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Original Article

# Effect of an oral antidiabetic, sitagliptin, a DPP-4 inhibitor, on bone remodeling: study in ovariectomized rats

## Efeito de um antidiabético oral sitagliptina, um inibidor da DPP-4, na remodelação óssea: estudo em ratas ovariectomizadas

**Luciana Muniz Pechmann*[I], Vicente Florentino Castaldo Andrade[I], Thais Andrade Costa Casagrande[II], Rafaela Ceron[III] Leticia Capote dos Santos[III], Edneia Amancio de Souza Ramos Cavalieiri[I], Gabriela Casani Cardoso[I], Regiane Stafim da Cunha[I] Carolina Aguiar Moreira[I]**

[I]Universidade Federal do Paraná, Curitiba, PR, Brasil
[II]Universidade Positivo, Curitiba, PR, Brasil
[III]Instituto Pró-Renal, Curitiba, PR, Brasil

***Autor Correspondente:**

Ellian Robert Vale Santos
Bacharel em Educação Física
ellian.robert@discente.ufma.br

Endereço para correspondência:
Universidade Federal do Maranhão
Departamento de Educação Física
Avenida dos Portugueses, nº 1966
Vila Bacanga, São Luís, Maranhão, Brasil
CEP: 65080-805

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## ABSTRACT

**Objective:** To evaluate the effects of sitagliptin, a DPP-4 inhibitor, on bone remodeling using dynamic histomorphometry and assess DPP4 gene expression in bone tissue of ovariectomized (OVX) rats, a model of hypoestrogenism. **Method:** Non-diabetic female Wistar rats were divided into five groups: OVX-S (ovariectomized, sitagliptin-treated, n=9), OVX (ovariectomized, saline-treated, n=7), SHAM-S (sham-operated, sitagliptin-treated, n=10), SHAM (sham-operated, saline-treated, n=7), and control (n=7). Sitagliptin (25 mg/kg) or saline was administered daily via gavage for 13 weeks post-surgery. Bone histomorphometry of the right tibia assessed structural (BV/TV, Tb.Th, Tb.Sp, Tb.N), remodeling static (OS/BS, O.Th, ES/BS) and dynamic (MS/BS, MAR, BFR/BS) parameters. DPP4 gene expression in the right femur was analyzed using RT-qPCR. Statistical analyses included ANCOVA and Kruskal-Wallis tests ($p<0.05$). **Results:** Sitagliptin mitigated bone resorption in OVX-S compared to OVX. Structural parameters showed lower BV/TV and Tb.N, and higher Tb.Sp in OVX and OVX-S versus SHAM, SHAM-S, and control, with OVX-S having smaller Tb.Sp than OVX ($p=0.012$). Static parameters indicated higher OS/BS in OVX-S versus SHAM-S ($p<0.04$). Dynamic parameters revealed that the ovariectomized (OVX) group demonstrated greater number of fluorescent labels and therefore a higher mineralized surface (MS/BS) than the OVX-S ($p<0.01$), indicative that sitagliptin effectively mitigated increased bone resorption associated with hypoestrogenism. Dynamic parameters also revealed greater BFR/BS in OVX compared to all groups ($p<0.001$). DPP4 expression was significantly lower in OVX-S and SHAM-S versus OVX ($p<0.01$). **Conclusion:** Sitagliptin reduces bone remodeling in hypoestrogenic states, likely by decreasing resorption, as shown by histomorphometry and reduced DPP4 expression, suggesting its potential to prevent bone loss in conditions like menopause.

