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Mechanical mandible competence in rats with nutritional growth retardation

Christian Esteban Lezón[a], Patricia Mabel Pintos[a], Clarisa Bozzini[a], Alan Agüero Romero[a], Patricia Casavalle[b,c], Silvia María Friedman[b], Patricia Mónica Boyer[a,*]

[a] Department of Physiology, School of Dentistry, University of Buenos Aires, Buenos Aires, Argentina
[b] Department of General and Oral Biochemistry, School of Dentistry, University of Buenos Aires, Buenos Aires, Argentina
[c] Department of Pediatrics, Nutrition Clinic, Clinical Hospital José de San Martin, School of Medicine, University of Buenos Aires, Buenos Aires, Argentina

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ABSTRACT

Objective: In order to provide a better understanding of the sympathetic nervous system as a negative regulator of bone status, the aim of the study was to establish the biomechanical mandible response to different doses of a β-adrenergic antagonist such as propranolol (P) in a stress-induced food restriction model of growth retardation.
Methods: Rats were assigned to eight groups: Control (C), C + P3.5 (CP3.5), C + P7 (CP7), C + P14 (CP14), NGR, NGR + P3.5 (NGRP3.5), NGR + P7 (NGRP7) and NGR + P14 (NGRP14). C, CP3.5, CP7 and CP14 rats were freely fed with the standard diet. NGR, NGRP3.5, NGRP7 and NGRP14 rats received, for 4 weeks (W4), 80% of the amount of controls food consumed. Propranolol 3.5, 7 and 14 mg/kg/day was injected ip 5 days per week in CP3.5 and NGRP3.5, CP7 and NGRP7, CP14 and NGRP14, respectively. At W4, zoometry, mandible morphometry, static histomorphometric and biomechanical competence were performed.
Results: A dose of Propranolol 7 mg/kg/day induced interradicular bone volume accretion reaching a mandible stiffness according to chronological age.
Conclusion: These findings evidenced that sympathetic nervous system activity is a negative regulator of mandible mechanical competence in the nutritional growth retardation model. Propranolol 7 mg/kg/day, under the regimen usage, seems to be appropriate to blockade SNS activity on mandible mechanical performance in NGR rats, probably associated to an effect on bone mechanostat system ability to detect disuse mode as an error.

1. Introduction

Bone mass and mechanical performance are associated to bone modeling and remodeling mechanisms during vertebrate life, both regulated by signalling and nervous mechanisms (Amling, Takeda, & Karsenty, 2000; Elefteriou, 2008).

Previous studies by others reported sympathetic innervation in bone tissue (Mach et al., 2002) and the presence of adrenergic receptors in osteoblasts and osteoclasts (Togari, 2002). Indeed, bone cells effectors express β-2 adrenergic receptor, which appears to be the main adrenergic receptor in bone cells, although β1, α1B, and α2B-adrenergic receptors could also participate in bone cell function (Bonnet, Pierroz, & Ferrari, 2008; Pierroz et al., 2012).

Mechanical properties in axial and appendicular bones in growing rats are mostly affected by nutritional status. Suboptimal chronic energy intake outcome in physiological, metabolic, cellular and behavioral responses considered as survival advantages of the adaptive response, ensuring energy fluxes to tissues that demand constantly energy and reduce morbo-morbility risk (Lifshitz & Moses, 1988; Friedman et al., 2006). Moreover, food restriction affected negatively bone quality. Indeed, previous studies performed in our laboratory showed impaired mechanical femoral and mandible competence, in nutritional growth restricted (NGR) rats. This could be the consequence of altered bone mass and architectural distribution rather than its intrinsic quality (Boyer et al., 2005; Compagnucci et al., 2005).

The nutritional stress model in growing male rats was developed by a 20% restricted balanced diet given for a long time. This model closely resembles the nutritional status in childhood

* Corresponding author at: Department of Physiology, School of Dentistry, University of Buenos Aires, Marcelo T. de Alvear 2142 3 A (C1122 AAH), Buenos Aires, Argentina.
E-mail address: pboyer59@hotmail.com (P.M. Boyer).

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which consume diets with insufficient total energy to sustain normal growth and weight gain (Friedman et al., 2006).

Considering hypothalamus as a centre involved in physiological, metabolic, autonomic, neuroendocrine and behavior functions, malnutrition during high growth rate velocity could outcome in different morpho-functional brain dysfunctions (Soto-Moyano, Belmar, Perez, Ruiz, & Hernandez, 1995); severity, that depends on the time of onset and/or the duration and degree of food restriction. Previously, we demonstrated that global sympathetic hypothalamic activity is enhanced in NGR rats (Olivera et al., 2008). Moreover, corticosterone serum levels were about 300% higher in NGR rats as compared to control rats, suggesting an increase in hypothalamic pituitary adrenal (HPA) axis activity (unpublished data). The impaired biomechanical bone performance observed in NGR rats (Boyer et al., 2005; Compagnucci et al., 2005) could be due, at least in part, to the overall increase of hypothalamic noradrenergic system and HPA axis activities, in response to mild chronic food restriction (Olivera et al., 2008).

Furthermore, β-adrenergic antagonist administration to NGR rats enhanced mechanical effectiveness of a weight-bearing bone like femur, due to an augment in cortical bone mass and its improvement in spatial distribution (Lezón et al., 2009; Lezón, Pintos, Olivera, Bozzini, & Boyer, 2012; Pintos, Lezón, Bozzini, Friedman, & Boyer, 2013; Lezon et al., 2016; Tasat et al., 2014).

