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Normal masticatory function partially protects the rat mandibular bone from estrogen-deficiency induced osteoporosis

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ABSTRACT

Background/aim: In a previous study we showed that mandibular alveolar (trabecular) bone appears to be less sensitive to estrogen deficiency than the proximal tibia spongiosa. We hypothesized that the mechanical loading of the alveolar process during mastication may protect the alveolar bone from the detrimental effects observed in other skeletal sites. To test this hypothesis we compared the effect of ovariectomy on the mandibular alveolar bone and the proximal tibia spongiosa of rats fed either a normal (hard) or a soft diet.

Methods: Forty six-month-old female Sprague–Dawley rats underwent trans-abdominal ovariectomy (OVX) or sham operation (SHAM). Half of the animals received their food in the usual form of pellets (hard consistency), while the other half received a soft, porridge-like, isocaloric diet of identical composition (soft consistency). Micro-computed tomographic histomorphometry was used to evaluate the trabecular micro-architecture. A two-factor analysis of variance was used to test for effects and interaction of ovariectomy and/or soft diet.

Results: OVX had a significantly negative effect on the proximal tibia spongiosa (all parameters under study except trabecular thickness; $p < 0.001$) and on the mandibular alveolar bone (trabecular number and spacing; $p < 0.05$). Soft diet led to a further decrease of mandibular BV/TV ($p < 0.01$), trabecular thickness ($p < 0.05$) and number ($p < 0.05$), as well as increase of separation ($p < 0.001$). A significant interaction was observed between OVX and soft diet concerning the mandibular BV/TV, as well as trabecular thickness and spacing ($p < 0.05$).

Conclusion: Normal (hard) diet limited significantly the negative effects of estrogen deficiency on mandibular alveolar bone micro-architecture four months after ovariectomy.

1. Introduction

Ovariectomy (OVX) in the rat results in bone loss and micro-architecture deterioration at various skeletal sites containing trabecular bone (Ammann et al., 1996) and is the most commonly used animal model of post-menopausal osteoporosis (for a review see Turner et al., 2001). As far as mandibular bone is concerned, there are some studies that have demonstrated a negative effect of OVX on the mandibular body (Elovic et al., 1995), condyle (Tanaka et al., 2000; Sakakura et al., 2001), and the alveolar process (bone surrounding the teeth) (Hara et al., 2001; Tanaka et al., 2003). However, many studies have failed to detect any effect or concluded that the effect of OVX on the mandibular condyle (Fujita et al., 2001; Yamashiro and Takano-Yamamoto, 1998; Tanaka et al., 1999) or the alveolar process (Johnson et al., 2002; Jiang et al., 2003) was significantly weaker in comparison to the long bones. Rat strain and site of measurement might partially explain these discrepancies (DeMoss and Wright, 1998).

Mastication is the first step in the process of digestion and is meant to prepare the food for swallowing and further processing in the digestive system (Van der Bilt et al., 2006). During mastication the bite force is regulated by central control mechanisms and sensory feedback from peripheral mechanoreceptors according to the size and the texture of food (Woda et al., 2006; Kohyama et al., 2003; Van der Bilt, 2010) in order to produce a smooth and efficient break down of the bolus before swallowing (Peyron et al., 2004; Mishellany et al., 2006). Food hardness is sensed during mastication and affects the masticatory force (Kohyama et al., 2004), the jaw muscle activity (Agrawal et al., 1998), and the

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mandibular functional movements (Peyron et al., 1997). Animal experimental studies have shown that changes of the masticatory muscle function induced by soft diet lead to a reduction of masticatory muscle strength and functional capacity (Kiliaridis and Shyu, 1988). The masticatory muscles have broadly distributed origins and insertions on the skull and mandible, while most muscle fibers attach to internal aponeuroses rather than to the bones directly (Herring, 2007). Masticatory hypofunction has been shown to result in changes of the mandibular morphology in both growing (Mavropoulos et al., 2004a, 2004b) and adult (Mavropoulos et al., 2004a, 2004b) rats.

There is significant evidence that the association between systemic and oral bone loss is very weak (Kribbs et al., 1989; Krall et al., 1996; Streckfus et al., 1997; Jeffcoat et al., 2000; Bollen et al., 2004; Wactawski-Wende et al., 2005; Ejiri et al., 2008). In a previous study we demonstrated that mandibular alveolar (trabecular) bone is less sensitive than the proximal tibia to estrogen-deficiency induced osteoporosis in the rat (Mavropoulos et al., 2007). We hypothesized that this might be due to the mechanical loading of the mandibular bone during mastication, which has been shown to influence the mandibular bone density and micro-architecture in the growing (Mavropoulos et al., 2004) and the adult rat (Mavropoulos et al., 2010). To test this hypothesis we compared the effect of estrogen depletion (ovariectomy) and reduced masticatory loading (soft diet), as well as their interaction, on the mandibular alveolar bone and the proximal tibia spongiosa in adult rats.