**Keywords:** Dipeptidyl Peptidase 4; Dipeptidyl Peptidase IV Inhibitors; Osteoporosis; Bone fractures; Bone

## RESUMO

**Objetivo:** Avaliar os efeitos da sitagliptina, um inibidor de DPP-4, na remodelação óssea usando histomorfometria dinâmica e avaliar a expressão do gene DPP4 no tecido ósseo de ratos ovariectomizados (OVX), um modelo de hipoestrogenismo. **Métodos** :Ratas Wistar fêmeas não diabéticas foram divididas em cinco grupos: OVX-S (ovariectomizadas, tratadas com sitagliptina, n=9), OVX (ovariectomizadas, tratadas com solução salina, n=7), SHAM-S (operadas sem retirada dos ovários, tratadas com sitagliptina, n=10), SHAM (operadas sem retirada dos ovários, tratadas com solução salina, n=7) e controle (n=7). Sitagliptina (25 mg/kg) ou solução salina foi administrada diariamente via gavagem por 13 semanas após a cirurgia. A histomorfometria óssea da tíbia direita avaliou parâmetros estruturais (BV/TV, Tb.Th, Tb.Sp, Tb.N), estáticos de remodelação (OS/BS, O.Th, ES/BS) e dinâmicos (MS/BS, MAR, BFR/BS). A expressão do gene DPP4 no fêmur direito foi analisada usando RT-qPCR. As análises estatísticas incluíram testes de ANCOVA e Kruskal-Wallis ($p<0,05$). **Resultados:** A sitagliptina atenuou a reabsorção óssea no grupo OVX-S em comparação com o OVX. Os parâmetros estruturais mostraram BV/TV e Tb.N mais baixos e Tb.Sp mais altos nos grupos OVX e OVX-S em comparação com SHAM, SHAM-S e controle, com o OVX-S tendo Tb.Sp menor que o OVX ($p=0,012$). Parâmetros estáticos indicaram maior OS/BS no OVX-S em comparação com SHAM-S ($p<0,04$). Parâmetros dinâmicos revelaram que o grupo ovariectomizado (OVX) apresentou maior número de marcações fluorescentes e, portanto, uma superfície mineralizada (MS/BS) maior que o OVX-S ($p<0,01$), indicando que a sitagliptina efetivamente atenuou o aumento da reabsorção óssea associado ao hipoestrogenismo. Parâmetros dinâmicos também revelaram maior BFR/BS no OVX em comparação com todos os grupos ($p<0,001$). A expressão de DPP4 foi significativamente menor em OVX-S e SHAM-S em comparação com OVX ($p<0,01$). **Conclusão**: A sitagliptina reduziu a remodelação óssea em estados hipoestrogênicos, provavelmente diminuindo a reabsorção, conforme demonstrado pela histomorfometria e expressão reduzida de DPP4, sugerindo seu potencial para prevenir a perda óssea em condições como a menopausa.


**Palavras-chave:** Dipeptidil Peptidase 4; Inibidores da Dipeptidil Peptidase IV; Osteoporose; Fraturas ósseas; Osso

## INTRODUCTION

Dipeptidyl peptidase-4 (DPP-4) is a serine peptidase that degrades various substrates, including cytokines, neuropeptides, and incretins such as gastric inhibitory polypeptide (GIP) and glucagon-like peptides 1 and 2 (GLP-1, GLP-2) (1). These substrates influence bone cells, cytokines, and immune cells within the skeletal system, suggesting a role for DPP-4 in bone metabolism beyond its established function in glucose regulation for type 2 diabetes mellitus (T2DM) management[2].

Sitagliptin, the first DPP-4 inhibitor approved for T2DM, has been widely used in patients with conditions such as cardiovascular disease, chronic kidney disease, and obesity due to its favorable safety profile and minimal side effects[3]. While other T2DM medications have emerged, DPP-4 inhibitors remain a preferred choice, particularly for elderly patients[4].

Bone remodeling is a dynamic process involving bone resorption by osteoclasts and bone formation by osteoblasts, tightly regulated by hormones and signaling molecules to maintain skeletal integrity[4]. In postmenopausal osteoporosis, estrogen deficiency disrupts this balance, leading to increased bone resorption, reduced bone mass, and elevated fracture risk[5]. Aging, particularly in the postmenopausal phase, is associated with a higher prevalence of T2DM, osteoporosis, and fractures[4].

Estrogen deficiency increases receptor activator of nuclear factor kappa-B ligand (RANKL) expression, promoting bone marrow-derived macrophage differentiation into osteoclasts[6]. Notably, DPP-4 is produced by osteoclasts in response to RANKL stimulation, reducing GLP-1 levels and suggesting a skeletal-pancreatic endocrine axis that regulates metabolic processes[7]. Inhibiting DPP-4 activity may restore bone metabolic balance and prevent bone loss[8] Preclinical studies have demonstrated that DPP-4 inhibitors, including sitagliptin, reduce bone resorption independently of glycemic control by decreasing osteoclast numbers and RANKL expression in ovariectomized rats, thereby preserving bone integrity[8,9,10].

Clinical studies in T2DM patients generally report an inverse association between DPP-4 inhibitor use and fracture risk[11,12,13]. However, some studies have noted negative or neutral effects on bone tissue[14,15,16]. These discrepancies may stem from small sample sizes, challenges in identifying fractures as endpoints, or the influence of other antihyperglycemic medications[17]. Additionally, DPP-4 is implicated in inflammatory and metabolic processes, including dyslipidemia and insulin resistance, which may further influence bone health[18,19].

Given these findings, this study aimed to evaluate the effects of sitagliptin on bone remodeling in ovariectomized female rats using histomorphometry, a gold-standard technique for measurement of bone remodeling, mainly the dynamic parameters, performed through previous use of fluorescents agents that label the mineralized front and indicate the intensity of bone remodeling by osteoblast activity and indirectly, the osteoclast effects and assess the impact of DPP-4 inhibition on DPP4 gene expression in bone tissue.

## MATERIAL AND METHODS

### Ethical Considerations

The study protocol was approved by the Animal Ethics Committee (CEUA-UP) under protocol number 22/2023. All experimental procedures were conducted according to the guidelines for animal experimentation established by the Brazilian National Council for the Control of Animal Experimentation (CONCEA).

