M., Carvalho, V.F., Rocha, F.S. and Zanetta-Barbosa, D. Influence of implant surfaces on osseointegration: a histomorphometric and implant stability study in rabbits. *Brazilian Dental Journal* **26**(5), (2015). 451–457. https://doi.org/10.1590/0103-6440201300411

Wang, T., Gou, Z., Wang, F., Ma, M. and Zhai, S. Comparison of GLP-1 analogues versus sitagliptin in the management of type 2 diabetes: systematic review and meta-analysis of head-to-head studies. *PloS One* **9**(8), 2014, e103798. https://doi.org/10.1371/journal.pone.0103798

Wilson, R. D. and Islam, M.S. Fructose-fed streptozotocin-injected rat: an alternative model for type 2 diabetes. *Pharmacological Reports: PR* **64**(1), 2012, 129–39. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/22580529

Xu, F., Dong, Y., Huang, X., Li, M., Qin, L., Ren, Y., et al., Decreased osteoclastogenesis, osteoblastogenesis and low bone mass in a mouse model of type 2 diabetes. *Molecular Medicine Reports* **10**(4), 2014, 1935–41. https://doi.org/10.3892/mmr.2014.2430

Yang, J., Huang, C., Wu, S., Xu, Y., Cai, T., Chai, S., et al. The effects of dipeptidyl peptidase-4 inhibitors on bone fracture among patients with type 2 diabetes mellitus: A network meta-analysis of randomized controlled trials. *PloS One* **12**(12), 2017, e0187537. https://doi.org/10.1371/journal.pone.0187537

Younis, W.H., Al-Rawi, N.H., Mohamed, M.A.-H. andYaseen, N.Y. Molecular events on tooth socket healing in diabetic rabbits. *The British Journal of Oral & Maxillofacial Surgery* **51**(8), 2013, 932–6. https://doi.org/10.1016/j.bjoms.2013.08.014

Zhong, Q., Itokawa, T., Sridhar, S., Ding, K.-H., Xie, D., Kang, B., et al. Effects of glucose-dependent insulinotropic peptide on osteoclast function. *American Journal of Physiology-Endocrinology and Metabolism* **292**(2), 2007, E543–E548. https://doi.org/10.1152/ajpendo.00364.2006

Zhou, W., Liu, Z., Yao, J., Chi, F., Dong, K., Yue, X., et al. The Effects of exenatide microsphere on serum BGP and ALP levels in ZDF rats after implantation. *Clinical Implant Dentistry and Related Research* **17**(4), 2015, 765–70. https://doi.org/10.1111/cid.12184

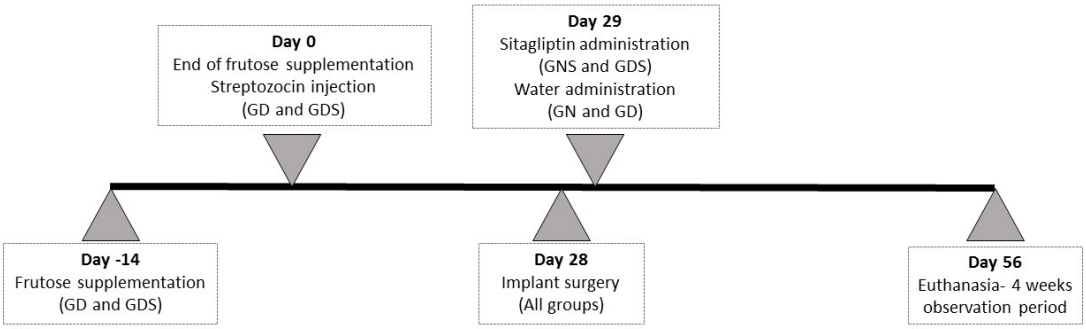

Figure 1: Experimental design. GN: Normoglycemic group; GNS: Normoglycemic treated with Sitagliptin group; GD: Diabetic group; GDS: Diabetic group treated with Sitagliptin