The mandible is not a weight-bearing bone as femur (Mavropoulos, Rizzoli, & Ammann, 2007; Van Eijden, 2000). It is considered as a "load-bearing bone" not influenced by body weight but by the mechanical masticatory loading. Indeed, previous studies by us showed different behavior in mandible and femur, in NGR rats (Compagnucci et al., 2005). In congruence, other authors showed similar results in rats under protein deficiency in quantity or quality (Bozzini, Champin, Alippi, & Bozzini, 2011; Alippi, Picasso, Huygens, Bozzini, & Bozzini 2012).

In order to provide a better understanding of the sympathetic nervous system (SNS) as a negative regulator of bone status, the aim of the study was to establish the biomechanical mandible response to different doses of a β-adrenergic antagonist such as propranolol (P) in a stress-induced food restriction model of growth retardation.

2. Materials and methods

2.1. Animals

Weanling male Wistar rats (mean initial body weight: 48.90 ± 1.60 g) were housed and kept under 12 h light–12 h dark cycles and maintained at 21 ± 1 °C with 50-60% humidity. The experiment was conducted in accordance with the principles and procedures outlined in the National Institute of Health Guide Lines for the Care and Use of Laboratory Animals (National Institute of Health, 1985, revised 1990National Institute of Health, 1985National Institute of Health, 1985, revised 1990) and approved by the University of Buenos Aires Ethic Committee.

2.2. Diet

Animals were fed with a standard diet (Purina chow, Gilardoni SA, Buenos Aires, Argentina) of the following composition (g/100 g): protein, 22.7; lipids, 7.09; fiber, 6.0; Ca, 1.3; P, 0.8; ashes, 6.50; water, 7.60; dextrin, balance.

2.3. Experimental design

Rats were randomly assigned to eight groups: Control (C), C + P3.5 (CP3.5), C + P7 (CP7), C + P14 (CP14), NGR, NGR + P3.5 (NGRP3.5), NGR + P7 (NGRP7) and NGR + P14 (NGRP14). C, CP3.5, CP7 and CP14 rats were fed freely with the standard diet. NGR, NGRP3.5, NGRP7 and NGRP14 rats received, for 4 weeks (W4), 80% of the amount of food consumed the previous day by their respectively control groups, corrected by body weight. All rats had free access to water. Propranolol (Richmond Laboratory, Buenos Aires, Argentina) 3.5, 7 and 14 mg/kg/day was injected ip 5 days per week, for four weeks in CP3.5 and NGRP3.5, CP7 and NGRP7, CP14 and NGRP14, respectively. C and NGR received saline injections at an identical dosage regimen. Propranolol regimen administration and doses concentration were chosen according previous studies (Takeda et al., 2002). C and NGR received saline injections at an identical dosage regimen. Body weight and dietary intake were recorded daily, and body length, every 4 days. A Mettler PC 4000 scale (Zurich, Switzerland) was used to measure body weight with an accuracy of ±1 mg. For length measurements, animals were anaesthetized light anesthesia (a mixture of 2% xylazine hydrochloride (0.5 mg/100 g i.p. Konig Laboratories, Buenos Aires, Argentina)) and 5% ketamine hydrochloride (5 mg/100 g i.p. Holliday-Scott SA. Buenos Aires, Argentina). Body length was determined with a scaled ruler in mm from the nose tip to the last hairs of the tail base. Food consumption was measured by using special feeders, which allowed the recovery of spilled food. Food intake was weighed daily with a Mettler scale (accuracy ±1 mg). Animals were euthanized under anesthesia: 0.1 ml of ketamin hydrochloride (100 mg/ml, Holliday Lab., Buenos Aires, Argentina)/100 g body weight was mixed with 0.02 ml of xylazine (100 mg/ml, Konig Lab, Buenos Aires, Argentina)/100 g body weight by intramuscular injection, after 4 weeks of experimental period (W4). The hemimandibles from each animal were then dissected, cleaned of adhering soft tissue, weighed in a Mettler scale and stored at −20 °C wrapped in gauze soaked with Ringer's solution in sealed plastic bags, in accordance with Turner and Burr (1993). Each bone was thawed at room temperature before analysis. Mandibular growth was estimated directly on the right hemimandible by taking measurements (to the nearest 0.05 mm) by the use of digital callipers according to Eratalay, Simmonds, Mofty, Rosenberg, and Nelson (1981) with some modifications (Alippi, Meta, Boyer, & Bozzini, 1999). A Mettler PE 600 scale (Zurich, Switzerland) was used to measure mandible weight expressed in g. Bones were used for mechanical and for histomorphometric studies.

2.4. Mandibular morphometric properties

Mandibular growth as a whole was estimated directly from mandibular weight and area on the right mandible (to the nearest 0.05) by using a digital calliper according to Eratalay et al. (1981) and modifications (Alippi et al., 1999) (Fig. 1) as follows: (1) mandibular area was calculated from a triangle formed between three stable points: the most superior posterior point of the coronoid process (B), the most posterior point of the angular process (C), and the most anterior inferior bone point of the interdental spine (O); (2) mandibular length was measured from the most anterior inferior bone point of the interdental spine to the furthest point on the articular surface of condyle (length OA); (3) the length of the base of the jaw was estimated by the distance OC; (4) length of incisor alveolar process: distance from the most anterior superior bone point of the interdental spine (i) to the point immediately anterior to the anterior surface of the first molar (K) (5) length of alveolar process: measured from K to the alveolar process immediately posterior to the posterior root of the third molar (L). The mandibular length was divided into anterior (KL + Ki) and posterior (OA-anterior part) parts by a vertical line drawn perpendicular to the oclusal plane of the molars immediately posterior to the posterior surface of the third molar. These specific measurements were chosen because they give information