### Animal Sourcing and Care

Non-diabetic female Wistar rats were maintained in autoclaved cages lined with wood shavings in a suitable room within the animal facility, under controlled conditions of 22°C temperature and a 12-hour dark/light cycle.

### Surgical Procedures

The animals were subjected to ovariectomy (OVX; n=16) or sham surgery (SHAM; n=17) at the age of 17 weeks. The ovaries, located were clamped and removed. The sham procedure followed the same steps, but the ovaries were left in place. After surgery, the animals in both groups received analgesia with tramadol hydrochloride (5 mg/kg) administered intramuscularly every 12 hours for 3 days.

### Intervention

The animals were further categorized into five groups according to the allocated intervention:

- OVX-S (n=9) and SHAM-S (n=10): included animals subjected to ovariectomy and sham surgery, respectively, that were allocated to receive sitagliptin.
- OVX (n=7) and SHAM (n=7): included animals subjected to ovariectomy and sham surgery, respectively, that were allocated to receive saline solution.
- Control (n=7): included animals that served as controls and were not subjected to ovariectomy or sham surgery and were not allocated to receive sitagliptin or saline solution.

Starting the day after surgery, the animals received sitagliptin 25 mg/kg (Januvia; Merck Sharp & Dohme) as 100 mg tablet diluted in 100 mL of saline solution or saline solution alone (1.5 mL). This intervention was administered daily via gavage between 8:00 am and 9:00 am for 13 weeks. The dose of sitagliptin was adjusted according to the animals' weights, which were measured at the surgery and at 7-day intervals thereafter.

At 8 and 3 days prior to euthanasia, the animals received two intraperitoneal injections of calcein (10 mg/mL) at a dose of 20 mg/kg to label bone tissue and assess dynamic parameters of bone remodeling via histomorphometry.

### Euthanasia

At the end of the 13-week intervention period the animals were then placed in a $CO_2$ chamber until they became unconscious.

### Bone Histomorphometry

In preparation for the histomorphometric analysis, the right tibia was removed from each animal by dissecting the right hind limb and immediately placing it in bottles containing

70% alcohol. These undecalcified bone samples were embedded in polymethylmethacrylate (Polysciences, Warrington, PA, USA), sliced longitudinally into 6-µm sections using a microtome (Leica RM2235, Leica Biosystems, Nußloch, Germany), and stained using toluidine blue (Merck KGaA, Darmstadt, Germany).

Bone histomorphometry was performed using a microscope (Nikon Labophot II, Tokyo, Japan) equipped with a high-resolution UV color video camera (Olympus DP71, Olympus America Inc., Center Valley, PA, USA) and analyzed with OsteoMeasure software (OsteoMetrics, Inc., Atlanta, GA, USA, version 3.3.0.2). All analyses were conducted by a single investigator.

The following histomorphometric parameters were analyzed:

- **Structural parameters:** bone volume per tissue volume (BV/TV; %), trabecular thickness (Tb.Th; µm), trabecular separation (Tb.Sp; µm), and trabecular number (Tb.N; n/mm).
- **Static bone formation parameters:** osteoid thickness (O.Th; µm), osteoid surface (OS/BS; %).
- **Static bone resorption parameter:** eroded surface (ES/BS;%).
- **Dynamic remodeling parameters:** mineral formation rate (MS/BS; %), mineral apposition rate (MAR; µm/d), bone formation rate per unit of bone surface (BFR/BS; $\mu m^3/\mu m^2/y$).

Histomorphometric parameters are reported following the nomenclature recommended by the ASBMR Histomorphometry Nomenclature Committee (20).

## DPP4 Gene Expression

Expression of the DPP4 gene was assessed in the right femur, which was collected aseptically using sterilized and cooled materials and maintained in liquid nitrogen in a -80 oC freezer. For gene expression analysis, the 2-ΔΔCT method was applied as described in the literature[21]. Oligonucleotides used in the reverse transcriptase quantitative polymerase chain reaction (RT-qPCR) technique are shown in Table 1.

Table 1 – Oligonucleotides used in the reverse transcriptase quantitative polymerase chain reaction (RT-qPCR) technique. The table lists forward and reverse primer sequences (5' to 3') for the DPP4 gene and the endogenous control gene GAPDH

| Gene | Forward 5'- 3' | Reverse 5'- 3' |
|---|---|---|
| DPP4 | TCCCAACTCCAGAGGACAAC | CAGGGCTTTGGAGATCTGAG |
| GAPDH* | GATGCTGGTGCTGAGTATGTCG | TGGTGCAGGATGCATTGCTGA |

*Endogenous control gene

### Statistical Analysis

Data were compiled and organized using Microsoft Excel (version 15.33, 2017). Descriptive statistics for quantitative variables were reported as means ± standard deviations. Inferential analyses were conducted using IBM SPSS Statistics (version 23; IBM Corp., Armonk, NY, USA) and Prism 8 (GraphPad Software, LLC, Boston, MA, USA).

