



Effect of strength training versus raloxifene on bone weight, blood glucose, lipid and antioxidant profile in ovariectomized rats

Marcos Antônio Pereira dos Santos¹, Maria do Carmo de Carvalho e Martins¹, Raquel Suelen Brito da Silva², Carolina Farias de Arruda Lopes² and Alexandre Sérgio Silva^{2*} 

¹Departamento de Biofísica e Fisiologia, Centro de Ciências da Saúde, Universidade Federal do Piauí, Teresina, Piauí, Brazil. ²Departamento de Educação Física, Centro de Ciências da Saúde, Universidade Federal da Paraíba, Cidade Universitária, S/N, 58059-900, João Pessoa, Paraíba, Brazil. *Author for correspondence. E-mail: alexandresegiorsilva@yahoo.com.br

ABSTRACT. We compared the effect of the treatment with strength training (ST) and raloxifene (RALOX) on bone weight, blood glucose, lipid, and antioxidant profile in ovariectomized rats. Twenty-four Wistar rats were distributed into four groups: ovariectomy + VEHICLE (control); ovariectomy + RALOX; ovariectomy + ST; ovariectomy + RALOX + ST. Thirty days after ovariectomy, the animals underwent the treatment with RALOX (750 µg day⁻¹) and/or ST (three sessions week⁻¹). Thirty days after, all groups were sacrificed, tibia and femur were weighed, and the blood was collected for analysis of the lipid profile, glucose, and antioxidants catalase (CAT) and glutathione (GSH). The ST group showed greater femur weight (0.82 ± 0.18 g) and RALOX + ST had greater tibia weight (0.61 ± 0.17 g) than CONTROL with femur weight of 0.65 ± 0.08 g and tibia of 0.49 ± 0.08 g with no differences between treatments ($p > 0.05$). ST group showed significantly higher catalase (181.7 ± 15.4 µM g⁻¹) compared to the other groups. In contrast, the GSH value was lower in ST group (89.2 ± 8.1 µM g⁻¹) compared to RALOX (175.9 ± 17.1 µM g⁻¹) and RALOX + ST (162.8 ± 12.1 µM g⁻¹), but the values of these two groups did not differ from CONTROL (115.3 ± 21.1 µM g⁻¹). Total cholesterol did not differ between groups ($p > 0.05$), but exercise alone (54.3 ± 2.5 mg dL⁻¹) or with RALOX (53.0 ± 1.5 mg dL⁻¹) resulted in higher HDL cholesterol than CONTROL (45.5 ± 2.5 mg dL⁻¹). Only RALOX+ST presented lower glucose (140.3 ± 9.7 mg dL⁻¹) values than CONTROL (201.7 ± 30.6 mg dL⁻¹). In conclusion, ST promotes similar benefits on bone and metabolic parameters compared to pharmacological treatment in ovariectomized rats.

Keywords: hormonal reposition; ovariectomy; strength training.

Received on June 26, 2021.
Accepted on January 16, 2023.

Introduction

Menopause is characterized as a progressive systemic change, of which stands out weight gain, increase in abdominal fat, dyslipidemia (Cardoso et al., 2018), and loss of bone microarchitecture associated to potential risk to fractures (Lupsa & Insogna, 2015). Genetics, hormonal status, eating habits, and physical activity level influence the rate of progression of this disorder (Marques & Liberali, 2012). There is already well-established positive association between these factors and chronic diseases, including hypertension and diabetes (Kabodi, Ajami, Zakiei, Zangeneh, & Saeidi, 2019).

Pharmacological hormone replacement therapies prevent further bone loss, reduce likelihood of future fractures, prevent the onset of cardiovascular disorders, and psychic disorders, resulting from hormone deficiency in postmenopausal women (Sousa, Alves, Rocha, Moraes, & Carvalho, 2007). Besides hormonal treatment, there has been several attempts to increase the effectiveness of estrogens through the selective estrogen receptor modulators. These second-generation drugs induced bone mineral gain from 2 to 4% at the lumbar spine, proximal femur, and total body in 602 newly menopause women, according to a European multicenter study (Delmas et al., 1997) and induces osteoblast activity with decreased osteoclast activity (Yogui et al., 2018). In particular the raloxifene (RALOX), a second-generation selective estrogen receptor modulator (SERM), is an example of a class of pharmacological compounds with agonist action on bone.

