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The effects of strength training and raloxifene on bone health in aging ovariectomized rats

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ABSTRACT

The aim of this study was to investigate the effects of strength training (ST) and raloxifene (Ral), alone or in combination, on the prevention of bone loss in an aging estrogen-deficient rat model. Aging Wistar female rats were ovariectomized at 14 months and allocated to four groups: (1) non-trained and treated with vehicle, NT-Veh; (2) strength training and treated with vehicle, ST-Veh; (3) non-trained and treated with raloxifene, NT-Ral; and (4) strength training and treated with raloxifene, ST-Ral. ST was performed on a ladder three times per week and Ral was administered daily by gavage (1 mg/kg/day), both for 120 days. Areal bone mineral density (aBMD), strength, microarchitecture, and biomarkers (osteocalcin, OCN; osteoprotegerin, OPG; and tartrate-resistant acid phosphatase, TRAP) were assessed. Immunohistochemistry was performed for runt-related transcription factor 2 (RUNX2), osterix (OSX), OCN, OPG, TRAP, and receptor activator of nuclear factor kappa-B ligand (RANKL). The rats that performed ST (ST-Veh) or were treated with Ral (NT-Ral) showed significant improvements in aBMD ($p = 0.001$ and 0.004), bone strength ($p = 0.001$), and bone microarchitecture, such as BV/TV (%) ($p = 0.001$), BS/TV (mm^2/mm^3) ($p = 0.023$ and 0.002), Conn.Dn ($1/mm^3$) ($p = 0.001$), Tb.N (1/mm) ($p = 0.012$ and 0.011), Tb.Th (1/mm) ($p = 0.001$), SMI ($p = 0.001$ and 0.002), Tb.Sp ($p = 0.001$), and DA ($p = 0.002$ and 0.007); there was also a significant decrease in plasma levels of OCN ($p = 0.001$ and 0.002) and OPG ($p = 0.003$ and 0.014), compared with animals in the NT-Veh group. Ral, with or without ST, promoted an increased immunolabeling pattern for RUNX2 ($p = 0.0105$ and $p = 0.0006$) and OSX ($p = 0.0105$), but a reduced immunolabeling pattern for TRAP ($p = 0.0056$) and RANKL ($p = 0.033$ and 0.004). ST increased the immunolabeling pattern for RUNX2 ($p = 0.0105$), and association with Ral resulted in an increased immunolabeling pattern for OPG ($p = 0.0034$) and OCN ($p = 0.0024$). In summary, ST and Ral administration in aged, estrogen-deficient Wistar female rats is associated with a decrease in bone turnover marker plasma levels, increased activity of cells that promote osteoblastogenesis, and decreased activity of cells that promote osteoclastogenesis; these are correlated with higher aBMD, bone strength, and bone microarchitecture at the femoral neck. The results indicate that strength training and Ral are potential tools to reduce the risk of fractures at clinically relevant sites.

1. Introduction

Osteoporosis is a bone disease characterized by increased risk of fracture. Its prevalence is increasing as a result of longer life expectancies and population aging; consequently, it is responsible for a rise in social and public health problems [1,2]. Osteoporosis arises from changes in bone homeostasis, which result in impairments to the remodeling cycle [3]. Hip fractures are one of the most common types of fracture that affect people with osteoporosis, and are associated with a higher level of disability and health care costs than all other osteoporotic fractures [4,5]. Fourteen percent of the total number of fractures in the USA are femoral neck fractures, which account for 72% of the total cost of treating osteoporotic fractures [6].

Among local and systemic factors that contribute to an imbalance in the activity of bone cells, estrogen has an important role to play in bone health as a result of both direct and indirect activity [7]. Postmenopausal

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women have an increased risk of fragility fractures related to changes in hormonal levels [8]; these changes result in a decrease in bone mineral density (BMD), due to an increase in bone resorption relative to bone formation [9]. Preventive and therapeutic interventions for maintaining bone health are therefore extremely important given the impact on functional independence and quality of life in older people. In recent years, several studies have identified pathways involved in the maintenance and formation of bone that can be used for targeting drug therapy, such as the use of anabolic agents (parathyroid hormone) [10], and antiresorptive treatments, such as immunological (RANKL antibody) [11], hormone replacement [12], and bisphosphonate therapy [13].

Raloxifene (Ral), a selective estrogen receptor modulator (SERM), is an example of a class of pharmacological compounds with beneficial effects on bone tissue [14] and that lower the risk of breast cancer [15]. Clinical studies have demonstrated that Ral prevents bone loss and lowers the risk of fracture by modulating bone turnover [16]. Ral has been described as an antiresorptive drug, but the presence of estrogen receptors in osteoblasts and bone marrow stromal cells suggests that Ral can have a direct role in the regulation of osteoblast lineage cells as well as an osteoblast stimulatory role [17,18]. However, the mechanism by which this SERM exerts its effect on bone cells has yet to be fully elucidated [17], and no in vivo study has documented an effect of Ral on osteoblasts.