To evaluate the effects of surgery and treatment on body weight, generalized linear models (GLMs) with repeated measures were used to assess within-group changes from baseline to euthanasia and between-group differences across the five groups (OVX, OVX-S, SHAM, SHAM-S, and control). For histomorphometric parameters, data met the assumption of independent observations. Homogeneity of variances was confirmed using Levene's test, and normality was assessed with the Shapiro-Wilk test. Although minor deviations from normality were observed for some variables, F-tests were considered robust under these conditions[22]. One-way analysis of covariance (ANCOVA) was used to compare histomorphometric outcomes across groups at the euthanasia time point, with body weight at euthanasia included as a covariate. Significant ANCOVA results were followed by Bonferroni post hoc tests for pairwise comparisons.

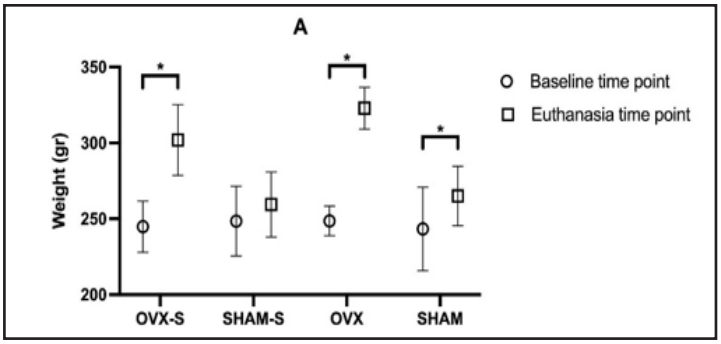
Expression of the *DPP4* gene was analyzed using the Kruskal-Wallis test in Prism 8 software. A significance level of $p < 0.05$ was applied for all statistical tests.

## RESULTS

### Changes in Weight from Baseline to the Euthanasia Time Point

Body weight increased significantly from baseline to euthanasia in the OVX ($p < 0.001$), OVX-S ($p < 0.001$), and SHAM ($p = 0.024$) groups, as determined by generalized linear models with repeated measures (Figure 1). No significant weight changes were observed in the SHAM-S or control groups. Between-group comparisons using Bonferroni post hoc tests revealed significant differences in weight gain, with the OVX group showing greater weight gain compared to the SHAM-S and control groups ($p < 0.05$).

Figure 1 – Bar graph illustrating changes in body weight from baseline to the euthanasia time point across all study groups




Open circles and squares denote mean values at each time point, with vertical lines representing standard deviations (error bars). Bars with asterisks linking groups signify statistically significant differences ($p < 0.05$) determined by the Bonferroni post hoc test. An asterisk (*) indicates a significant within-group change ($p < 0.05$) from baseline to the euthanasia time point. Abbreviations: CON, control group; OVX, ovariectomized group receiving saline solution; OVX-S, ovariectomized group receiving sitagliptin; SHAM, sham surgery group receiving saline solution; SHAM-S, sham surgery group receiving sitagliptin.

### Bone Histomorphometric Parameters at the Euthanasia Time Point

Body weight at euthanasia was included as a covariate in all histomorphometric analyses and did not significantly influence outcomes ($p > 0.05$ for all parameters).

### Structural Histomorphometric Parameters

Significant differences were observed among groups for bone volume per tissue volume (BV/TV), trabecular separation (Tb.Sp), and trabecular number (Tb.N) ($p < 0.001$ for each, one-way ANCOVA; Table 2). Post hoc analysis with Bonferroni correction showed:

**BV/TV**: The OVX and OVX-S groups exhibited significantly lower BV/TV compared to the SHAM, SHAM-S, and control groups ($p < 0.05$). No significant difference was found between OVX and OVX-S groups (Figure 2A).

**Tb.Sp**: The OVX group had significantly greater trabecular separation than the OVX-S, SHAM, SHAM-S, and control groups ($p < 0.05$). The OVX-S group also showed greater Tb.Sp than the SHAM, SHAM-S, and control groups, but less than the OVX group ($p = 0.012$; Figure 2D).

**Tb.N**: The OVX and OVX-S groups had significantly fewer trabeculae than the SHAM, SHAM-S, and control groups ($p < 0.05$).

**Tb.Th**: No significant differences in trabecular thickness were observed among groups ($p = 0.22$).

### Static Bone Formation Parameters

Significant differences were observed for osteoid surface per bone surface (OS/BS; $p = 0.03$, ANCOVA). Post hoc analysis showed that the OVX-S group had a higher OS/BS than the SHAM-S group ($p < 0.04$; Figure 2B). No significant differences were found for osteoid thickness (O.Th; $p = 0.17$; Table 2).

### Static Bone Resorption Parameters

No significant differences were observed for eroded surface per bone surface (ES/BS; $p = 0.11$) among groups (Figure 2C; Table 2).