Concomitantly, studies claim that physical exercises aiming hypertrophy and muscle strength gain have positive correlation with bone mineral density (Moreira et al., 2014; Watson et al., 2018). Moreover, it is well

established that dynamic aerobic exercise training improves lipid and glucose profile, and further improves the antioxidant activity, thus preventing effects associated with aging and oxidative stress that are increased after menopause (Marques et al., 2011; Tobias et al., 2014). However, the effect of strength training (ST) on metabolic profile is still little studied.

This scenario allows us to assume that strength training can help improve postmenopausal bone health and promote other metabolic benefits not granted by estrogen, contributing to the overall health of menopausal females. However, in the absence of previous research evaluating the influence of RALOX and ST on metabolic profile, the present study was initially carried out with an animal model in order to subsidize future interventions in humans.

Therefore, the aim of this study was to verify the effect of treatments with RALOX and ST alone or in association on bone weight, antioxidant activity, lipid profile, and blood glucose in ovariectomized rats.

Material and methods

Animals

This is an experimental study with animal model. We used 24 virgin female Wistar rats (200-300 g), 90 days old, from the vivarium of the Center for Agricultural Sciences-FUPI, fed rodent chow/water ad libitum and maintained in standard conditions at room temperature $26 \pm 2^{\circ}\text{C}$, relative humidity: 44-56%; cycle 12h light: 12 hours dark). The animals were distributed into 4 groups ($n = 6/\text{group}$): 1- ovariectomy + VEHICLE (control); 2- ovariectomy + RALOX; 3- ovariectomy + ST; 4- ovariectomy + RALOX + ST. All procedures were performed according to ethical principles in animal experimentation by the National Council for the Control of Animal Experimentation (CONCEA n. 097/12) and national legislation for pet section in vigor (Federal Law 11794 of 08 October 2008).

Procedures

The experimental model of ovariectomy was based in previous studies (Gomes et al., 2018; Kawakami et al., 2018). The ovariectomy was performed at age of 50 days, following anesthesia with intraperitoneal ketamine at a dose of 40 mg kg^{-1} and xylazine at a dose of 5 mg kg^{-1} bodyweight solution. The ovaries were exposed and was then held ovarian pedicle ligation using 5-0 catgut chrome wire needle (ETHICON - chrome catgut 5-0) for excision of ovarian after proper hemostasis. This was followed by the intramuscular administration of antimicrobial $0.1 \text{ ml } 100 \text{ g}^{-1}$ and anti-inflammatory compound (benzylpenicillin G benzathine and procaine, dihydrostreptomycin and piroxicam), as well as appropriate packaging for post anesthesia care of the animal.

Thirty days after the ovariectomy, the animals of the drug-treated groups started to receive selective estrogen receptor modulators (RALOX, $750 \mu\text{cg}$) diluted in 0.5 ml propylene glycol daily for 30 consecutive days accordingly. This dosage, as well as the period of 30 days enough to start the osteopenic phenomenon, was based on a previous study by Carvalho and Cliquet Jr. (2004). The animals of the exercise group started a strength training following a protocol previously adopted by Brito et al. (2018). Strength training consisted of jumping in a PVC cylinder, 30 cm in diameter and 70 cm high, containing water heated at about 32°C . After a two-day period of adjustment in the liquid environment, one hour a day, the animals went through a protocol of three sessions (alternated days) per week of strength training for four-week. Each session consisted of four sets of ten jumps with one-minute interval between sets and with overhead of 50% of body weight. The load was placed on the animals' chests through a special vest that involved the neck and back, but kept their feet free to perform the movements. The body weight of the animals was determined at baseline and during the training protocol for adjusting the training load.

Twenty-four hours after training and drug protocols and after 12h fast, the animals were anesthetized and euthanized (Rocha & Massone, 2006). The right tibia and right femur were removed by knee and tibio-tarsal disarticulation and the joint in the anteroposterior direction using an analog caliper for clinical assessment and measurement of bone weight. These bones were chosen because the femoral head and hip fractures are the most prevalent nonvertebral fractures (Söderqvist et al., 2009) and are associated with a higher risk of mortality after fractures caused by osteoporosis (Johnell et al., 2004), in addition to the fact that the animals underwent strength exercises for lower limbs.