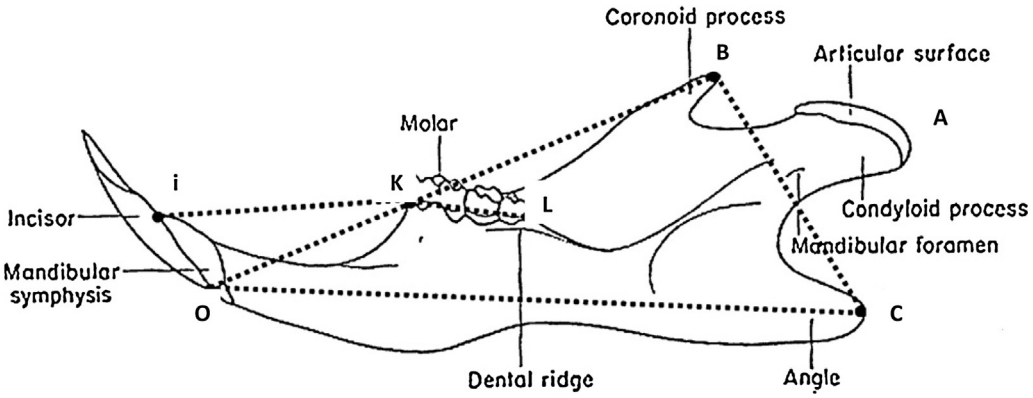

Fig. 1. Medial aspects of the right hemimandible showing the bony points between which measurements were taken.
A: the most posterior point of the condyloid process;
B: the most superior point of the coronoid process;
C: the most posterior point of the angular process;
I: the most anterior superior bone point of the interdental spine;
K: bone point on the alveolar process immediately anterior to the anterior surface of the first molar;
L: bone point immediately posterior to the posterior surface of the third molar;
O: the most anterior inferior bone point of the interdental spine.

on the growth of the bone as a whole without considering its morphological units (Moore, 1973).

Only one calibrated observer performed mandible morphometric measurements and the coefficient of variation (CV) was 3.397 ± 2.341 (mean $\pm$ SD) and CI95: 2.364–5.509.

2.5. Mandible histomorphometry

The left hemimandible of each animal was resected, fixed in 4% buffered formalin at room temperature, decalcified in 10% ethylenediaminetetraacetic acid (EDTA) pH 7 during 25 days, dehydrated and embedded in paraffin. Mesio-distally oriented sections of the lower first molar were obtained and stained with hematoxylin-eosin. Histomorphometric evaluation of the decalcified bone section was performed on digitalized microphotographs by employing an Olympus Photomicroscope CX31, Infinity 1 Camera and Infinity Software 5.0.3. Interradicular bone volume was expressed as% of total bone volume (BV/TV; %).

2.6. Biomechanical tests on mandible

Mechanical properties of the rat hemimandible were determined using a three-point bending mechanical test (Hogan, Groves, & Sampson, 1999). Each bone was placed on two lowers supports (11 mm span) with the lateral aspect facing down and centred along its length. Loads were applied transversally to the bone axis at a point immediately posterior to the posterior surface of the third molar. The test machine (Instron model 4442, Instron Corp., Canton, MA, USA) was operated in stroke control at a rate of 5.00 mm/min, which is useful to describe the static properties of the bone structure. The plots of load *v.* deformation (W/d) obtained were analysed to determine the following structural mechanical properties: Load at fracture (Wf, N) that represents the value of the load at fracture and expresses directly the resistance of the whole bone to fracture, incorporating both the elastic and the plastic behaviours; Load at yielding (Wy, N) that represents the end point of the elastic deformation (yielding point) and defines a threshold