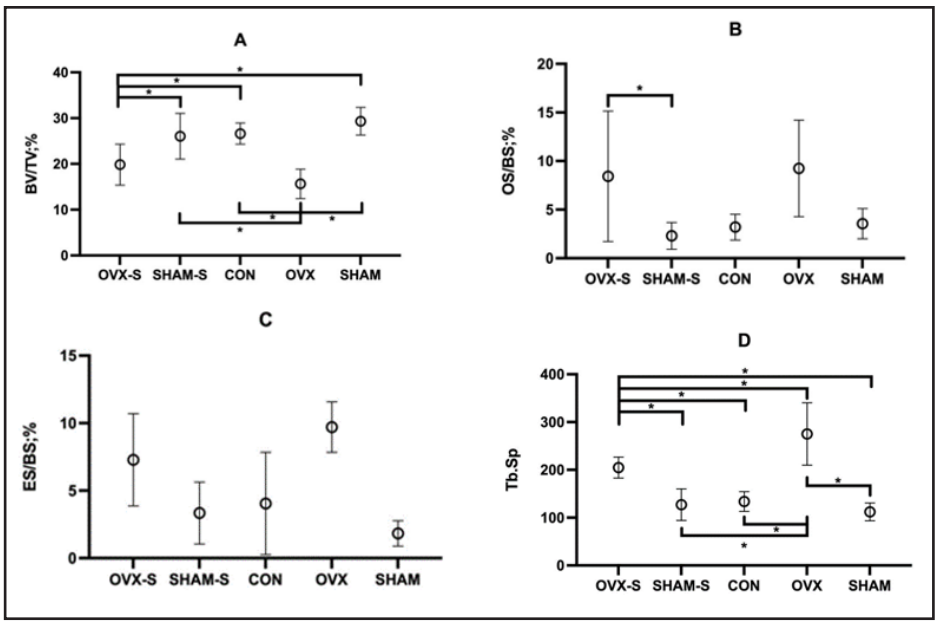
### Dynamic Bone Remodeling Parameters

Significant differences were observed for mineralized surface per bone surface (MS/BS) and bone formation rate per bone surface (BFR/BS) ($p < 0.001$ for both, ANCOVA). Post hoc analysis revealed that the OVX group had significantly higher MS/BS and BFR/BS compared to all other groups ($p < 0.05$; Figures 3A, 3B). The OVX group also exhibited more double-labeled and single-labeled surfaces than the OVX-S group, indicating higher bone remodeling activity (Figure 4). No significant differences were found for mineral apposition rate (MAR; $p = 0.06$; Figure 3C).

### DPP4 Gene Expression Analysis

Expression of the DPP4 gene was significantly lower in the sitagliptin-treated groups (OVX-S and SHAM-S) compared to the OVX group ($p < 0.01$, Kruskal-Wallis test). The OVX group exhibited the highest DPP4 expression levels (Figure 5).

Figure 2 – Bar graph depicting histomorphometric results for structural bone parameters in each study group




Open circles represent mean values, with vertical lines indicating standard deviations (error bars). Bars with asterisks connecting groups denote statistically significant differences ($p < 0.05$) based on the Bonferroni post hoc test. Panels: (A) BV/TV (bone volume per tissue volume); (B) OS/BS (osteoid surface per bone surface); (C) ES/BS (eroded surface per bone surface); (D) Tb.Sp (trabecular separation). Abbreviations: BV/TV, bone volume per tissue volume; CON, control group; ES/BS, eroded surface per bone surface; OS/BS, osteoid surface per bone surface; OVX, ovariectomized group receiving saline solution; OVX-S, ovariectomized group receiving sitagliptin; SHAM, sham surgery group receiving saline solution; SHAM-S, sham surgery group receiving sitagliptin; Tb.Sp, trabecular separation

### Static Bone Formation Parameters

In the ANCOVA model, only one bone formation measurement, OS/BS, was significantly different among the groups. This difference was because the OVX-S group had a higher OS/BS value than the SHAM-S group. ($p < 0.04$) (Figure 2B; Table 2).

### Static Bone Resorption Parameter

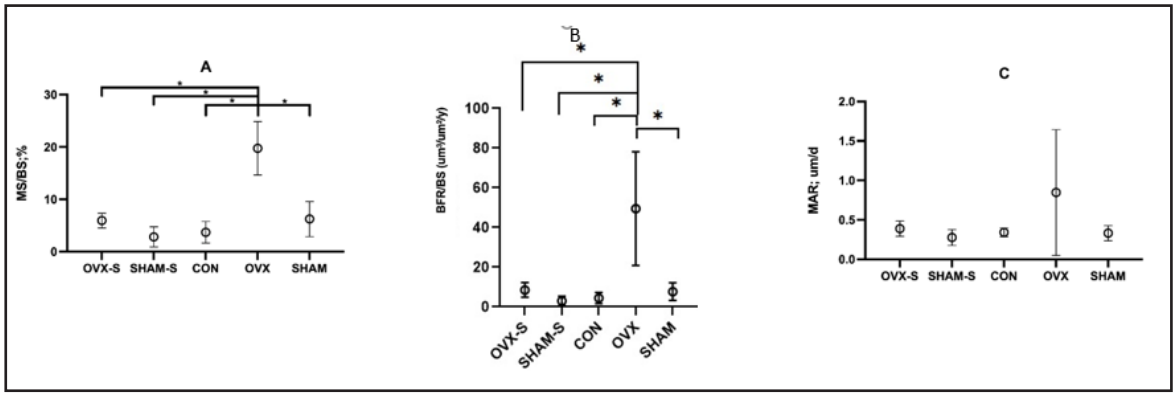
No significant difference was found among the groups regarding ES/BS ($p = 0.087$) (Figure 2C).