It was collected 3 mL of blood sample from each animal through puncturing of the caudal vena cava in order to dose the serum levels of total cholesterol, HDL cholesterol triglycerides (TG), and glucose by using the automatic immunochemical analyzer Labmax 240 premium using kits of the Labtest (Minas Gerais,

Brazil). Reduced glutathione (GSH) was determined by 1 ml of liver tissue supernatant (0.5 ml homogenate precipitated by 2 ml) of 5% trichloroacetic acid (TCA) and 0.5 ml of Ellman's reagent (0.0198%) DTNB in 1% sodium citrate and 3 ml of phosphate buffer (pH 8.0). The color developed was read at 412 nm. Catalase were quantified through the enzyme decomposition of hydrogen peroxide by the decrease in optical density at 230 nm, 37°C. For this, liver tissue homogenate was prepared; 100 mg ml⁻¹ of tissue in 0.1 M phosphate buffer (pH 7.0) and then centrifuged at 5800 rpm for 10 min under cooling at 4°C. The supernatant was diluted 50-fold with 1.0 M Tris-HCl buffer and 5 mM EDTA (pH 8.0). Then, an aliquot of 10 L was subjected to a reaction medium containing H₂O₂ in 10 mM Tris HCl 1.0 M and 5 mM EDTA (pH 8.0). The reduction of H₂O₂ was monitored spectrophotometrically for 6 minutes (Beers Jr. & Sizer, 1952).

Statistical analyses

Data is presented as mean and standard error of the mean (SEM). After confirming data normality and homogeneity through Shapiro Wilk and Levene tests respectively, One-way ANOVA was performed followed by Tukey post-test for comparison between groups. The level of significance was set at $p < 0.05$. The statistical procedures were performed in GraphPad InStat version 3.03 software (GraphPad, San Diego, CA, USA).

Results

The animals in the four groups maintained their body weight without statistically significant changes, although descriptive reduction was noted in the trained (5.3%), trained + raloxifene (3.7%) and in the group without any treatment (4.5%). Moreover, the group treated only with raloxifene showed an increase of 1.3% in body weight.

ST group was the only which the femur weight was significantly higher than control. This was noted both in the analysis of absolute bone weight (Figure 1) and in the analysis of bone weight normalized by body weight (0.34 ± 0.07 g 100 g⁻¹ of body weight for the ST group; 0.26 ± 0.06 g 100 g⁻¹ of body weight for the CONTROL; 0.30 ± 0.05 g 100 g⁻¹ of body weight for RALOX; 0.27 ± 0.03 g 100 g⁻¹ of body weight for the RALOX + ST group). No difference was observed among treated groups.

Regarding tibia weight, only RALOX + ST was statistically higher than control for absolute weight (Figure 1). However, this difference was not observed for bone weight normalized by body weight (0.26 ± 0.04 g 100 g⁻¹ body weight for the RALOX + ST group; 0.20 ± 0.03 g 100 g⁻¹ body weight for CONTROL; 0.24 ± 0.03 g 100 g⁻¹ body weight to ST; 0.21 ± 0.04 g 100 g⁻¹ body weight for RALOX).

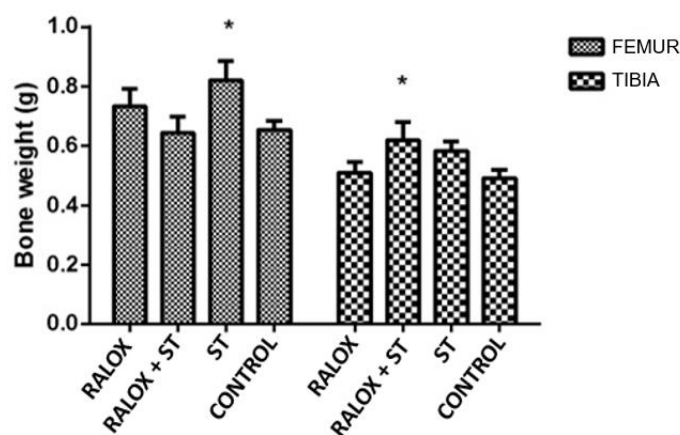


Figure 1. Effect of strength exercise isolated and associated to hormone therapy (RALOX) on femur and tibia weight. $n = 6$ for all groups. * = $p < 0.05$ to control group (ANOVA one way).

Regarding serum catalase, it can be observed in Figure 2 that animals of the ST group (181.7 ± 15.4 Mm g⁻¹) had significantly higher values than those of RALOX (108.9 ± 9.4 μ M g⁻¹), RALOX + ST (94.6 ± 11.7 μ M g⁻¹) and CONTROL (128.8 ± 11.2 μ M g⁻¹). In contrast, the serum activity of GSH was significantly lower in ST group (89.2 ± 8.1 μ M g⁻¹) compared to RALOX + ST (162.8 ± 12.1 μ M g⁻¹) and RALOX (175.9 ± 17.1 μ M g⁻¹) groups (Figure 2). Despite showing higher GSH levels in relation to ST group, the values of groups RALOX + ST and RALOX did not differ from CONTROL group (115.3 ± 21.1 μ M g⁻¹).

