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Effects of isometric strength training followed by no exercise and *Humulus lupulus* L–enriched diet on bone metabolism in old female rats

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Abstract

We investigated in female rats the effects on bone metabolism of a prolonged no-training period, subsequent to an isometric exercise program, performed during young adulthood and those of a long-term consumption of *Humulus lupulus* L–enriched diet (genistein 1.92 and daidzein 1.24 mg/kg diet) combined or not with isometric training. Forty-eight rats (4 weeks old) were randomly divided into 4 groups: trained (C-Tr) or nontrained rats (C-NTr) fed with control diet and trained (H-Tr) or nontrained rats (H-NTr) fed with *Humulus lupulus* L–enriched diet. The diets lasted 100 weeks. Training was followed over a 25-week period. Bone parameters were measured at week 100. Our results showed that no significant difference was observed among the 4 groups in uterine relative weight, calcium (Ca) intake, fecal Ca, urinary Ca excretion, net Ca absorption, plasma Ca, and bone Ca content. Calcium balance was significantly enhanced in H-NTr rats in comparison with C-NTr and C-Tr rats. Isometric strength training led to a significant increase in total bone mineral density (BMD), diaphyseal BMD, and osteocalcin-deoxypyridinoline ratio in C-Tr rats compared with the other groups. The main findings of the present study indicate that in female rats, a 25-week isometric strength training performed during young adulthood followed by a prolonged no-training period increases BMD values and osteocalcin-deoxypyridinoline ratio, whereas long-term consumption of *Humulus lupulus* L–enriched diet does not improve bone parameters. It suggests that bone gains induced by exercise do not decrease immediately after cessation of training and also confirms the importance of the practice of physical activity during puberty and young adulthood to maximize the achieved peak bone density.

1. Introduction

Peak bone mass is an important determinant of risk for osteoporosis: it may account for more than half of the variation in bone mass until at least 65 years of age [1]. There are evidences that suggest that exercise maximizes bone mineral density (BMD) during the younger years, as well as improves bone density by reducing the rate of skeletal attrition during aging [2]. For children, the most efficient exercise interventions have exposed young developing skeletons to dynamic impact loads, such as those induced by jumping [3]. Although this training mode may serve to increase peak skeletal strength and thereby may serve to prevent the development of osteopenia, it may not be available to all subjects. In these cases, resistance training probably represents an attractive exercise modality. A vast literature supports that resistance training is positively associated with high BMD in both young and older adults and that the effect of resistive exercise is relatively site specific to the working muscles and the bones to which they attach [4-12]. These observations in humans were supported by animal studies. In 1999, Lac and Cavalie [13] validated a

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rat model of resistance training and reported that a 10-week isometric strength training increased both trabecular and cortical femoral bone mass in growing male rats. Nevertheless, it was not clear whether the benefits of physical activity would be maintained after cessation of the training. Human studies showed that athletes maintain the positive effects of training into adulthood, even after cessation of training [14-17]. On the contrary, other investigations have suggested that ongoing training is needed to keep the bone mass [18-20]. Because literature data are rather contradictory, the bone response to cessation of training deserves more attention.

Besides, targeting healthy life pattern, there is evidence that the human diet contains, in addition to essential macronutrients, a complex array of naturally occurring bioactive molecules, phytochemicals endowed with antioxidant and anti-inflammatory properties. Indeed, several epidemiological studies have shown that consumption of foods rich in such polyphenolic compounds is associated with beneficial effects in the prevention of osteoporosis [21]. This is why there is an increasing rationale to focus on phytoestrogens (PE) because emerging data support the hypothesis that these weakly estrogenic compounds present in plants may represent a potential alternative therapy for a range of hormone-dependent conditions. Hops, a dioecious plant of the Cannabaceae family, is a rich source of such compounds [22]. The female flowers of hops (*Humulus lupulus* L) have been used primarily as a preservative and a flavoring agent in beer. This is the alcoholic beverage of choice in many parts of the world and may represent a vehicle to increase the consumption of natural products with antioxidant and other health-promoting properties [23] because beer is rich in amino acids, peptides, B vitamins, and phenolic compounds derived from hops and malts [24]. Moreover, hops is known to exhibit estrogen-like activities. In this light, Tobe et al [25] isolated estrogenic compounds from hops extract for beer brewing and reported that one of these active components (humulone) was a strong inhibitor of bone resorption. Miyamoto et al [26] have shown that, in ovariectomized rats, 8-isopentenylnaringenin (a prenylflavonoid present in hops) suppresses gonadectomy-induced bone resorption in a manner similar to estrogens. More recently, Effenberger et al [27] investigated the effects of several hops-derived compounds on cultured osteoblast-like cells and demonstrated that these specific PE compounds exerted estrogen-like activities and displayed positive effects on bone metabolism. Of interest, hops must be considered as a plant rich in estrogens that could be interesting for its bone-sparing effects.