### Dynamic Remodeling Parameters

The OVX group had significantly more double-labeled and single-labeled surfaces than the OVX-S group, indicating a higher bone remodeling throughout formation rate values (Figures 3 and 4).

The analysis showed significant differences in dynamic bone parameters among the groups for MS/BS and BFR/BS values. *Post hoc* analysis revealed the OVX group had significant higher MS/BS and BFR/BS compared to all other groups (Figure 3A and 3B). Although the OVX group had the highest MAR values, these differences weren't significant. (Figure 3C).

Figure 3 – Bar graph illustrating histomorphometric results for dynamic bone remodeling parameters in each study group




Open circles represent mean values, with vertical lines indicating standard deviations (error bars). Bars with asterisks connecting groups denote statistically significant differences ($p < 0.05$) based on the Bonferroni post hoc test. Panels: (A) MS/BS (mineralized surface per bone surface); (B) BFR/BS (bone formation rate per bone surface); (C) MAR (mineral apposition rate). Abbreviations: BFR/BS, bone formation rate per unit of bone surface; CON, control group; MAR, mineral apposition rate; MS/BS, mineralized surface per bone surface; OVX, ovariectomized group receiving saline solution; OVX-S, ovariectomized group receiving sitagliptin; SHAM, sham surgery group receiving saline solution; SHAM-S, sham surgery group receiving sitagliptin.

Table 2 – Results of bone histomorphometric parameters across study groups

| | OVX-S (n = 9) | OVX (n = 7) | SHAM-S (n = 10) | SHAM (n = 7) | Control (n = 7) | P value |
|---|---|---|---|---|---|---|
| BV/TV* | 19.85 ± 4.49 | 15.64 ± 3.21 | 26.05 ± 5.01 | 29.33 ± 3.00 | 26.64 ± 2.32 | <0.001 |
| OS/BS* | 8.43 ± 6.71 | 9.24 ± 4.97 | 2.30 ± 1.37 | 3.56 ± 1.55 | 3.20 ± 1.33 | 0.03 |
| ES/BS | 7.28 ± 3.42 | 9.72 ± 1.87 | 3.35 ± 2.29 | 1.83 ± 0.94 | 4.05 ± 3.98 | 0.11 |
| Tb.Th | 50.21 ± 10.06 | 49.05 ± 3.51 | 43.17 ± 5.34 | 46.03 ± 5.14 | 48.22 ± 5.94 | 0.22 |
| O.Th | 2.07 ± 0.64 | 2.52 ± 0.48 | 1.74 ± 0.65 | 1.77 ± 0.51 | 1.89 ± 0.60 | 0.17 |
| Tb.Sp* | 204.84 ± 31.87 | 275.20 ± 65.37 | 127.14 ± 33.02 | 112.16 ± 18.81 | 133.76 ± 20.63 | <0.001 |
| Tb.N* | 3.98 ± 0.50 | 3.19 ± 0.65 | 6.06 ± 1.07 | 6.43 ± 0.94 | 5.56 ± 0.86 | <0.001 |
| MS/BS* | 5.95 ± 1.42 | 19.76 ± 5.10 | 2.83 ± 1.92 | 6.25 ± 3.55 | 3.72 ± 2.06 | <0.001 |
| MAR | 0.39 ± 0.10 | 0.85 ± 0.80 | 0.28 ± 0.10 | 0.33 ± 0.10 | 0.34 ± 0.03 | 0.06 |
| BFR/BS* | 8.58 ± 3.71 | 49.55±28.6 | 3.06 ± 2.41 | 7.75 ± 4.49 | 4.45 ± 2.82 | <0.001 |

Note: Data are presented as mean ± standard deviation. Asterisks (*) indicate statistically significant differences ($p < 0.05$) based on analysis of covariance (ANCOVA), with body weight at euthanasia as a covariate. Abbreviations: BFR/BS, bone formation rate per unit of bone surface; BV/TV, bone volume per tissue volume; CON, control group; ES/BS, eroded surface per bone surface; MAR, mineral apposition rate; MS/BS, mineralized surface per bone surface; N.Oc/B.Pm, number of

osteoclasts per bone perimeter; OS/BS, osteoid surface per bone surface; O.Th, osteoid thickness; OVX, ovariectomized group receiving saline solution; OVX-S, ovariectomized group receiving sitagliptin; SHAM, sham surgery group receiving saline solution; SHAM-S, sham surgery group receiving sitagliptin; Tb.N, trabecular number; Tb.Th, trabecular thickness; Tb.Sp, trabecular separation