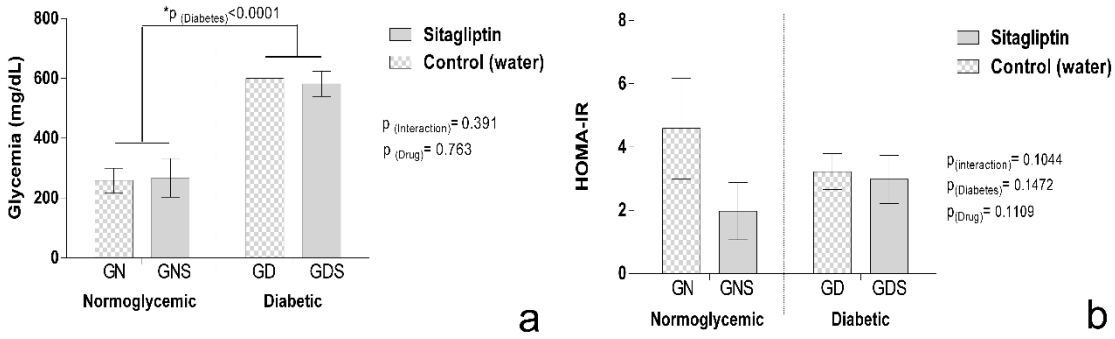

Figure 2: Plots of mean and standard deviation of glycemia and HOMA-IR. a) Glycemia. The main effect “Diabetes” showed statistically significant difference, with normoglycemic animals presenting lower values of glycemia than diabetic animals. b) HOMA-IR. There was no statistically significant difference in "interaction effect", main effect "diabetes" or main effect "drug" for HOMA-IR. GN: Normoglycemic group; GNS: Normoglycemic treated with Sitagliptin group; GD: Diabetic group; GDS: Diabetic group treated with Sitagliptin

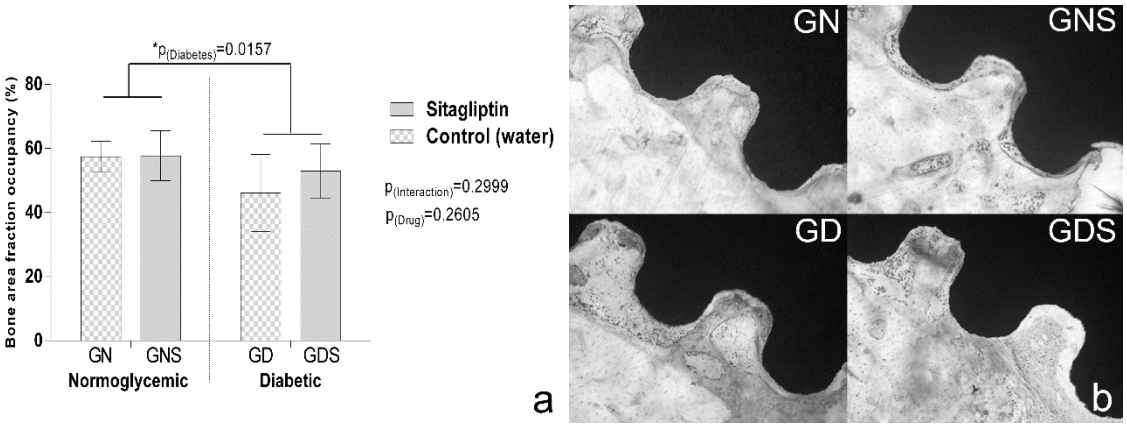

Figure 3: Plots of means and standard deviation of bone area fraction occupancy (BAFO). a) A significant statistical difference was observed on main effect "Diabetes" (F=6.643; p=0.0157) after two-way ANOVA test, with diabetic animals presenting lower values of BAFO than normoglycemic animals. b) Representative photomicrographs of bone tissue area from each group. Original magnification of 200x, toluidine blue staining. GN: Normoglycemic group; GNS: Normoglycemic treated with Sitagliptin group; GD: Diabetic group; GDS: Diabetic group treated with Sitagliptin