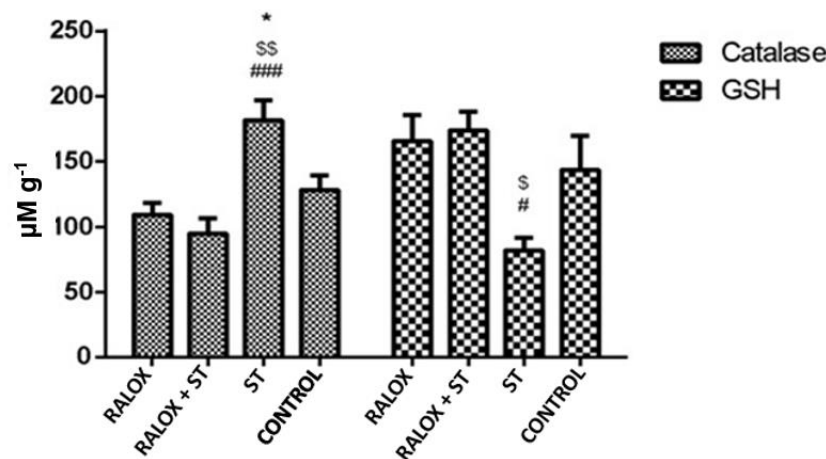


Figure 2. Effect of strength exercise isolated and associated to hormone therapy (RALOX) on the antioxidant enzymes catalase and glutathione. $n = 6$ for all groups. * = $p < 0.05$ to CONTROL group, ** = $p < 0.001$ to RALOX, *** = $p < 0.001$ to RALOX + ST (ANOVA one way).

Animals treated with hormone therapy or exercise showed total cholesterol levels similar to those of the control group (Table 1). Strength training alone or associated with estrogen resulted in higher HDL cholesterol compared to control or estrogen treated animals. For glucose, only animals of the control group showed values higher than 200 mg dL^{-1} . Treated animals presented values around 25% smaller, but only RALOX + ST group presented statistically lower glycemia levels in relation to the control group.

Table 1. Biochemical characteristics of the groups at the post-intervention.

Variables	RALOX	RALOX + ST	ST	Control
Total cholesterol (mg dL^{-1})	101.2 ± 7.3	93.9 ± 10.5	102.2 ± 8.9	98.7 ± 6.2
HDL cholesterol (mg dL^{-1})	43.4 ± 1.9	$53.0 \pm 1.5^{*\$}$	$54.3 \pm 2.5^{*\$}$	45.5 ± 2.5
Triglycerides (mg dL^{-1})	51.0 ± 8.9	42.3 ± 7.3	45.5 ± 7.8	53.7 ± 7.6
Glucose (mg dL^{-1})	147.2 ± 7.2	$140.3 \pm 9.7^*$	156.8 ± 7.4	201.7 ± 30.6

Values are means \pm SEM. * difference from control group; \$ = difference from RALOX group; $p < 0.05$.

Discussion

Data from this study showed that strength exercise results in higher bone weight and increased antioxidant activity of the catalase. Additionally, strength training promotes increase in HDL cholesterol, and associated with hormone therapy, promotes decrease in blood glucose.

Unlike our study, which used the ovariectomy model, Stringhetta-Garcia et al. (2016) conducted a strength training protocol in aged Wistar rats (physiologically menopausal) and found an increase in osteoblast cell activity and a decreased cell osteoclastic activity, resulting in increased bone strength and micro architecture increment in the neck of the femur. Souza, Rosa, Ricardo, Moraes and Rocha (2005) verified that estrogen repairs tibia of rats submitted to oophorectomy, with statistically significant increase in bone mineral density of 21.8% in twenty-eight days at dose of $3 \text{ mg kg}^{-1} \text{ day}^{-1}$. Thus, our study corroborates to this previous data. It can be noted that in either model (aged or ovariectomized rats), both raloxifene and strength training treatment are effective according to data from our study (for physical training) and previous literature (for raloxifene).

Results of the clinical trials are more discrete. Reginster et al. (2004) showed a decrease in biochemical markers of bone turnover in women with osteoporosis after the use of estrogen. However, estrogen improved bone mineral density of the lumbar spine, femur and spine only by 2-4% (Sousa et al., 2007; Marques & Liberali, 2012). McClung (2005) found increased mineral density by 1.6% in both lumbar spine and total hip, as well as an increase of 1.2% in the femur neck after two years of treatment with raloxifene at a clinical dose of 60 mg per day. The data from our study indicate that the physical exercise associated with selective estrogen receptor modulators is more promising, at least in animal model, since strength training was the only treatment that resulted in greater weight femur, and selective estrogen receptor modulators was only able to result in higher weight of the tibia when associated with physical training.