Figure 4 – Representative images from dynamic histomorphometry demonstrating calcein labeling in trabecular bone

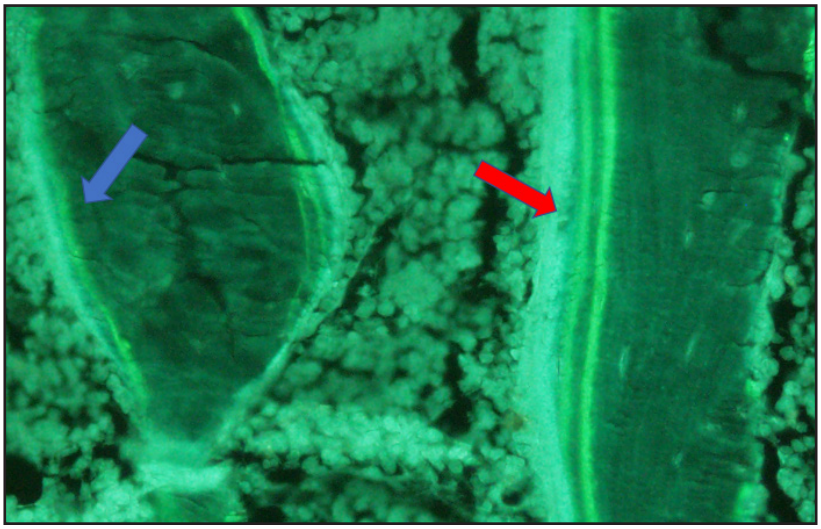
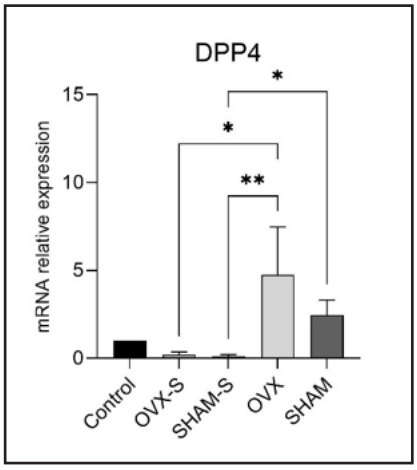


Image shows double labeling (red arrow). and single labeling (blue arrow) indicative of active bone formation in the in the OVX group Abbreviations: OVX, ovariectomized, ovariectomized group

## DPP4 Gene Expression Analysis

The expression of the *DPP4* gene was significantly lower in the groups treated with sitagliptin (OVX-S and SHAM-S). The highest *DPP4* expression level was observed in the OVX group (Figure 5).

Figure 5 – Bar graph comparing DPP4 gene expression levels across study groups




Symbols denote statistical significance: *p < 0.05; **p < 0.01 (based on Kruskal-Wallis test). Abbreviations: CON, control group; OVX, ovariectomized group receiving saline solution; OVX-S, ovariectomized group receiving sitagliptin; SHAM, sham surgery group receiving saline solution; SHAM-S, sham surgery group receiving sitagliptin.

## FINAL CONSIDERATIONS

This study presents novel and impactful findings demonstrating that sitagliptin significantly mitigates bone remodeling in ovariectomized rats, primarily by reducing bone resorption associated with hypoestrogenism. The histomorphometric analysis, considered the gold standard for assessing bone remodeling, revealed that sitagliptin treatment had a positive impact on bone tissue in both structural and remodeling parameters indicating a suppression of the heightened bone remodeling typical of estrogen deficiency. Additionally, a significant decreasing in DPP4 gene expression in bone tissue observed in the sitagliptin-treated ovariectomized animals, suggesting a direct mechanistic link between DPP-4 inhibition and reduced osteoclastic activity[9,11]. These findings are particularly noteworthy as they provide evidence of relationship between glucose and bone metabolism.

Estrogen deficiency may also influence osteoclastogenesis by increasing the production of reactive oxygen species (ROS), which is one of the main pathophysiological factors in postmenopausal osteoporosis[5]. In cases of estrogen deficiency, there is heightened ROS production by osteoclasts and pre-osteoclasts. Additionally, ROS play a critical role in RANKL-induced osteoclastogenesis, functioning as intracellular signaling mediators that activate mitogen-activated protein kinases (MAPKs), which are essential for the differentiation, activation, and survival of osteoclasts[9]. The reduction in bone remodeling seen in our study aligns with prior studies that showed an anti-osteoclastic effect of sitagliptin as demonstrated *in vitro* by Wang et al., who reported that this medication inhibits the intracellular signaling

cascade of protein kinase B (AKT), extracellular signal-regulated kinase (ERK), and c-Fos induced by RANKL, resulting in the suppression of osteoclast-specific transcription factors such as nuclear factor of activated T-cells, cytoplasmic 1 (NFATc1) and the formation of F-actin ring, both of which are essential for bone resorption by mature osteoclasts[9,10].