Thus, the purpose of the present study was to investigate in old female rats the effects on bone metabolism of an isometric exercise program performed during young adulthood, followed by a prolonged no-exercise period, and those of a long-term consumption of hops (*Humulus lupulus* L) containing genistein and daidzein (1.92 and 1.24 mg/kg diet) combined or not with isometric strength training.

2. Materials and methods

2.1. Animals

Forty-eight female Sprague-Dawley rats (Charles River Laboratories, L'Arbresle, France), 4 weeks old and weighing 100 to 125 g, were housed 4 animals to a stainless steel cage (45 × 30 × 20 cm, Tecniplast Gazzada, Buguggiate, Italy) in a room with controlled temperature (22°C ± 2°C), relative humidity (40%-60%), and a 12-hour:12-hour light-dark cycle.

After a 1-week acclimation period, rats were randomly divided into 4 groups (n = 12 rats per group): trained rats (C-Tr) and nontrained rats (C-NTr) fed with semisynthetic control diet, and trained rats (H-Tr) and nontrained rats (H-NTr) fed with *Humulus lupulus* L powder–enriched semisynthetic diet. Rats were examined daily for general conditions during the experimental protocol, which lasted 100 weeks. This protocol was approved by the European Community for the care and the use of animals (L 358-86/609EEC).

2.2. Training program

Exercise training was carried out every morning, 5 days a week, for a total of 25 weeks. The exercise regimen, which has been validated by Lac and Cavalie [13], consisted of an isometric strength training during which duration and intensity were gradually increased as detailed in Table 1. To increase the exercise intensity, progressive loads were

Table 1
Schedule of the training program

Week	Training program
1: day 1	6 repetitions (10 s)
Day 2	6 repetitions (4 × 10 and 2 × 30 s)
Day 3	6 repetitions (2 × 10 and 4 × 30 s)
Day 4	6 × 30 s
Day 5	6 × 30 s
2-3	6 × 30 s
4-5	6 × 30 s with a 25-g load
6-7	6 × 30 s with a 50-g load
8-9	(6 × 30 s and 4 × 30 s) with a 50-g load. RT: 20 min between serials
10-11	6 × 30 s with a 75-g load
12-13	(6 × 30 s and 2 × 30 s) with a 75-g load. RT: 10 min between serials
14-15	(6 × 30 s and 2 × 30 s) with a 100-g load. RT: 10 min between serials
16-17	(6 × 30 s and 4 × 30 s) with a 100-g load. RT: 20 min between serials
18-19	2 × (6 × 30 s) with a 100-g load. RT: 10 min between serials
20-21	2 × (4 × 30 s and 1 × 40 s) with a 100-g load. RT: 10 min between serials
22-23	2 × (4 × 30 s and 2 × 40 s) with a 100-g load. RT: 10 min between serials
24-25	2 × (4 × 30 s and 1 × 1 min) with a 100-g load. RT: 10 min between serials

Each repetition (from 10 seconds to 1 minute) was separated by resting for 20 seconds. RT indicates rest time.

Table 2
Composition of diets (per kilogram diet)

	Semisynthetic control diet	*Humulus lupulus* L powder–enriched semisynthetic diet
Corn starch	400 g	363 g
Cellulose	60 g	15 g
Casein	200 g	185 g
Sucrose	210 g	210 g
Mineral mix	70 g	70 g
Vitamin mix	10 g	10 g
Peanut oil	25 mL	22 mL
Corn oil	25 mL	25 mL
Humulus lupulus L powder	–	100 g
Ca	10.47 g	13.83 g
Genistein	–	1.92 mg
Daidzein	–	1.24 mg

placed around the abdomen of the rat by means of an elastic band added since week 4. The size of the elastic band was adapted to the rat morphology so as to avoid the compression of the rat torso. Rats were set on the horizontal floor of a box, which was then put in a vertical position. The animals gripped with their claws and remained in climbing position because the floor is made with a wire netting. Nontrained animals had no access to food throughout the training session periods of the exercised animals. The exercise regimen was followed by a 75-week no-training period.

2.3. Diet

Animals were fed ad libitum with either a semisynthetic control diet or a semisynthetic *Humulus lupulus* L powder–enriched diet (Laboratoire ADP, Reventin Vaugris, France) (100 g/kg diet, Table 2). All other compounds were purchased from SAFE (Epinay-Sur-Orge, France). Diets were prepared each month, stored at room temperature, protected from the light, and distributed throughout the study.