In addition to demonstrating benefits of the association of strength exercise and RALOX on tibia weight, the other finding of this study was that strength training has the additional benefit of improving

aspects of the metabolic profile involved in menopause (antioxidant activity, lipidic profile and glycaemia). The beneficial influence on these metabolic profile indicators is relevant because the lack of estrogen production in the menopause is accompanied by disorders of the lipid and glycemic profile, in addition to systemic inflammation and oxidative stress (Wang et al., 2018). On the other hand, several studies support the benefits of physical activity on lipid metabolism and oxidative stress, thus preventing cardiovascular disease (Mann, Beedie, & Jimenez, 2014; Poblete-Aro et al., 2018; Abdelbasset, Tantawy, Kamel, Alqahtani, & Soliman, 2019).

This study provides a further indication that strength training combined with therapy of selective estrogen receptor modulators is better than just one or the other isolated intervention.

Although estrogen receptors are present in several other tissues besides bone tissue (Stringhetta-Garcia et al., 2016) and are reported to improve the lipidic profile (Reginster et al., 2004), our study corroborates the findings from Reginster et al. (2004), Martyn-St James e Carroll (2008) and Takada, Iba, Yoshizaki and Yamashita (2012), which failed to verify beneficial effect of RALOX on HDL cholesterol. In our study, only strength training was able to increase HDL, alone or associated to raloxifene. The training effect in increasing HDL lipoproteins is something well established. In their studies, Karolkiewicz et al. (2009), Costa et al. (2018), and Nassef et al. (2019) indicated an effectivity of the aerobic training to increase HDL, but the training load should be relatively high and precise, so that it could result in energy expenditure of 1500 kcal week⁻¹. On the other hand, data with strength exercise are scarce, particularly in menopausal populations, which evidences that our finding is relatively unknown.

While Motahari-Tabari, Shirvani, Shirzad-E-Ahoodasthy, Yousefi-Abdolmaleki and Teimourzadeh (2014), Glouzon, Barsalani, Lagacé and Dionne (2015), and Mendoza et al. (2016) reported that aerobic training improved insulin sensitivity, data regarding strength training are scarcer, although Panveloski-Costa et al. (2011) have already reported that strength training reduces blood glucose and inflammatory markers in obese animals, but not under conditions of induced menopause. These findings suggest two important practical implications. The first is that increased blood glucose and diabetes are associated with menopause and exogenous estrogen and progestogens (Stuenkel, 2017), which is in accordance with the higher glycemic values of rats in the control group of our study. The second is that the data from this study reinforce the possibility that the combination of RALOX and strength training is effective in preventing post-menopausal blood sugar increase.

The main limitation of this study was that the effect of treatments on bone health was evaluated only by weight bone. Bone mineral density, as well as its architecture and biochemical markers of osteoblastic / osteoclast activities deserve to be evaluated in future studies, considering the paucity of these data in training programs with strength exercise. The main highlight of this study was the finding of improvement of important metabolic aspects resulting from strength training alone or associated with RALOX.

Taking into consideration the potentials and limitations of the present study, the data collaborate to strengthen a body of evidence indicating potential benefits of physical training in the prevention and treatment of osteopenia determined menopause. While Müller, Keiler, Kräker, Zierau and Bernhardt (2018) found an association between the motivation of rats to exercise with estrogen levels, Gomes et al. (2018) demonstrated that strength training reversed the increase in glycemia, insulin and prevented sarcopenia in ovariectomized rats. To end, Stunes et al. (2020), validated that strength training protocol alone or combined with metformin improved the trabecular microarchitecture of ovariectomized rats. Although aerobic training is the most studied modality, our data and these other studies are forming an amount of evidence for benefits promoted by strength training under experimental menopause.

Conclusion

In summary, this study shows that the combination of RALOX + ST ensures greater benefit in the bone weight and glycaemia of ovariectomized rats. Moreover, exercise promotes an additional improvement of the lipid profile and increase of the antioxidant activity. These results seem potentially relevant by the fact that the deterioration of the lipid profile, blood glucose and reduced antioxidant capacity are the effects of menopause occurring before the symptoms of osteopenia, so that combining these two forms of treatment is potentially more robust for health promotion after menopause.