Another study, with a methodology very similar to ours, revealed that sitagliptin treatment significantly reduced malondialdehyde and increased catalase levels, indicating reduced oxidative stress. It also adjusted runt-related transcription factor 2 (RUNX2) and AKT expression and downregulated RANKL/osteoprotegerin(OPG) pathways. These molecular changes correlated with improved bone density and lessened histopathological alterations associated with osteoporosis. These findings collectively suggest that sitagliptin promotes osteoblast function and reduces osteoclast activity[23].

The significant decrease in *DPP4* expression supports a skeletal-pancreatic axis, where DPP-4 inhibition may enhance incretin levels (e.g., GLP-1, GLP-2), promoting bone formation and reducing resorption[7]. The lack of significant changes in static resorption parameters (e.g., ES/BS) suggests that sitagliptin's effects are more pronounced on dynamic remodeling processes, warranting further exploration.

Discrepancies with studies like Cusick et al.[24] which reported no histomorphometric changes with sitagliptin, may stem from methodological differences. The current study's use of dynamic histomorphometry with calcein labeling enabled precise assessment of bone formation rates, unlike static methods used previously. The lower sitagliptin dose (25 mg/kg vs. 500 mg/kg) in Cusick et al, suggests efficacy at clinically relevant doses, minimizing off-target effects. Weight gain in OVX and OVX-S groups was controlled as a covariate, confirming that skeletal effects were independent of body weight, consistent with Jin et al (25).

The study's strengths include the use of dynamic histomorphometry for precise remodeling assessment and *DPP4* gene expression analysis for mechanistic insights. The statistical rigor, including ANCOVA and Bonferroni post hoc tests, enhances reliability.

In conclusion, this study demonstrated that sitagliptin treatment significantly reduces bone remodeling in ovariectomized rats, as evidenced by histomorphometric analysis, and decreases *DPP4* gene expression in bone tissue. These findings suggest the inhibition of DPP-4 could be an adjuvant therapy to mitigate bone loss in hypoestrogenic conditions, such as menopause, by attenuating excessive bone resorption frequently seen in this period of life. The reduction in *DPP4* expression provides a mechanistic basis for these effects, reinforcing the interplay between bone and glucose metabolism.

### Limitations of the study

Limitations include the lack of additional molecular analyses (e.g., Western blotting) and tartrate-resistant acid phosphatase (TRAP) staining to directly assess osteoclast activity. Also, a wider range of sitagliptin doses to determine the optimal anti-osteoporotic dose could provide more detailed results.

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## DECLARATIONS

## Authorship Contributions

**Luciana Muniz Pechmann**

Endocrinologist, PhD student in Internal Medicine at Universidade Federal do Paraná

https://orcid.org/0000-0002-2965-5268 • lucianapechmann@gmail.com

Contribution: Conceptualization, Data Curation, Formal Analysis, Investigation, Methodology, Project Administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing

**Vicente Florentino Castaldo Andrade**

Endocrinologist, PhD in Health Sciences and Internal Medicine from Universidade Federal do Paraná

https://orcid.org/0000-0001-6101-4359 • vicentefcandrade@gmail.com

Contribution: Supervision, Validation, Visualization, Writing – review & editing

**Thais Andrade Costa Casagrande**

PhD in Veterinary Surcial Clinical from Universidade de São Paulo

https://orcid.org/0000-0001-9437-4043 • thais.costa@up.edu.br

Contribution: Methodology

**Rafaela Ceron**

Master's student in Internal Medicine at Universidade Federal do Paraná

https://orcid.org/0009-0001-9015-0736 • rafaelaceron@gmail.com

Contribution: Methodology

**Leticia Capote dos Santos**

Bachelor's degree in Dentistry from Universidade Tuiuti do Paraná

https://orcid.org/0000-0002-6655-639X • dra.leticiacapotedossantos@gmail.com

Contribution: Methodology

**Edneia Amancio de Souza Ramos Cavalieiri**

PhD in Microbiology, Parasitology and Pathology from Universidade Federal do Paraná

https://orcid.org/0000-0002-8855-5988 • edneiaama@ufpr.br

Contribution: Methodology

**Gabriela Casani Cardoso**
PhD in Microbiology, Parasitology and Pathology from Universidade Federal do Paraná
https://orcid.org/0000-0002-5658-7029 • gabrielacasani@gmail.com
Contribution: Methodology

**Regiane Stafim da Cunha**
PhD in Microbiology, Parasitology and Pathology from Universidade Federal do Paraná
https://orcid.org/0000-0002-7925-7698 • regidacunha@gmail.com
Contribution: Methodology

**Carolina Aguiar Moreira**
PhD in Internal Medicine from Universidade Federal do Paraná
https://orcid.org/0000-0002-9908-4907 • carolina.aguiar.moreira@gmail.com
Contribution: Conceptualization, Data Curation, Formal Analysis , Investigation, Methodology, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing

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