2.4. Body weight, systolic blood pressure, and heart rate assay

Rats were weighed once a month and just before sacrifice. Resting systolic blood pressure (SBP) and heart rate (HR) were monitored in conscious, restrained, and previously warmed rats using the indirect tail cuff method by a sphygmomanometer (PE-3000; Narco-Biosystems, Houston, TX) before the beginning of treatments (T0) and at T + 14, T + 27, T + 46, T + 67, T + 89, and T + 100 weeks.

2.5. Sampling of feces and urine

During the last week of the study, rats were transferred into individual metabolism cages (22 × 22 × 18 cm, Tecniplast Gazzada), allowing separate collection of feces and urine. These samples were collected during a 24-hour period to measure fecal calcium (Ca), urinary deoxypyridinoline (DPD; a marker for bone resorption), and urinary Ca excretion. The volumes of urine and feces samples for each rat were recorded. Food intake was estimated during this metabolism period by the difference in weight of food put in the cage minus spilled food.

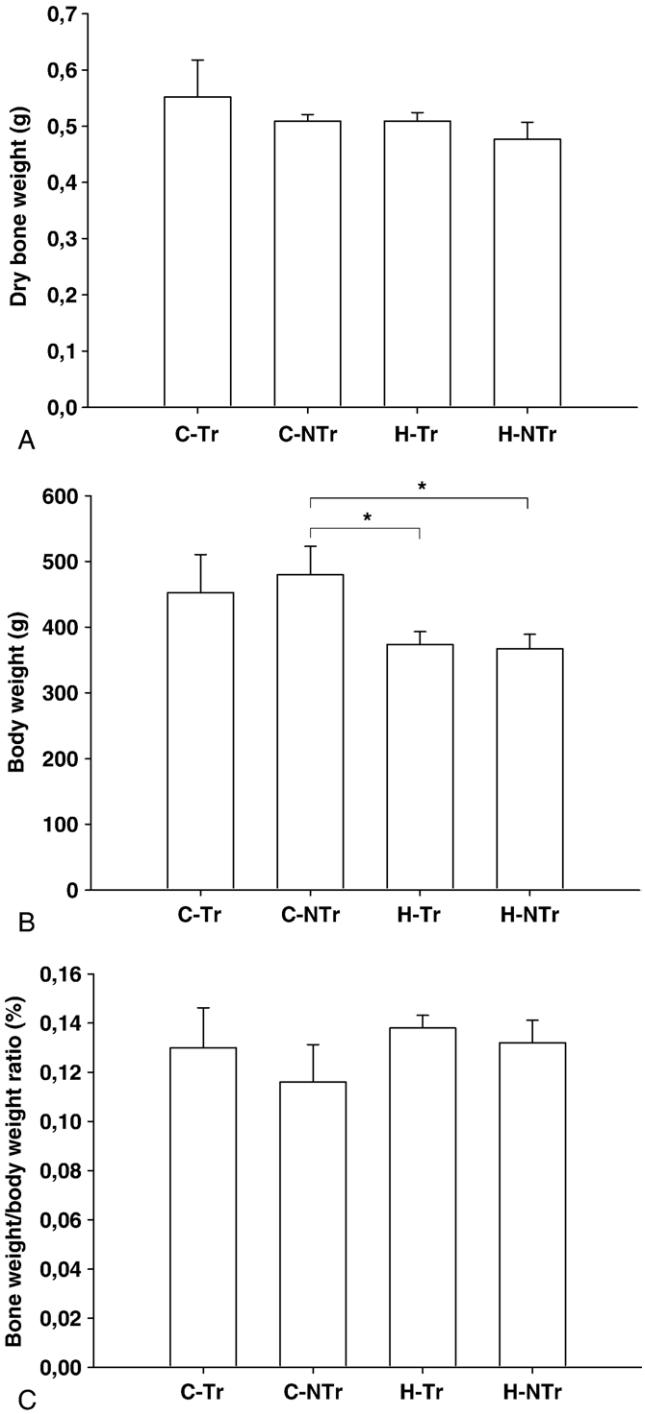

Fig. 1. Dry bone weight of right tibia (A), body weight (B), and bone weight–body weight ratio (C) in rats after experimental protocol. Values are expressed as mean ± SEM. n = 8 (C-Tr), 9 (C-NTr), 12 (H-Tr), and 9 (H-NTr). Statistically significant at $*P < .05$.