2. Materials and methods

2.1. Animals and protocol

Forty six-month-old female Sprague–Dawley rats (Novartis, Basel, Switzerland) underwent trans-abdominal ovariectomy (OVX) or sham operation (SHAM), and were pair-fed isocaloric hard or soft diet (Novartis Nutrition, Bern, Switzerland). For half of the animals the food was given in the usual form of pellets (hard consistency), while for the other half it had a soft, porridge-like, consistency. The experiment lasted 16 weeks. Body weight was recorded every week. Blood was collected at the tip of the tail and 12 hours urine collections were obtained at the end of the study in metabolic cages. The study received the approval of the Animal Ethics Committee of the Faculty of Medicine of the University of Geneva.

2.2. Microtomographic histomorphometry by microcomputed tomography (μCT)

Micro-computed tomographic histomorphometry of the secondary spongiosa of the proximal tibia and the mandibular alveolar process was performed with a high-resolution μCT system (μCT 40; Scanco Medical AG, Bassersdorf, Switzerland) as previously described (Muller et al., 1996; Mavropoulos et al., 2004a, 2004b). Three-dimensional images of each proximal tibia and hemi-mandible were acquired with a voxel size (nominal resolution) of 16 μm in all spatial directions. Samples were secured in a cylindrical sample holder in air. Resolution was set to medium (500 projections with 1024 samples each). The trabecular and cortical parts of the tibia were separated with semi-automatically drawn contours. For the rat hemi-mandible (Fig. 1A), the volume of interest (VOI) was drawn on a slice-based method starting from the first slice containing the crown of the first molar and moving dorsally one hundred slices in the area of the alveolar process between the roots of the molars and the root of the incisor. Trabecular bone was carefully contoured on the first and the last slice, while the intermediate slices were first interpolated by morphing (Fig. 1B). Each slice was subsequently visually inspected and the contour was modified where necessary.

Micro-tomographic histomorphometric indices were calculated directly from the binarized VOI. Total volume (TV) was the volume of the whole sample examined. Bone volume (BV) and surface (BS) were calculated using tetrahedrons corresponding to the enclosed volume of the triangulated surface. Mean trabecular thickness (Tb.Th.) was determined from the local thickness at each voxel representing bone. Trabecular number (Tb.N.) was calculated by taking the inverse of the mean distance between the middle axes of the structure. The complete acquiring procedure was repeated a further four times with four mandibles chosen at random to calculate the coefficient of variation (CV; range, 1.57–2.51% for Tb.Th and BV/TV, respectively).

2.3. Biochemical parameters

Plasma osteocalcin and IGF-I were measured using radioimmunoassay with reagents from Biomedical Technologies (Stoughton, MA, USA) for the former and with a kit from Nichols Institute (San Juan Capistrano, CA, USA) after extraction by acid-ethanol and cryoprecipitation for the latter. Total urinary deoxypyridinoline was determined after acid hydrolysis using a kit from Metra-Biosystems (Mountain View, CA, USA).

2.4. Statistical analysis

All data are presented as mean and standard error of the mean (in parentheses). A two-way analysis of variance was employed to investigate the main effects of both experimental factors (OVX and soft diet) as well as their interaction. All statistical analyses were performed using the SPSS statistical package (SPSS 19.0.0, SPSS Inc., IBM Corp., USA). A result was considered as statistically significant at $p < 0.05$.

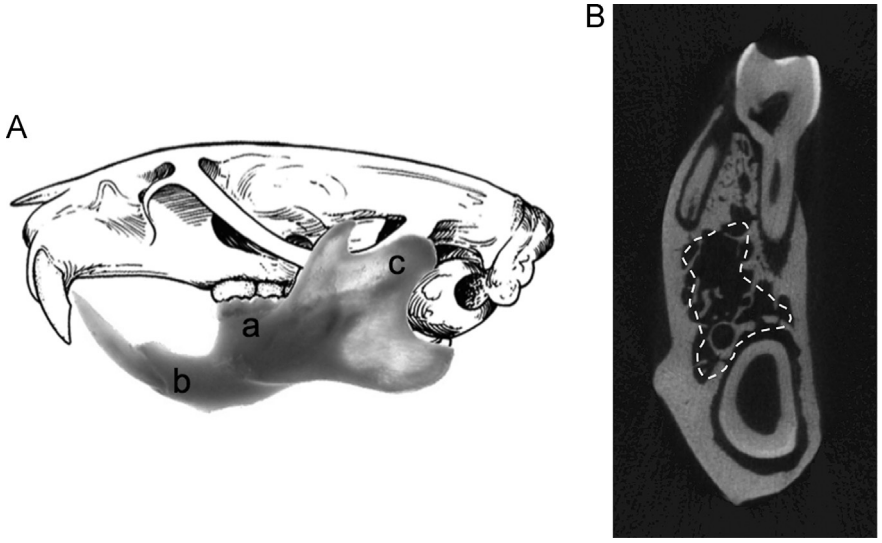

Fig. 1. (A) Lateral photograph of a rat mandible superimposed on a drawing of the skull (a) the alveolar process that houses the molars and is the equivalent of the alveolar process in humans; (b) not to be confused with the incisor alveolar process, which does not exist in the human mandible; and (c) the condylar process, which articulates with the skull, and (B) micro-CT scan (the dashed line represents the volume of interest (VOI) as it appears in one layer).

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