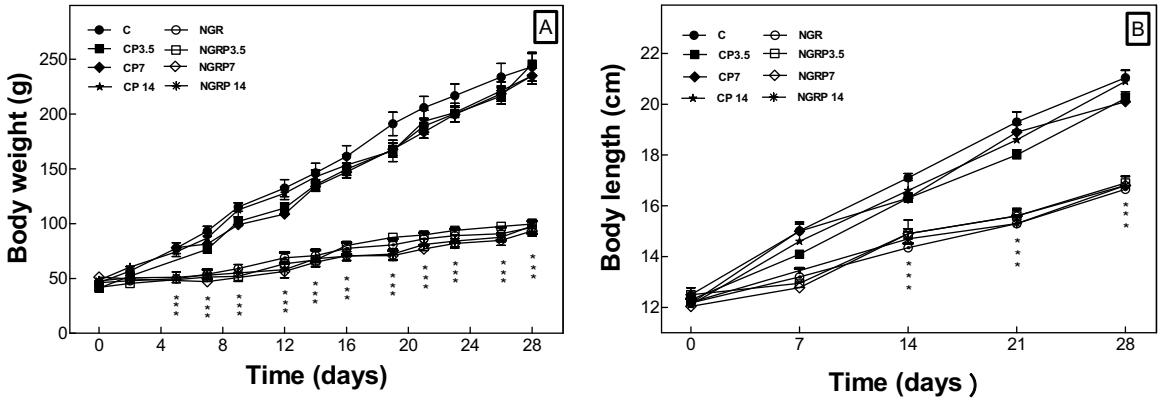

Fig. 2. (A) Body weight over time in control (C), control + propranolol 3.5 mg/kg/day (CP3.5), control + propranolol 7 mg/kg/day (CP7), control + propranolol 14 mg/kg/day, nutritional growth retardation (NGR), NGR + propranolol 3.5 mg/kg/day (NGRP3.5), NGR+ propranolol 7 mg/kg/day (NGRP7),NGR + propranolol 14 mg/kg/day (NGRP14) groups.
Mean values and SD for 10 animals per group. One way ANOVA and Student-Newman-Keuls test were performed at every four days among all groups.
***: indicates significant differences between NGR, NGRP3.5, NGRP7, NGRP14 vs. C, CP3.5, CP7, CP14, respectively ($p < 0.001$).
(B) Body length over time in control (C), control + propranolol 3.5 mg/kg/day (CP3.5), control + propranolol 7 mg/kg/day (CP7), control + propranolol 14 mg/kg/day, nutritional growth retardation (NGR), NGR + propranolol 3.5 mg/kg/day (NGRP3.5), NGR + propranolol 7 mg/kg/day (NGRP7), NGR + propranolol 14 mg/kg/day (NGRP14) groups.
Mean values and SD for 10 animals per group. One way ANOVA and Student-Newman-Keuls test were performed at every seven days among all groups.
***: indicates significant differences between NGR, NGRP3.5, NGRP7, NGRP14 vs. C, CP3.5, CP7, CP14, respectively ($p < 0.001$).

Table 1
Morphometry and static histomorphometry of bone mandible.

Parameter	C	NGR	CP3.5	NGRP3.5	CP7	NGRP7	CP14	NGRP14
Hemimandible weight (g)	0.38 ± 0.01^{a}	0.28 ± 0.02^{b}	0.36 ± 0.01^{a}	0.28 ± 0.01^{b}	0.37 ± 0.01^{a}	0.30 ± 0.01^{b}	0.35 ± 0.01^{a}	0.26 ± 0.01^{b}
Mandible area (mm^2)	104.14 ± 2.13^{a}	83.39 ± 1.27^{b}	106.30 ± 2.86^{a}	86.20 ± 2.55^{b}	103.16 ± 2.97^{a}	89.00 ± 1.85^{b}	105.67 ± 2.45^{a}	86.76 ± 1.91^{b}
Mandible length (mm)	23.71 ± 0.32^{a}	21.70 ± 0.14^{b}	23.10 ± 0.18^{a}	21.70 ± 0.21^{b}	23.11 ± 0.21^{a}	21.88 ± 0.24^{b}	23.26 ± 0.37^{a}	21.33 ± 0.28^{b}
Mandible anterior part (mm)	13.76 ± 0.15^{a}	13.31 ± 0.12^{a}	13.50 ± 0.09^{a}	13.30 ± 0.16^{a}	13.71 ± 0.14^{a}	13.28 ± 0.11^{a}	13.76 ± 0.09^{a}	13.32 ± 0.16^{a}
Mandible posterior part (mm)	9.88 ± 0.27^{a}	8.40 ± 0.16^{b}	9.70 ± 0.15^{a}	8.30 ± 0.19^{b}	10.09 ± 0.11^{a}	8.51 ± 0.16^{b}	9.77 ± 0.16^{a}	8.01 ± 0.20^{b}
BV/TV (%)	44.0 ± 1.50^{a}	32.5 ± 1.30^{b}	42.9 ± 1.50^{a}	34.1 ± 1.20^{b}	42.1 ± 2.40^{a}	43.3 ± 2.80^{a}	44.8 ± 2.60^{a}	33.7 ± 1.53^{b}

Control (C), control + propranolol 3.5 mg/kg/day (CP3.5), control + propranolol 7 mg/kg/day (CP7), control + propranolol 14 mg/kg/day, nutritional growth retardation (NGR), NGR + propranolol 3.5 mg/kg/day (NGRP3.5), NGR+ propranolol 7 mg/kg/day (NGRP7), NGR + propranolol 14 mg/kg/day (NGRP14) groups.
BV/TV (%): Bone volume (BV/TV) was measured in the interradicular region of the first lower molar (B). H&E, 40×. Arrows show the alveolar bone.
Mean values and SD for 7 animals per group.
Different letters mean significant differences between groups ($p < 0.001$).

about which unrecoverable permanent deformation occurs, with the first appearance of the first microcracks that occur on the periosteal surface of the bone; Yielding deformation (dy, mm) at the yielding point, and structural stiffness or bone rigidity that represents the rigidity of the bone or the resistance to deformation (Wy/dy, N/mm).

Geometric properties were also measured. They are: (1) bone length and diameters; (2) cross-sectional area (CSA, mm^2): using an Isomet low-speed diamond saw (Buheler, Lake Bluff, IL, USA) the fracture section was regularised to perform micromorphometrical determinations of the vertical (load direction) and horizontal (right angle to load direction) outer (VOD, HOD) and inner (VID,