2.6. Collection of blood and tissues

At the end of the experimental protocol, food was withheld overnight. The following morning, 24 hours after the last exercise training period, the rats were killed under pentobarbital anesthesia (60 mg/kg body weight intraperitoneally; Ceva Santé animale, Libourne, France) between 8:00 and 9:00 AM; and blood was collected via the abdominal aorta using 10-mL plastic syringes fitted with 23-gauge needles. Blood samples were immediately centrifuged at 4000*g* for 10 minutes at 4°C. Plasma was removed and stored at −80°C until analysis. Afterward, the right tibia and the left femur were entirely and quickly excised and cleaned from adjacent tissue. The tibia was frozen in liquid nitrogen and stored at −80°C until analysis. The femur was stored in ethanol 70% until analysis. Finally, uteri were removed from each rat and weighed.

2.7. Biochemical analysis

Calcium levels in diets, feces, urine, plasma, and ashed bone samples (tibiae, dissolved in 10 mol/L HCl and diluted in 0.5% lanthanum chloride) were measured by atomic absorption spectrophotometry (Perkin Elmer 3300; Perkin Elmer, Courtaboeuf, France).

Plasma concentrations of osteocalcin (OC), a marker of bone formation, were measured by a specific radioimmunoassay (RIA) kit (Rat Osteocalcin RIA kit; Biomedical Technologies, Stoughton MA) using a rat I^{125}-labeled OC, goat anti–rat OC antibody, and donkey anti–goat second antibody. Sensitivity is 0.01 ng/mL. Intra- and interassay precisions are 6.8% and 8.9%, respectively.

Free urinary DPD, a marker of bone resorption, was measured by a competitive RIA using anti-DPD monoclonal antibody coated to the inner surface of a polystyrene tube and I^{125}-labeled DPD (Gamma-BCT DPD; IDS, Boldon, United Kingdom). The DPD values were expressed as nanomoles of DPD per millimoles of creatinine [28]. Creatinine was measured with a creatinine assay kit (kit Biomerieux SA, Narcy l'Etoile, France), which is a quantitative, colorimetric assay based on a modified Jaffe method in which picric acid forms a colored solution in the presence of creatinine. Sensitivity is 2 nmol/L. Intra- and interassay precisions are 4% and 6%, respectively.

2.8. Physical measurements

Total left femoral BMD was determined by dual x-ray absorptiometry using a Hologic QDR 4500A x-ray densitometer (Hologic, Massy, France). Furthermore, the BMDs of 2 subregions, corresponding to the proximal metaphyseal zone (M-BMD), which is rich in cancellous bone, and to the diaphyseal zone (D-BMD), mainly cortical bone, were assessed as previously described by Pastoureau et al [29]. All BMD values were expressed as grams per square centimeters. Intra- and interassay variations measured on 10 femoral samples are 0.22% and 0.24%, respectively.

2.9. Statistical analysis

Values for all results were expressed as means ± SEM. Significance of differences among the 4 groups was determined by 1-way analysis of variance (ANOVA), followed by Fisher protected least significant difference post hoc test. When normality test failed, a 1-way ANOVA on ranks was performed. The effects of training, diet, and interaction of both interventions (training × diet) were analyzed by 2-way ANOVA, followed by Fisher protected least significant difference post hoc test. The level of significance was set at $P < .05$. Tests were carried out using Sigma Stat 2.03 (St Louis, MO).

3. Results

3.1. Mortality rate

First deaths appeared during week 67 of the protocol. Total mortality rate represented 20.75%: the highest mortality rate was observed in trained rats fed with control diet (33%), whereas it was naught in trained rats fed with

Table 3
Effects of isometric exercise training and *Humulus lupulus* L–enriched diet on Ca metabolism

Groups	Food intake (g/d)	Ca intake (mg/d)	Fecal Ca (mg/d)	Urinary Ca (mg/d)	Ca balance (mg/d)	Net Ca absorption (%/d)	Plasma Ca (g/dL)	Right tibia Ca content (mg/g dry bone)
C-Tr	13.7 ± 1.66	143.2 ± 17.40	60.7 ± 3.87	5.3 ± 0.66	77.1 ± 15.21	54.9 ± 4.66	9.9 ± 0.31	283.2 ± 8.36
C-NTr	15.1 ± 2.92	158.0 ± 30.61	67.5 ± 4.74	8.8 ± 2.01	81.6 ± 26.56	46.3 ± 11.73	9.7 ± 0.81	292.3 ± 16.8
H-Tr	11.3 ± 1.84	156.7 ± 25.41	47.9 ± 3.21*,†	4.2 ± 1.26	104.6 ± 21.60	65.7 ± 4.60	9.8 ± 0.15	273.4 ± 10.37
H-NTr	14.0 ± 0.93	193.6 ± 12.87	55.05 ± 3.84**	6.6 ± 0.94	131.9 ± 13.61	70.5 ± 3.65	10.3 ± 0.73	284.7 ± 12.29
P value training	NS	NS	NS	$P < .05$	NS	NS	NS	NS
P value diet	NS	NS	$P < .01$	NS	NS	$P < .05$	NS	NS
P value interaction (training × diet)	NS	NS	NS	NS	NS	NS	NS	NS