Strength training (ST) has been proposed as an alternative to drug therapy in the prevention and treatment of osteoporosis, as a means of preventing deterioration of bone mass. ST exercises are known to increase bone strength and bone mass [19], and improve bone microarchitecture [20], whereas a sedentary lifestyle has been associated with increased bone resorption [21]. Mechanical loading also influences a range of tissues, including muscle, tendons, and ligaments, and represents a means of protecting skeletal integrity in a nonpharmacological fashion [22]. In the postmenopausal period, there is decreased activity of estrogen receptor α in bone cells, with a concurrent decline in the responsiveness of bone to mechanical load, which further exacerbates deterioration of bone quality [23–25]. This is a possible explanation for bone loss in postmenopausal women in amounts comparable to that associated with disuse [26].

Considering bone fragility in old age, it is essential to propose interventions that can minimize bone loss and validate preventive measures for primary osteoporosis. Thus, the aim of this study was to investigate the effects of ST and Ral, alone or in combination, on the prevention of bone loss in an aged estrogen-deficient rat model.

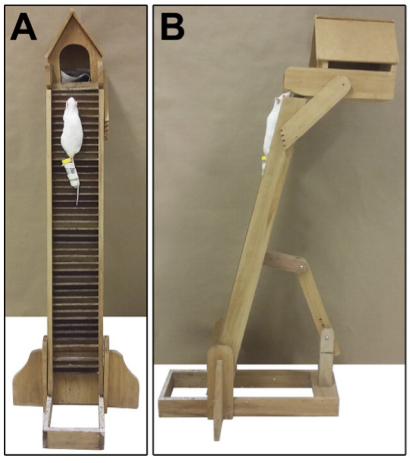
2. Material and methods

2.1. Animals

All animal procedures were approved (Process number 001,397–2010) by the Institutional Animal Care and Use Committee of the Faculty of Dentistry (Univ. Estadual Paulista – UNESP, Araçatuba, SP, Brazil) and complied with the Guide for Care and Use of Laboratory Animals.

Female Wistar rats aged 13–18 months were obtained from the central animal facility of the Faculty of Dentistry of Araçatuba, and were housed at 22 °C (±2 °C) under a 12:12 h light:dark cycle. The animals were allowed free access to water and a commercial pellet diet (Presence® Ratos e Camundongos, Paulínia, SP, Brazil). All female rats were multiparous as this was an inclusion criterion. The reproductive life of these animals began on average at 2 to 8 months of age, reaching 3 to 4 pregnancies. The general health of the rats was monitored on a daily basis and the estrus cycle was checked during the 13th month, to determine acyclicity. In female rats, the incidence of regular estrous cyclicity decreases progressively during aging and their estrous cycles tend to become irregular, usually with prolonged estrus and diestrus, characterizing peri-estropause. This period is similar to perimenopause in women [27–29]. Thus, rats were ovariectomized to decrease estradiol plasma concentrations even more, similar to what occurs at menopause, and to study changes at the beginning of this new phase in naturally aging female rats.

At 14 months of age, acyclic animals were submitted to a bilateral ovariectomy [30]. For the ovariectomy procedure, animals were anesthetized with ketamine (Vetaset®, Fort Dodge Saude Animal Ltda, Brazil) at a dose of 50 mg/kg of body weight, in combination with xylazine (Coopazine, Coopers Brazil Ltda) at a dose of 25 mg/kg of body weight, by intraperitoneal injection. Incisions were made at the side of the abdomen to enable removal of the ovaries. Animals were sutured and received a prophylactic intramuscular dose of antibiotic (1 mL/kg; Veterinary Pentabiotic; Fort Dodge Animal Health Ltd.). Ten days after surgery, ovariectomized (OVX) rats were randomly assigned to one of four groups: (1) non-trained and treated with vehicle (NT-Veh, n = 10), (2) strength training and treated with vehicle (ST-Veh, n = 10), (3) non-trained and treated with raloxifene (NT-Ral, n = 10), and (4) strength training plus raloxifene (ST-Ral, n = 10). After 120 days, the animals were sacrificed with anesthetic overdose; the uterus was collected and weighed to verify the success of ovariectomy.



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Age (months/weeks)	Weeks ST	Load (%)
14/1	1	Adaptation
14/2	2	20
14/3	3	30
14/4	4	40
15/1	5	50
15/2	6	60
15/3	7	70
15/4	8	80
16-18 / 1-4	9-16	80

Fig. 1. Strength training in rats. Ladder used in strength training and animal performing strength training (**A-B**). Loading weight program in relation to duration of strength training, and percentage of loading weight (**C**).