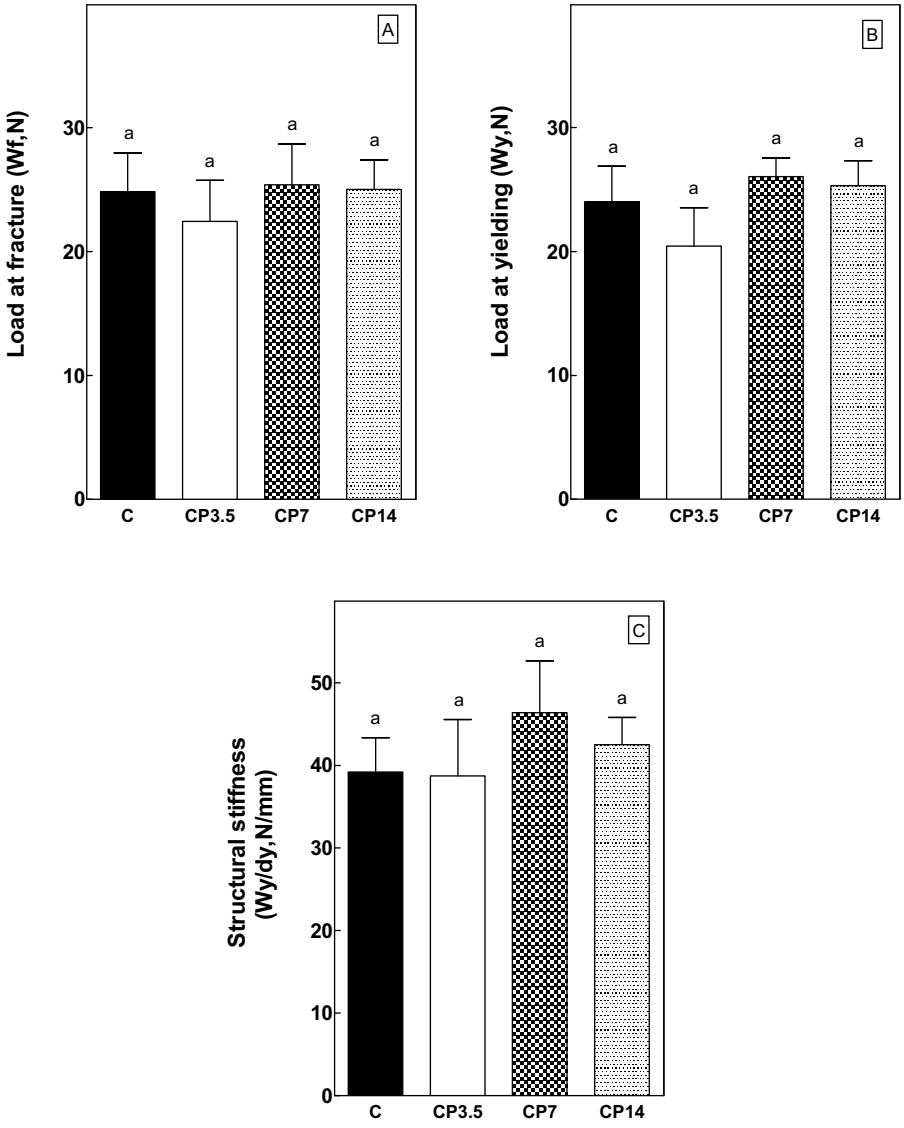

Fig. 3. Mandible structural properties in control groups.
Load at fracture (A), Load at yielding (B) and Structural stiffness (C) of the mandible of control (C), control + propranolol 3.5 mg/kg/day (CP3.5), control + propranolol 7 mg/kg/day (CP7), control + propranolol 14 mg/kg/day (CP14) groups. Mean values and SD for 10 animals per group. Different letters mean significant differences between groups ($p < 0.05$).

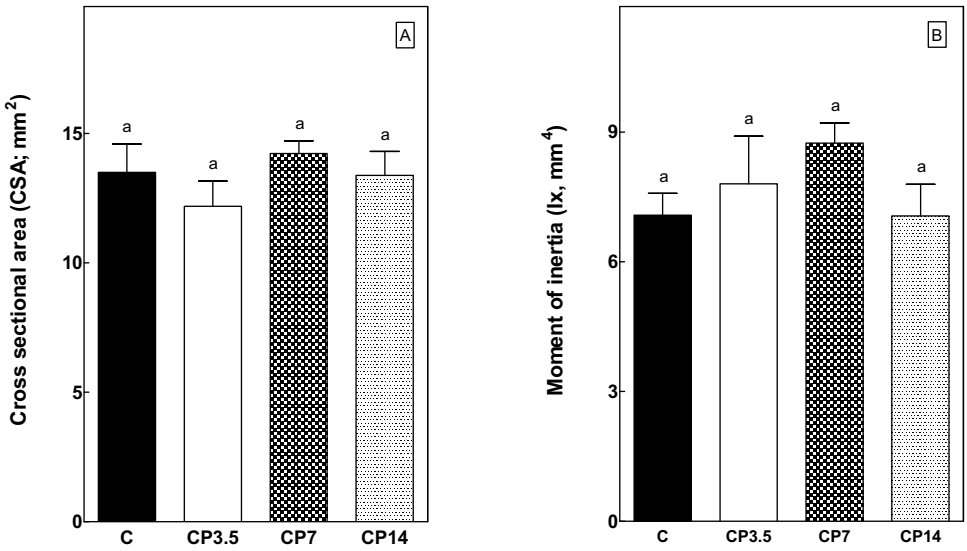

Fig. 4. Mandible geometrical properties in control groups.
Cross sectional area (A) and Moment of inertia (B) of the mandible of control (C), control + propranolol 3.5 mg/kg/day (CP3.5), control + propranolol 7 mg/kg/day (CP7), control + propranolol 14 mg/kg/day (CP14) groups. Mean values and SD for 10 animals per group. Different letters mean significant differences between groups ($p < 0.05$).

HID) diameters of the fracture sections. Measurements were taken directly using a stereomicroscope (Stenu DV4, Carl Zeiss Microimagen, Gottingen, Germany) with an accuracy of ± 0.001 mm. CSA was calculated by applying the equation: CSA = 3.14 (VOD × VID − HOD × HID)/4. (3) moment of inertia of cortical bone (with reference to the anterior-posterior bending axis, Ix, mm^4) as estimated by the equation: Ix = (3.14 [$VOD^3 \times HOD - VID^3 \times HID$/64]). I× captures both bone mass and distribution on the cross section. Bone material properties (intrinsic properties of the mineralised tissue) were calculated from structural and geometric properties as follow: Young's modulus or modulus of elasticity (E, mm^2) or bone material stiffness, calculated by the formula: $E = WyL^3/48\,dy\,Ix$ (Wy = load at the yielding point, L = distance between supports, dy = yielding deformation or maximal stress deflection, Ix = moment of inertia of the cross-section in relation to the horizontal axis).