Values are expressed as mean ± SEM. n = 8 (C-Tr), 9 (C-NTr), 12 (H-Tr), and 9 (H-NTr). NS indicates no significant difference.

* Statistically significant at $P < .05$ as compared with C-NTr group.

** Statistically significant at $P < .01$ as compared with C-NTr group.

† Statistically significant at $P < .05$ as compared with C-Tr group.

Humulus lupulus L diet and corresponded to 25% in nontrained rats fed with control or *Humulus lupulus* L diet (data not shown).

For all following parameters, no statistically significant interaction between isometric exercise training and *Humulus lupulus* L–enriched diet was observed.

3.2. Body weight, SBP, HR

During the test period, all the animals gained weight compared with their initial weights. After 25 weeks of treatment (end of training period), no statistical difference was observed among the 4 groups ($P = .061$). At the end of the experimental period, body weight was significantly decreased in H-Tr (373 ± 19 g) and H-NTr rats (367 ± 22 g) compared with that of C-NTr group (480 ± 43 g, $P < .05$, Fig. 1). On the other hand, the different treatments did not modify resting SBP and HR values (data not shown).

3.3. Uterine relative weight

No statistical difference was observed among the 4 groups. The uterine relative weights (in grams per 100 grams body weight) were 0.24 ± 0.06, 0.21 ± 0.05, 0.22 ± 0.07, and 0.27 ± 0.04 in C-Tr, C-NTr, H-Tr, and H-NTr rats, respectively.

3.4. Calcium metabolism

The effects of exercise training and/or *Humulus lupulus* L–enriched diet on Ca metabolism are shown in Table 3. Isometric strength training and *Humulus lupulus* L–enriched diet, alone or in combination, had no effect on Ca intake, urinary Ca excretion, net Ca absorption ([{Ca intake − fecal Ca} ÷ Ca intake] × 100), Ca balance (Ca intake − [fecal Ca + urinary Ca]), and plasma Ca. However, fecal Ca excretion was significantly decreased in nontrained or trained rats fed with *Humulus lupulus* L–enriched diet in comparison with nontrained rats fed with control diet ($P < .05$ and $P < .01$, respectively). Moreover, it was significantly lower in trained rats fed with *Humulus lupulus* L–enriched diet in comparison with trained rats fed with control diet ($P < .05$).

3.5. Bone Ca content and bone weight–body weight ratio

Bone Ca content and bone weight–body weight ratio, measured in right tibiae, were affected neither by exercise training nor by diet nor by the combination of both (Table 3 and Fig. 1, respectively).

3.6. Bone mineral density

Isometric strength training (C-Tr) led to a significant increase in total left femoral BMD (Fig. 2A) compared with that observed in C-NTr ($P < .05$), H-Tr ($P < .01$), and H-NTr rats ($P < .05$). Whereas proximal M-BMD was identical among the 4 groups (Fig. 2B), isometric strength training significantly enhanced D-BMD, with higher D-BMD values in C-Tr group than those measured in C-NTr ($P < .01$), H-Tr ($P < .001$), and H-NTr groups ($P < .001$, Fig. 2C).