References

- Abdelbasset, W. K., Tantawy, A. S., Kamel, D. M., Alqahtani, B. A., & Soliman, G. S. (2019). A randomized controlled trial on the effectiveness of 8-week high-intensity interval exercise on intrahepatic triglycerides, visceral lipids, and health-related quality of life in diabetic obese patients with nonalcoholic fatty liver disease. *Medicine*, 98(12), e14918. DOI: <https://doi.org/10.1097/MD.00000000000014918>
- Beers Jr., R. F., & Sizer, I. W. (1952). A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. *Journal of Biological Chemistry*, 195(1), 133-140. DOI: [https://doi.org/10.1016/S0021-9258\(19\)50881-X](https://doi.org/10.1016/S0021-9258(19)50881-X)
- Brito, A. F., Silva, A. S., Souza, A. A., Ferreira, P. B., Souza, I. L. L., Araújo, L. C. C., ... Silva, B. A. (2018). Aortic response to strength training and spirulina platensis dependent on nitric oxide and antioxidants. *Frontiers in Physiology*, 9, 1522. DOI: <https://doi.org/10.3389/fphys.2018.01522>
- Cardoso, G. A., Silva, R. S. B., Sena, K. F., Ferreira, A. P. U., Moura, S. K. M. S. F., & Silva, A. S. (2018). Influence of menopause on body fat induced by aerobic training. *Motriz*, 24(1), e1018147. DOI: <https://doi.org/10.1590/S1980-6574201800010012>
- Carvalho, D. C. L., & Cliquet Jr., A. (2004). The action of Low-intensity pulsed ultrasound in bones of osteopenic rats. *Artificial Organs*, 28(1), 114-118. DOI: <https://doi.org/10.1111/j.1525-1594.2004.07091.x>
- Costa, R. R., Pilla, C., Buttelli, A. C. K., Barreto, M. F., Vieiro, P. A., Alberton, C. L., ... Kruel, L. F. M. (2018). Water based aerobic training successfully improves lipid profile of dyslipidemic women: a randomized controlled trial. *Research Quarterly for Exercise and Sport*, 89(2), 173-182. DOI: <https://doi.org/10.1080/02701367.2018.1441485>
- Delmas, P. D., Bjarnason, N. H., Mitlak, B. H., Ravoux, A., Shah, A. S., Huster, W. J., ... Christiansen, C. (1997). Effects of raloxifene on bone mineral density, serum cholesterol concentrations, and uterine endometrium in postmenopausal women. *New England Journal of Medicine*, 337(23), 1641-1647. DOI: <https://doi.org/10.1056/NEJM199712043372301>
- Glouzon, B. K., Barsalani, R., Lagacé, J. C., & Dionne, I. J. (2015). Muscle mass and insulin sensitivity in postmenopausal women after 6-month exercise training. *Climacteric*, 18(6), 846-851. DOI: <https://doi.org/10.3109/13697137.2015.1083002>
- Gomes, R. M., Ferreira Júnior, M. D., Francisco, F. A., Moreira, V. M., Almeida, D. L., Saavedra, L. P. J., ... Moraes, S. M. F. (2018). Strength training reverses ovariectomy-induced bone loss and improve metabolic parameters in female Wistar rats. *Life Sciences*, 213, 134-14. DOI: <https://doi.org/10.1016/j.lfs.2018.10.032>
- Johnell, O., Kanis, J. A., Odén, A., Sernbo, I., Redlund-Johnell, I., Pettersson, C., ... Jönsson, B. (2004). Mortality after osteoporotic fractures. *Osteoporosis International*, 15(1), 38-42. DOI: <https://doi.org/10.1007/s00198-003-1490-4>
- Kabodi, S., Ajami, E., Zakiei, A., Zangeneh, A., & Saeidi, S. (2019). Women's quality of life in menopause with a focus on hypertension. *Journal of Obstetrics and Gynaecology of India*, 69(3), 279-283. DOI: <https://doi.org/10.1007/s13224-018-1172-y>
- Karolkiewicz, J., Michalak, E., Pospieszna, B., Deskur-Smielecka, E., Nowak, A., & Pilaczyńska-Szcześniak, Ł. (2009). Response of oxidative stress markers and antioxidant parameters to an 8-week aerobic physical activity program in healthy, postmenopausal women. *Archives of Gerontology and Geriatrics*, 49(1), e67-71. DOI: <https://doi.org/10.1016/j.archger.2008.09.001>
- Kawakami, M., Yokota-Nakagi, N., Uji, M., Yoshida, K., Tazumi, S., Takamata, A., ... Morimoto, K. (2018). Estrogen replacement enhances insulin-induced AS160 activation and improves insulin sensitivity in ovariectomized rats. *American Journal of Physiology, Endocrinology and Metabolism*, 315(6), E1296-E1304. DOI: <https://doi.org/10.1152/ajpendo.00131.2018>
- Lupsa, B. C., & Insogna, K. (2015). Bone health and osteoporosis. *Endocrinology and Metabolism Clinics of North America*, 44(3), 517-530. DOI: <https://doi.org/10.1016/j.ecl.2015.05.002>
- Mann, S., Beedie, C., & Jimenez, A. (2014). Differential effects of aerobic exercise, resistance training and combined exercise modalities on cholesterol and the lipid profile: review, synthesis and recommendations. *Sports Medicine*, 44(2), 211-221. DOI: <https://doi.org/10.1007/s40279-013-0110-5>