2.7. Statistical analysis

Results are expressed as mean ± standard error of the mean (SEM). Data were analysed by one-way ANOVA followed by the Student–Neuman–Keuls test. Differences were considered significant if $p < 0.05$ (Sokal & Rohlf, 1994). Statistical analysis was performed with the Graphpad Prism (version 5.0) statistical package (Graphpad Software, San Diego, CA, USA).

3. Results

As shown in Fig. 2A and B, food restriction induced a highly significant ($p < 0.01$) negative effect on body growth in NGR, NGRP3.5, NGRP7 and NGRP14 rats as compared to their respectively control groups. Like body size, mandibular weight, length and area (an index of mandibular size) were significantly lower in all food-restricted groups at the end of the experimental period (Table 1) ($p < 0.001$).

When the length of the bone was divided into an anterior and posterior part by a vertical line drawn immediately posterior to the posterior surface of the third molar, only the posterior part was negative affected in food-restricted rats treated or untreated with P ($p < 0.001$). Nevertheless, anthropometric and bone morphometric parameters were no affected by P (Fig. 2 and Table 1, respectively) ($p > 0.05$).

Interradicular bone of the first lower molar at w4 is shown in Table 1 and in microphotographs. Static histomorphometric assessment showed a significant decrease in bone volume in NGR as compared to C group ($p < 0.01$). BV/TV (%) was found to increase 33.2% in NGRP7 group as compared to NGR rats ($p < 0.01$), reaching C and CP7 values. However, NGRP3.5 and NGPR14 rats showed no significant differences as compared to NGR group ($p > 0.05$). Propranolol had no effects on bone volume in control animals at any of the given doses use.

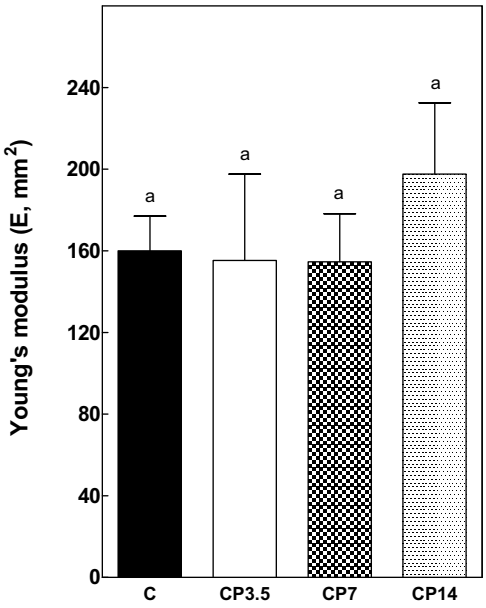

Fig. 5. Mandible material property in control groups.
Young's modulus of the mandible of control (C), control + propranolol 3.5 mg/kg/day (CP3.5), control + propranolol 7 mg/kg/day (CP7), control + propranolol 14 mg/kg/day (CP14) groups. Mean values and SD for 10 animals per group. Different letters mean significant differences between groups ($p < 0.05$).