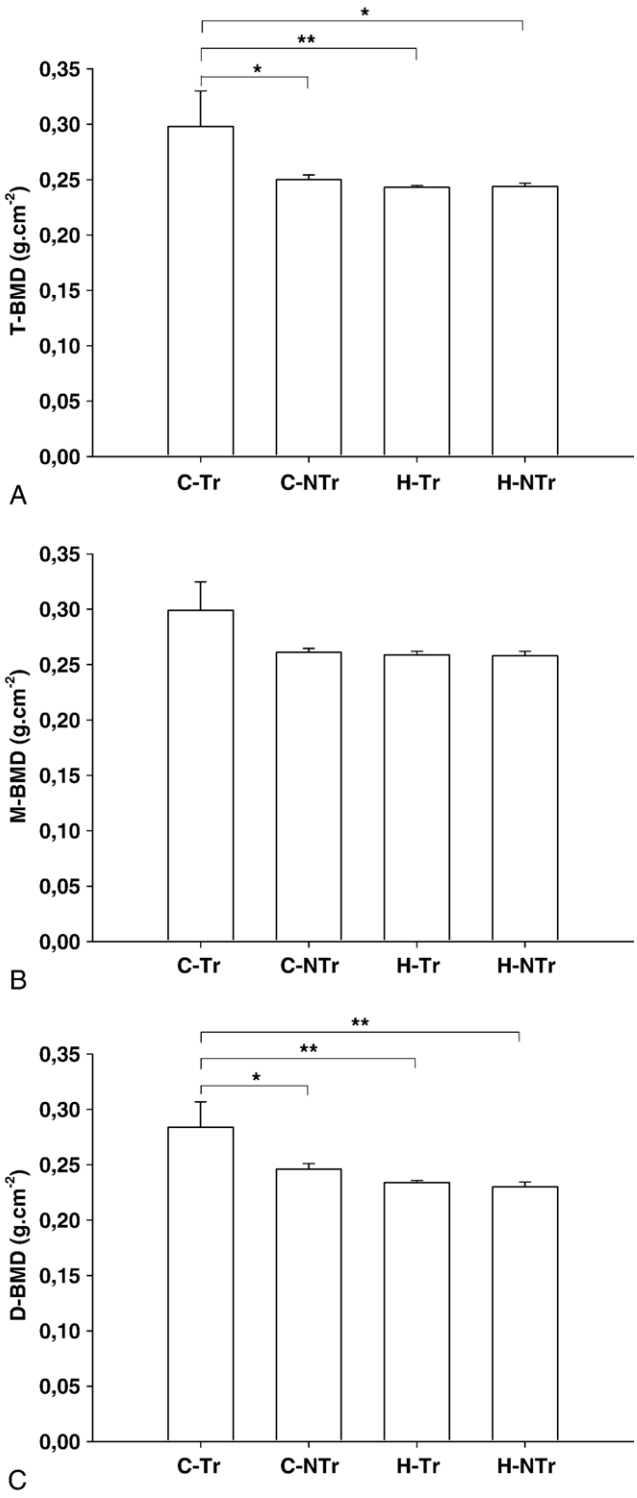

Fig. 2. Total left femoral BMD (A), M-BMD (B), and D-BMD (C). Values are expressed as mean ± SEM. n = 8 (C-Tr), 9 (C-NTr), 12 (H-Tr), and 9 (H-NTr). Statistically significant at $*P < .05$ and $**P < .01$. T-BMD indicates total left femoral bone mineral density.

3.7. Markers of osteoblastic and osteoclastic activity

As shown in Fig. 3A and B, OC and DPD concentrations were not significantly different among the 4 studied groups.

However, the OC/DPD ratio (Fig. 3C) was significantly greater in C-Tr than in C-NTr ($P < .05$), H-Tr ($P < .05$), and H-NTr rats ($P < .01$).