- Marques, E. A., Wanderley, F., Machado, L., Sousa, F., Viana, J. L., Moreira-Gonçalves, D., ... Carvalho, J. (2011). Effects of resistance and aerobic exercise on physical function, bone mineral density, OPG and RANKL in older women. *Experimental Gerontology*, 46(7), 524-32. DOI: <https://doi.org/10.1016/j.exger.2011.02.005>
- Marques, G. C., & Liberali, R. (2012). Consumo de proteínas na prática do treinamento de força - revisão sistemática. *Revista Brasileira de Nutrição Esportiva*, 6(32), 158-164.
- Martyn-St James, M., & Carroll, S. (2008). Meta-analysis of walking for preservation of bone mineral density in postmenopausal women. *Bone*, 43(3), 521-531. DOI: <https://doi.org/10.1016/j.bone.2008.05.012>
- McClung, M. R. (2005). New management options for osteoporosis with emphasis on SERMs. *Climacteric*, 18, 56-61. DOI: <https://doi.org/10.3109/13697137.2015.1104010>
- Mendoza, N., Teresa, C., Cano, A., Godoy, D., Hita-Contreras, F., Lapotka, M., ... Sánchez-Borrego, R. (2016). Benefits of physical exercise in postmenopausal women. *Maturitas*, 93, 83-88. DOI: <https://doi.org/10.1016/j.maturitas.2016.04.017>
- Moreira, L. D., Oliveira, M. L., Lirani-Galvão, A. P., Marin-Mio, R. V., Santos, R. N., & Lazaretti-Castro, M. (2014). Physical exercise and osteoporosis: effects of different types of exercises on bone and physical function of postmenopausal women. *Arquivos Brasileiros de Endocrinologia e Metabologia*, 58(5), 514-522. DOI: <https://doi.org/10.1590/0004-2730000003374>
- Motahari-Tabari, N., Shirvani, M., Shirzad-E-Ahoodasthy, M., Yousefi-Abdolmaleki, E., & Teimourzadeh, M. (2014). The effect of 8 weeks aerobic exercise on insulin resistance in type 2 diabetes: a randomized clinical trial. *Global Journal of Health Science*, 7(1), 115-121. DOI: <https://doi.org/10.5539/gjhs.v7n1p115>
- Müller, S. T., Keiler, A. M., Kräker, K., Zierau, O., & Bernhardt, R. (2018). Influence of estrogen on individual exercise motivation and bone protection in ovariectomized rats. *Laboratory Animals*, 52(5), 479-489. DOI: <https://doi.org/10.1177/0023677218756455>
- Nassef, Y., Lee, K. J., Nfor, O. N., Tantoh, D. M., Chou, M. C., & Liaw, Y. P. (2019). The impact of aerobic exercise and badminton on HDL cholesterol levels in adult Taiwanese. *Nutrients*, 11(3), 515. DOI: <https://doi.org/10.3390/nu11030515>
- Panveloski-Costa, A. C., Pinto Jr., D. A. C., Brandão, B. B., Moreira, R. J., Machado, U. F., & Seraphim, P. M. (2011). Resistive training reduces inflammation in skeletal muscle and improves the peripheral insulin sensitivity in obese rats induced by hyperlipidic diet. *Arquivos Brasileiros de Endocrinologia e Metabologia*, 55(2), 155-63. DOI: <https://doi.org/10.1590/S0004-27302011000200008>
- Poblete-Aro, C., Russell-Guzmán, J., Parra, P., Soto-Muñoz, M., Villegas-González, B., Cofré-Bolados, C., & Herrera-Valenzuela, T. (2018). Exercise and oxidative stress in type 2 diabetes mellitus. *Revista Medica de Chile*, 146(3), 362- 3732. DOI: <https://doi.org/10.4067/s0034-98872018000300362>
- Reginster, J. Y., Sarkar, S., Zegels, B., Henrotin, Y., Bruyere, O., Agnusdei, D., & Collette, J. (2004). Reduction in PINP, a marker of bone metabolism, with raloxifene treatment and its relationship with vertebral fracture risk. *Bone*, 34(2), 344-351. DOI: <https://doi.org/10.1016/j.bone.2003.10.004>
- Rocha, L. M. S., & Massone, F. (2006). Estudo anatomo-anestesiológico do segmento lombar (L1 a L6) em cães. *Brazilian Journal of Veterinary Research and Animal Science*, 43(2), 167-177. DOI: <https://doi.org/10.11606/issn.1678-4456.bjvras.2006.26496>
- Söderqvist, A., Ekström, W., Ponzer, S., Pettersson, H., Cederholm, T., Dalén, N., ... Tidermark, J. (2009). Prediction of mortality in elderly patients with hip fractures: a two-year prospective study of 1,944 patients. *Gerontology*, 55(5), 496-504. DOI: <https://doi.org/10.1159/000230587>
- Sousa, A. G. V., Alves, L. A. C., Rocha, R. F., Moraes, M. E. L., & Carvalho, V. A. P. (2007). Efeitos da terapia de reposição hormonal com raloxifeno e risedronato na reparação óssea de ratas com osteopenia. *Ciência Odontológica Brasileira*, 10(3), 81-89. DOI: <https://doi.org/10.14295/bds.2007.v10i3.295>
- Souza, D. M., Rosa, L. P., Ricardo, L. H., Moraes, L. C., & Rocha, R. F. (2005). Avaliação óssea alveolar de *Rattus norvegicus* por meio dos métodos radiográfico e morfométrico. *Ciência Odontológica Brasileira*, 8(4), 77-84. DOI: <https://doi.org/10.14295/bds.2005.v8i4.402>
- Stringhetta-Garcia, C. T., Singulani, M. P., Santos, L. F., Louzada, M. J. Q., Nakamune, A. C. S., Chaves-Neto, A. H., ... Dornelles, R. C. M. (2016). The effects of strength training and raloxifene on bone health in aging ovariectomized rats. *Bone*, 85, 45-54. DOI: <https://doi.org/10.1016/j.bone.2015.11.023>