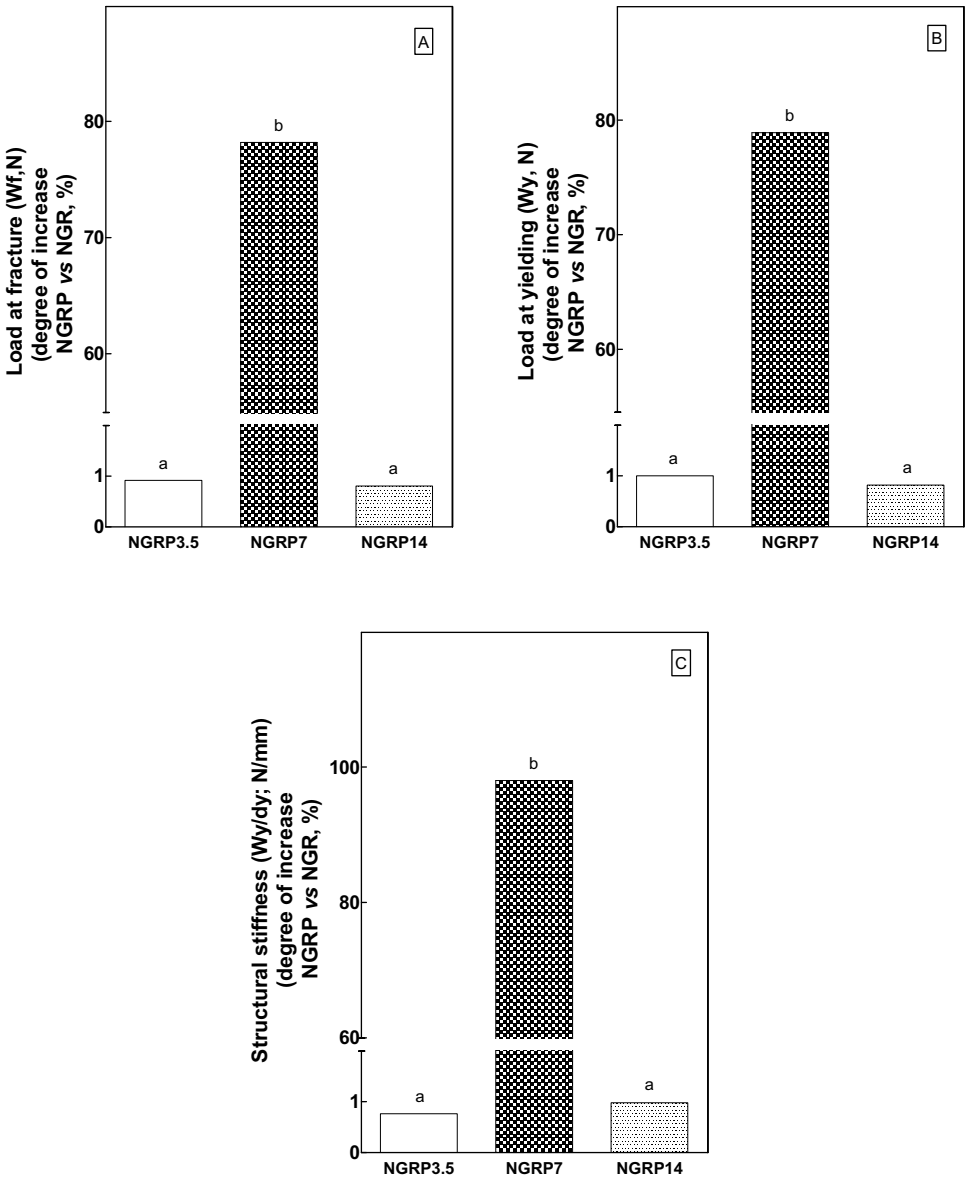

Fig. 6. Mandible structural properties in experimental groups.
Load at fracture (A), Load at yielding (B) and Structural stiffness (C) of the mandible of nutritional growth retardation (NGR), NGR + propranolol 3.5 mg/kg/day (NGRP3.5), NGR + propranolol 7 mg/kg/day (NGRP7), NGR + propranolol 14 mg/kg/day (NGRP14) groups expressed as percentage of increase respect to NGR. Mean values and SD for 10 animals per group. Different letters mean significant differences between groups ($p < 0.01$).

In well-nourished rats, β-blocker did not modify biomechanical competence parameters when propranolol was administered at any of the doses use in the present study as compared to C (Figs. 3–5). In fact, Wf, Wy, Wy/dy, CSA, I× and E were not affected by P ($p = 0.9095$; $p = 0.4606$; $p = 0.7574$: $p = 0.4290$; $p = 0.4176$ and $p = 0.7944$, respectively).

In food-restricted rats, chronic administration of 7 mg/kg/day of Propranolol induced significant changes in both structural (Fig. 6) and geometrical properties (Fig. 7) which values were significantly higher than NGR rats ($p < 0.01$). However, NGRP3.5 and NGPR14 rats showed no significant differences as compared to NGR group ($p > 0.05$). Moreover, significant differences in material bone quality were not evident between food restricted groups (Fig. 8) ($p = 0.6136$).

4. Discussion

Bone mechanical competence is determined by bone strength and stiffness (Martin, 1991). Frost suggested that bones can self-control their structural stiffness by modeling and remodeling. In fact, bone design can optimise through a permanent re-distribution of the mineralised tissue by a feedback mechanism. This homeostatic regulation of bone stiffness is called "bone mechanostat" (Frost, 1987, 1996).

During growth, nutritional status (Boyer et al., 2005; Ferretti et al., 1988, 1991) and mechanical stimuli (Schoenau and Fricke, 2008) are mainly contributor factors that determine bone quality.

In the present study, food restriction was severe enough to decrease normal growth rate in NGR, NGRP3.5, NGRP7 and NGRP14 rats. In mandible a similar behavior was observed in malnourished groups. Then, both the final mandibular weight and its morphometry were negative affected by food restriction in NGR, NGRP3.5, NGRP7 and NGRP14 rats. When propranolol was administered, no significant differences were observed on anthropo-morphometric bone parameters between CP3.5, CP7 and CP14 *v.* C and NGRP3.5, NGRP7 and NGRP14 *v.* NGR groups, respectively; and within groups.