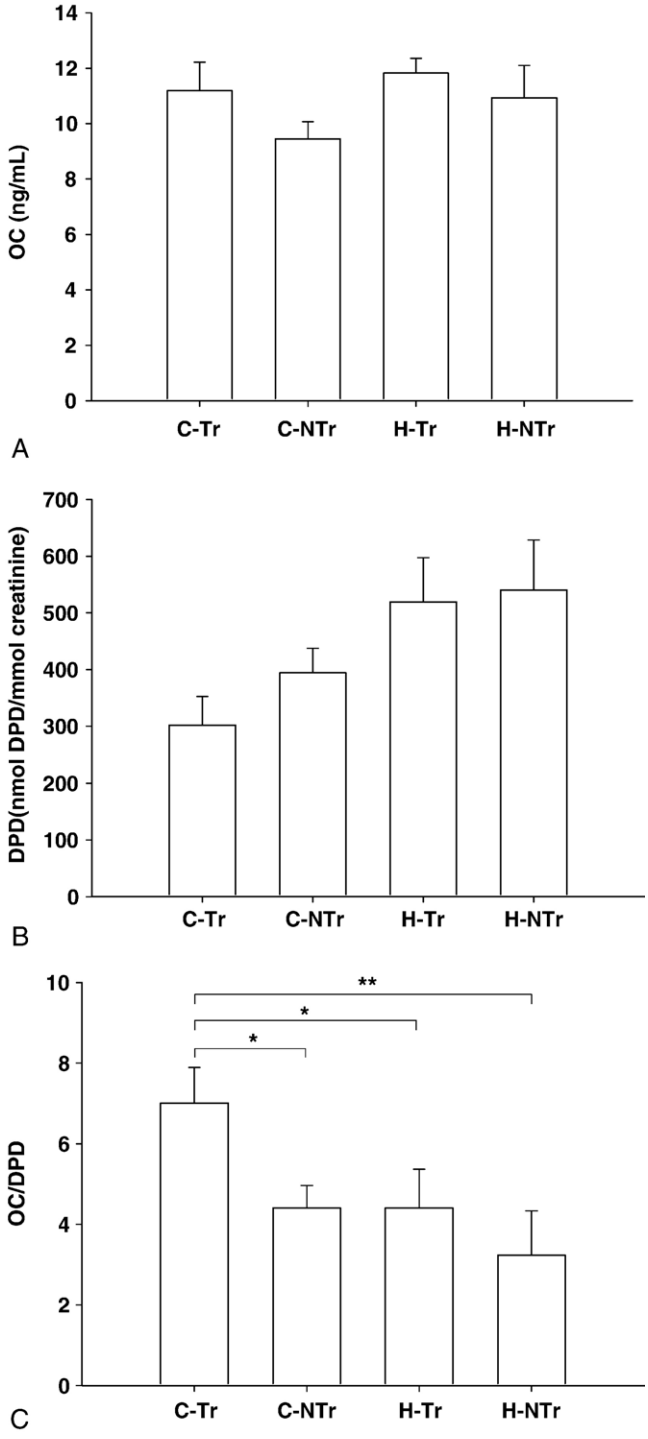

Fig. 3. Effects of isometric exercise training and *Humulus lupulus* L–enriched diet on OC (A) and DPD (B) levels and on OC/DPD ratio (C). Values are expressed as mean ± SEM. n = 8 (C-Tr), 9 (C-NTr), 12 (H-Tr), and 9 (H-NTr). Statistical significant at $*P < .05$ and $**P < .01$.

4. Discussion

The main findings of the present study indicate that in female rats, a 25-week isometric strength training performed during young adulthood followed by a prolonged no-training period increases BMD values and OC/DPD ratio, whereas long-term consumption of *Humulus lupulus* L–enriched diet does not improve bone parameters.

Isometric training is defined as the sustained contraction of a muscle over a certain period [30]. It often constitutes a part of training in nonspecific preparation of athletes and is generally called resistance or strength training. Hurley and Roth [31] reviewed the effects of strength training on risk factors for age-related diseases or disabilities in elderly human subjects and suggested in particular that strength training prevents the loss of BMD with age. In the present study, although plasma OC level, a marker of bone formation, was not significantly different among the 4 groups, urinary DPD excretion, a marker of bone resorption, tended to decrease in trained rats (C-Tr), resulting in a higher OC/DPD ratio in this exercised group. Similar effects upon bone metabolism have already been reported in male rats after a treadmill running exercise [32-35]. In the same way, after a 10-week progressive isometric strength training, Cavalie et al [30] showed a lower urinary DPD excretion in trained rats compared with resting rats, without difference in plasma OC concentration. Moreover, in our experimental conditions, isometric strength–trained animals fed with control diet presented greater total and D-BMD values than nontrained animals. Thus, inhibition of bone resorption largely occurred on cortical bone because D-BMD was higher in trained rats. If enhancement of BMD after exercise training is well established, bone regions affected by exercise-induced mechanical stress seem to vary according to the type of imposed exercise. Indeed, endurance training, such as treadmill running, primarily increased M-BMD (trabecular bone) but had no significant effects on D-BMD (cortical bone) [32], whereas isometric strength training, as used in our study, elicited greater M-BMD and D-BMD values [30].

In the present study, although dry bone mass of right tibia tended to increase in trained rats, parameters of Ca metabolism were not modified with exercise. The lack of isometric training effects on these parameters is certainly due to the cessation period following exercise. Here, it is interesting to notice that, on the contrary, possible changes in OC, DPD, and BMD induced by a 25-week isometric training persisted after the training cessation. Thus, our study shows the beneficial effects of resistance training on bone tissue metabolism but especially highlights the fact that these positive effects are preserved after a long no-exercise period. Our results are in accordance with previous rodent studies that demonstrated that exercise-induced bone benefits were maintained even after stopping exercise training [36,37].

Nevertheless, it is worth mentioning that opposite results were also reported, suggesting that continued exercise is required to maintain the positive effects of youth exercise into adulthood [18,38,39]. These discrepancies may be related to the no-training-period modality used in our study. Indeed, during this 75-week deconditioning period, animals were housed in free cage activity. One could also suppose that their level of activity was sufficient to maintain the exercise-induced bone gains, contrary to what one could have observed using restricted cage activities. In 2000, Yao et al [40] showed that a simple daily activity, such as bipedal stance for feeding or drinking, partially prevented castration-induced bone loss and increased bone mass in intact male rats. Thus, further studies should focus on evaluating the minimal level of activity needed to maintain exercise-induced bone benefits.