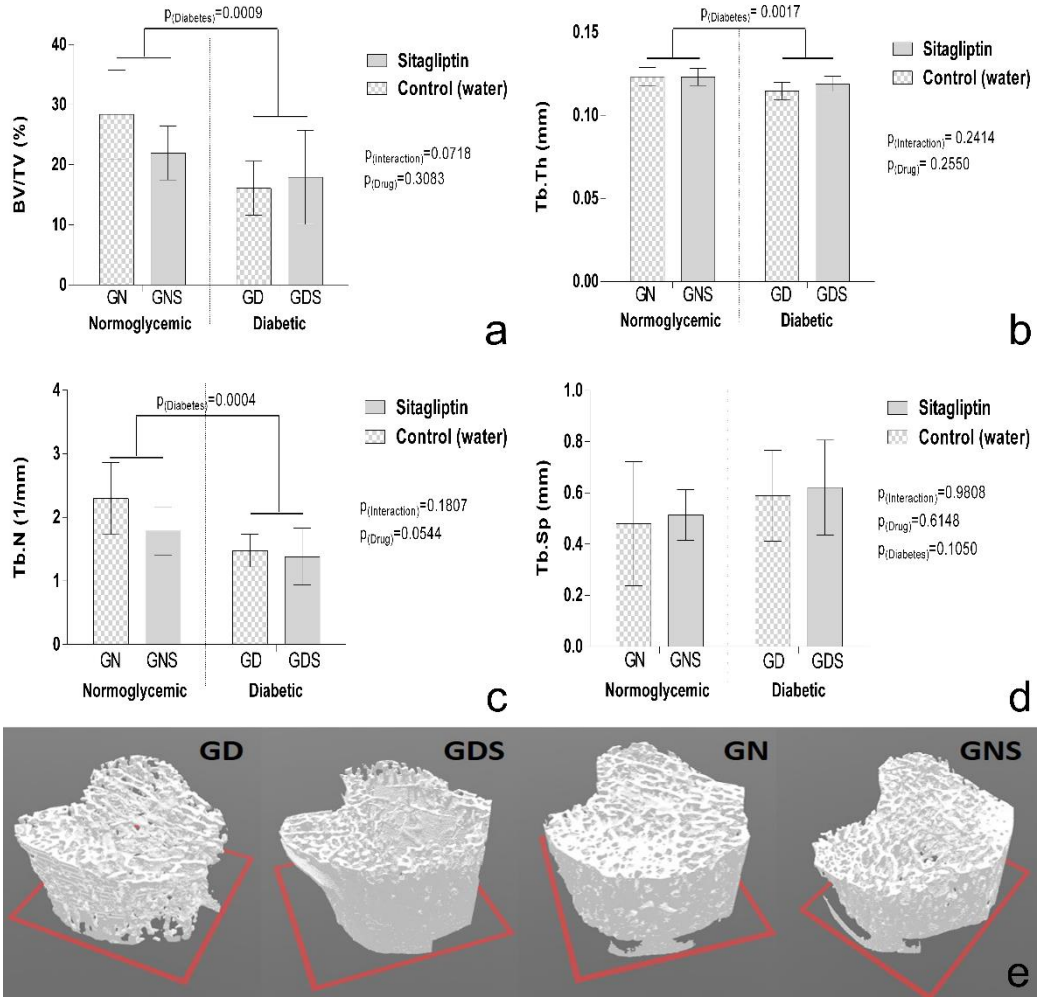

Figure 4: Plots of mean and standard deviation of micro-computed tomography results. After two-way ANOVA test, a significant main effect "Diabetes" was found in BV/TV (F=13.72; p= 0.0009) (a), Tb.Th (F=11.99, p=0.0017) (b), Tb.N (F=16.27, p=0.0004) (c), with lower values in diabetic animals, demonstrating the influence of diabetes on bone parameters. No statistical difference was found in Tb.Sp (F= 2.806; p = 0.1050) (d). (e) Tridimensional reconstruction of the volume of interest of one representative specimen from each group. Deterioration of bone microarchitecture can be seen in diabetic groups. GN: Normoglycemic group; GNS: Normoglycemic treated with Sitagliptin group; GD: Diabetic group; GDS: Diabetic group treated with Sitagliptin.