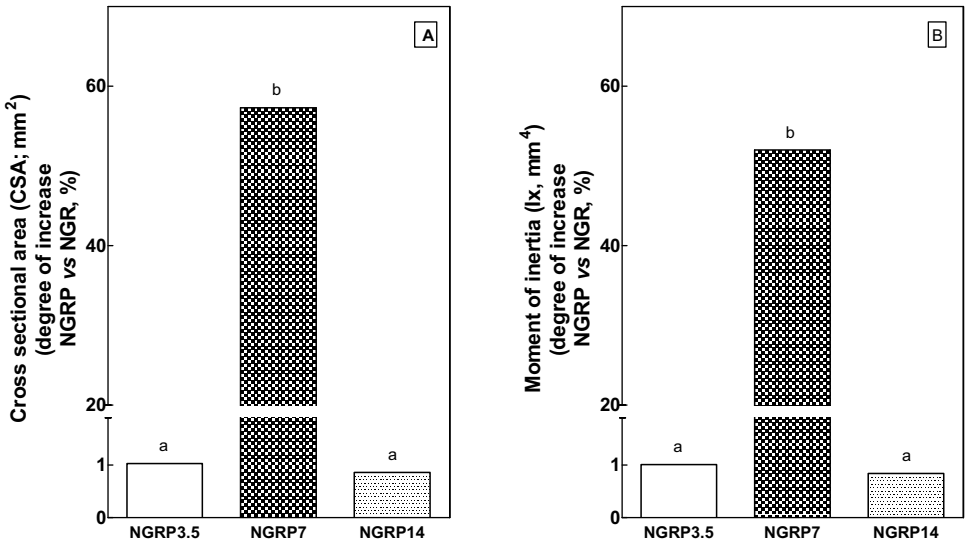

Fig. 7. Mandible geometrical properties in experimental groups.
Cross sectional area (A) and Moment of inertia (B) of the mandible of nutritional growth retardation (NGR), NGR + propranolol 3.5 mg/kg/day (NGRP3.5), NGR + propranolol 7 mg/kg/day (NGRP7), NGR + propranolol 14 mg/kg/day (NGRP14) groups expressed as degree of increase vs NGR (%). Mean values and SD for 10 animals per group. Different letters mean significant differences between groups, where NGR = a ($p < 0.01$).

By drawing a vertical line perpendicular to the oclusal plane of the molars immediately posterior to the posterior surface of the third molar, mandibular length can be partitioned into anterior and posterior parts. The present study started when the growth of the anterior part of the mandible was practically finished (Olivera, Bozzini, Meta, Bozzini, & Alippi, 2003); therefore, no significant differences were encountered between NGR and C rats, consistent with our previous studies (Boyer et al., 2000). Moreover, in well-nourished and food restricted rats treated with P, the incisor alveolar process and alveolar length were not affected during the experimental period. Results that are in agreement with Alippi et al. (1999).

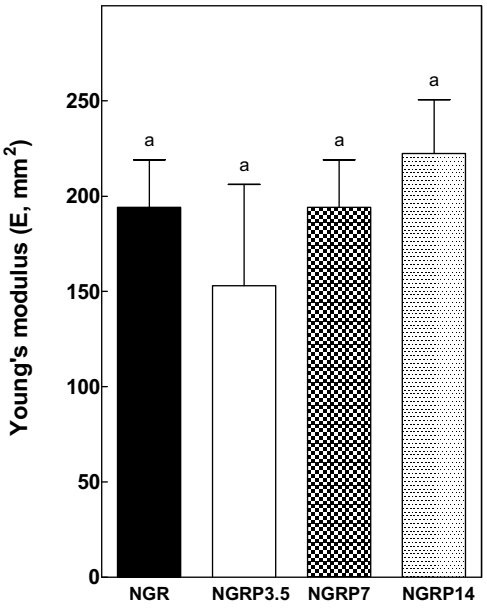

Fig. 8. Mandible material property in experimental groups.
Young's modulus of the mandible of nutritional growth retardation (NGR), NGR + propranolol 3.5 mg/kg/day (NGRP3.5), NGR+ propranolol 7 mg/kg/day (NGRP7), NGR + propranolol 14 mg/kg/day (NGRP14) groups. Mean values and SD for 10 animals per group. Different letters mean significant differences between groups, where NGR = a ($p < 0.05$).

However, the posterior part bone growth was negative affected in NGR rats, and cannot be reverted by P at any dose, indicating that food restriction induced a mandible deformation over age.

Static histomorphometric studies showed that P 7 mg/kg/day rise interradicular bone of the first lower molar, a craniofacial bone formed by intramembranous ossification, in NGRP7 (Nanci, 2003). Since we previously observed that propranolol increase femoral cortical mass without effect on cartilage plate in NGRP7 rats (Lezón et al., 2009), it is possible to hypothesize that β-blocker exert a positive stimulus on intramembranous ossification by bone apposition, under nutritional stress condition.

Additionally, the skeleton structural and mechanical efficiency are controlled by a homeostatic mechanism of bone quality (Frost, 1995).

Because the natural response of the bone mechanostat system is to adequate bone stiffness to mechanical requirement (Frost, 1995, 1996), and it is known that the main physiological loads on mandible are caused by muscle contractions during mastication (Schoenau, 2005), a close relationship between bone strength and muscle force or mass is proposed. Our results suggest that propranolol does not improve bone stiffness in NGRP7 rats throughout an effect on mechanical forces during mastication. In fact, food consumption was similar in both NGR and NGRP7 rats during the experimental period (data not shown). Moreover, at the end of the experiment, there were no differences in masseter mass between NGRP7 and NGR, being both significantly smaller than CP and C, respectively (0.39 ± 0.03 g vs 0.43 ± 0.03 g; 0.74 ± 0.02 g vs 0.70 ± 0.05 g, $p < 0.001$). These results are in congruence with our previous observations of unaffected total lean body mass in NGRP rats as compared to NGR group (Pintos et al., 2013). According to our results, the β-blocker could exert an effect on bone mechanostat functional efficiency through disturbances in endocrine-metabolic environment, modifying the ability of the system to detect disuse mode as an error due to changes in osteoclasts and osteoblasts sensitivity.

On the other hand, propranolol 3.5 mg/kg/day and 14 mg/kg/day did not prevent the negative effect of food restriction on mandible bone mechanical competence. This could be a consequence of an insufficient dose in the former, and in the latter, an inverse agonist activity of the β-blocker and/or changes in adrenoceptor sensitivity.

In summary, these findings evidenced that sympathetic nervous system activity is a negative regulator of mandible mechanical competence in the nutritional growth retardation model. Propranolol 7 mg/kg/day, under the regimen usage, seems to be appropriate to blockade SNS activity on mandible mechanical performance in NGR rats, probably associated to an effect on bone mechanostat system ability to detect disuse mode as an error.

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Ethical approval

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Conflicts of interest

The authors declare no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.archoralbio.2017.03.009.

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