The current results showed that dietary intake of *Humulus lupulus* L powder (100 g/kg diet) decreased fecal Ca excretion compared with control diet but did not modify markers of bone turnover (OC and DPD) or BMD values. Moreover, it had no effect on urinary Ca, net Ca absorption, Ca balance, plasma Ca, right tibia Ca content, and uterine relative weight. Contrary to what we expected, the 100-week hops supplementation did not improve bone status. In fact, the bone effect of PE might vary according to the menopause status, a proxy for endogenous hormonal milieu. Given that PE receptor interactions will necessarily compete with those of the cognate ligand, the result will likely depend in part on concentrations of endogenous sex steroids. Indeed, the bone-sparing effect of PE has been consistently demonstrated in the ovariectomized rat. As an example, using ovariectomized rats as osteoporosis model, Kondo [24] showed that the femoral bone loss caused by ovariectomy was significantly inhibited by 4 weeks of beer consumption, whereas no inhibitory effect was seen with beer brewed without hops, suggesting that the active ingredients in beer were derived from hops. In addition, other authors demonstrated that specific PE compounds found in hops extracts exerted estrogen-like activities on bone metabolism [27,41,42]. Our results are also in disagreement with the aforementioned studies. One could suppose that the supplementation imposed to our estrogen-replete rats was inadequate to produce beneficial effects on bone. However, despite an estrogen-depleted state, the findings in the study by Grainge et al [43] do not show a beneficial effect of heavy beer drinking on BMD in postmenopausal women. The high Ca intake of 1% to 1.4% in this study may have attenuated or prevented the ability to find significant effects of *Humulus lupulus* L enrichment. This suggests that our findings might be different if Ca intake was near the nutritional recommended daily intake. On the other hand, it is noteworthy that body weight was significantly lower in rats fed with *Humulus lupulus* L diet than in the control groups. Therefore, we could suggest that this phenomenon would have also attenuated the ability to find positive effect of *Humulus lupulus* L on bone because lower body weight is typically associated with less bone mass. To our knowledge, no study reported such results. Thus, further investigations are needed to confirm that *Humulus lupulus* L diet could be important in the management of body weight.

Finally, our results showed that isometric strength training combined with a dietary supplementation with *Humulus lupulus* L decreased fecal Ca excretion but modified neither other Ca metabolism parameters nor bone markers and BMD of old female rats. To our knowledge, hops-derived PE have never been used in association with exercise training for optimally increasing bone mass and preventing osteoporotic fractures. Nevertheless, because studies of animal models have demonstrated that the effectiveness of exercise for increasing bone mass in female subjects can be reduced if estrogens levels are diminished [44,45], some authors reported that the combination of estrogens and exercise may be more effective in increasing BMD in older women as compared with either treatment alone [46-48]. However, while debate over the risks and benefits of estrogen replacement is ongoing, other estrogen-like plant compounds such as PE may be preferred to combine with exercise training. Four animal studies to date indicated that the combined intervention of moderate exercise and genistein or soybean isoflavone administration showed an additive effect in preventing osteoporosis-related bone loss [49-52]. On the other hand, in postmenopausal women, recent investigation [53] suggested that intervention of isoflavones combined with walking did not show any efficacy on BMD. Here, it should also be noticed that in these later studies, the authors examined the cooperative effects of exercise training and predominant PE found in plants such as genistein and daidzein. Thus, the lack of literature data available on the effects of combined intervention of exercise training and dietary *Humulus lupulus* L on bone metabolism makes the comparison between our results with those of other authors very difficult.

To conclude, this study shows for the first time that isometric strength training performed during the first part of life, followed by a prolonged no-exercise period, induced positive effects on markers of bone turnover and BMD in old female rats. It suggests that bone gains induced by exercise do not decrease immediately after cessation of training and also confirms the importance of the practice of physical activity during puberty and young adulthood to maximize the achieved peak bone density and consequently to limit age-related bone loss and lower fracture risk for the future. Finally, diet rich in *Humulus lupulus* L alone or combined with isometric strength training does not seem to play an important role in prevention of age-related bone loss. Because phenolic compounds derived from hops and malts present in beer may have some health benefits, further investigations are needed to thoroughly evaluate the potential effects of long-term *Humulus lupulus* L consumption on bone metabolism.

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