- Stuenkel, C. A. (2017). Menopause, hormone therapy and diabetes. *Climacteric*, 20(1), 11-21.
DOI: <https://doi.org/10.1080/13697137.2016.1267723>
- Stunes, A. K., Erben, R. G., Schöler, C., Eriksen, E. F., Tice, M., Vashishth, D., ... Mosti, M. P. (2020). Skeletal effects of plyometric exercise and metformin in ovariectomized rats. *Bone*, 132, 115193.
DOI: <https://doi.org/10.1016/j.bone.2019.115193>
- Takada, J., Iba, K., Yoshizaki, T., & Yamashita, T. (2012). Correlation between a bone resorption marker and structural geometry of the proximal femur in osteoporotic women treated with raloxifene. *Journal of Orthopaedic Surgery*, 20(2), 209-213. DOI: <https://doi.org/10.1177/230949901202000215>
- Tobias, J. H., Gould, V., Brunton, L., Deere, K., Rittweger, J., Lipperts, M., & Grimm, B. (2014). Physical activity and bone: may the force be with you. *Frontiers in Endocrinology*, 5, 1-5.
DOI: <https://doi.org/10.3389/fendo.2014.00020>
- Wang, Q., Week, D. L. S., Nelson, S. M., Sattar, N., Ala-Korpela, M., & Lawlor, D. A. (2018). Metabolic characterization of menopause: cross-sectional and longitudinal evidence. *BMC Medicine*, 16(1), 17.
DOI: <https://doi.org/10.1186/s12916-018-1008-8>
- Watson, S. L., Weeks, B. K., Weis, L. J., Harding, A. T., Horan, S. A., & Beck, B. (2018). High-intensity resistance and impact training improves bone mineral density and physical function in postmenopausal women with osteopenia and osteoporosis: the LIFTMOR randomized controlled trial. *Journal of Bone and Mineral Research*, 33(2), 211-220. DOI: <https://doi.org/10.1002/jbmr.3284>
- Yogui, F. C., Momesso, G. A. C., Faverani, L. P., Polo, T. O. B., Ramalho-Ferreira, G., Hassumi, J. S., ... Okamoto, R. (2018). A SERM increasing the expression of the osteoblastogenesis and mineralization-related proteins and improving quality of bone tissue in an experimental model of osteoporosis. *Journal of Applied Oral Science*, 26, e20170329. DOI: <https://doi.org/10.1590/1678-7757-2017-0329>