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Biomechanical properties of the mandible, as assessed by bending test, in rats fed a low-quality protein

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ARTICLE INFO

Article history:
Accepted 13 August 2012

Keywords:
Mandible
Wheat gluten
Casein
Growth
Bone biomechanics

ABSTRACT

Objective: The present study describes the effects of feeding growing rats with diets containing increasing concentrations of wheat gluten (a low quality protein, G) on both the morphometrical and the biomechanical properties of the mandible.
Design: Female rats were fed one of six diets containing different concentrations (5–30%) of G between the 30th and 90th days of life. Control rats were fed a diet containing 20% casein (C), which allows a normal growth and development of the bone. Mandibular growth was estimated directly on excised and cleaned bones by taking measurements between anatomical points. Mechanical properties of the right hemimandibles were determined by using a three-point bending mechanical test to obtain a load/deformation curve and estimate the structural properties of the bone. Bone material properties were calculated from structural and geometric properties. The left hemimandibles were ashed and the ash weight obtained. Calcium content was determined by atomic energy absorption. Results were summarised as means ± SEM. Comparisons between parameters were performed by ANOVA and post-test.
Results: None of the G-fed groups could achieve a normal growth performance as compared to the C-fed control group. Like body size, age-related increments in mandibular weight, length, height and area (index of mandibular size) were negatively affected by the G diets, as was the posterior part of the bone (posterior to molar III). The cross-sectional geometry of the mandible (cross-sectional area and rectangular moment of inertia) as well as its structural properties (yielding load, fracture load, and stiffness) were also severely affected by the G diets. However, material properties (Young's modulus and maximum elastic stress) and calcium concentration in ashes and the degree of mineralisation were unaffected.
Conclusions: The differences in strength and stiffness between treated and control rats seemed to be the result of an induced loss of gain in bone growth and mass, in the absence of changes in the quality of the bone mineralised material.

1. Introduction

The skeleton of vertebrates has developed an important property, the resistance to deformation, and indirectly to fracture. Bone strength depends on both the structural and the material properties of bone. Fractures occur when the load on a bone exceeds the ability of the bone to carry that load. They occur when the load applied creates a stress that exceeds the strength of the organ.[1,2] Bones are adapted to the physiological mechanical demands to withstand ordinary stress (body weight, skeletal muscle contraction, masticatory loading) to which skeletal components are subjected.

It is assumed that the "load-carrying behaviour of bone" or "mechanical properties" of bones integrated as organs (*structural properties*) is directly related to both the amount (*bone mass*) and the architectural distribution of the mineralised

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http://dx.doi.org/10.1016/j.archoralbio.2012.08.007

tissue (*geometric properties*), and to the mechanical quality of bone material (*material properties*). The structural properties are the *strength* (assessable as the bone's ability to support loads) and the *stiffness* (measurable as the load/deformation relationship). While structural properties are dependent on bone size and shape, material properties are not. The latter are usually evaluated by assessing two important properties, namely the *stiffness of the mineralised tissue* (Young's modulus of elasticity) and its *maximum elastic stress*.[3,4] These properties are determined by matrix mineralisation as well as by other, mineralisation-unrelated, microstructural factors, such us crystal size and packing and disposition of collagen fibres.[5] The structural stiffness, and indirectly the strength of bones, is thought to be controlled by a "*bone mechanostat*".[6] This is a feedback mechanism that optimises the bone design through a permanent re-distribution of the mineralised tissue.

Both body weight and somatic muscles contractions can be considered as the most important "*mechanical factors*" in the determination of bone strength in the so called "*weight-bearing bones*", such as the axial or appendicular skeletal bones. The mandible is both morphologically and functionally different from the other bones of the axial skeleton. It also arises from a different embryonic germ layer (neuroectoderm) instead of bones of the axial and appendicular, which arise from the mesoderm. It has been shown that the mechanical loading of the mandible during mastication has an impact on the mass, density, and microarchitecture of the mandibular alveolar bone.[7,8] The mandible is not a weight-bearing bone. However, since it is influenced by mechanical masticatory loading, it can be considered as a "*load-bearing bone*" that presents similarities with the weight-bearing bone from the mechanical point of view.

As shown, mechanical factors are the primary ones in the determination of bone strength.[9] However, other "*non-mechanical factors*" also exist that can modulate bone physiology, by either establishing or maintaining the mechanical competence of bones. Dietary protein is one of them. In this sense, we have recently reported[8] that chronic protein malnutrition imposed on rats from infancy to early adulthood induces a significant reduction of strength and stiffness of the mandible that seem to be the result of an induced loss of gain in bone structural properties as a consequence of a correlative loss of gain in both growth and mass, yet not in bone material properties.

It has been repeatedly demonstrated that dietary protein concentration is an important determinant of the body growth rate, as it is the quality of the protein given to experimental animals.[10–19] We have demonstrated recently[20] that the quality of the protein given to growing rats during 60 d is important to determine the structural mechanical properties of the femur shaft (a weight-bearing bone) as it is its concentration in the diet. The present study describes in the same animals used in the prior study the effects of feeding growing rats with diets containing increasing concentrations of wheat gluten (a low quality protein) on the biomechanical properties of the mandible. The effects were compared to those observed in rats fed a diet containing 20%-casein, which allows a normal growth and development of the bone.[21] The main purpose of the study was to establish whether mandibular bone and axial or peripheral skeleton respond similarly from the biomechanical point of view to nutritional factors, as the quality of dietary proteins. Femur is a weight-bearing bone, while the mandible is a "load-bearing bone", not influenced by body weight but by the mechanical loading during mastication.

2. Materials and methods

Seven groups of 7 female Sprague-Dawley rats aged 30 d and weighing about 58 g at the start of the experiment were housed in stainless-steel cages under natural light–dark photoperiod and in a temperature controlled (23 °C) room. Rats were fed freely with one of 6 diets containing wheat gluten (BV = 64.0) at six different concentrations (5, 10, 15, 20, 25 and 30% = G diets). The control group was given a "standard" diet containing 20% casein (BV = 77.0) (C diet). The latter has been previously shown to meet all necessary requirements to allow normal skeletal and mandibular growth in the rat.[21] All the diets were isocaloric and protein was included in a protein-free diet by substituting an equivalent amount of dextrin. The protein-free diet contained 7% corn oil, 88% dextrin, 1% vitamin (AIN Vitamin Mixture 76, MP Biomedicals, Ohio, USA), 3.5 minerals (AIN-76 Mineral Mixture), and 0.5% choline. It should be pointed out, as mentioned above, that the experimental animals used in the present study were the ones from a prior study[20] in which the effects of G was determined in the femoral shaft. Thus, differences and similarities could be established between two bones having different physiological functions in the body.

The experimental period lasted 60 d. At this end, final body weight and length were established. Body length was taken as the distance between nose and tip of tail. Rats were then sacrificed by ether overdose. The hemimandibles 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.[22]

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 et al.[23] with some modifications.[24]

Dimensions were as follows (Fig. 1): (a) *mandibular area* was calculated from a triangle formed between three points: the most anterior inferior bone point of the interdental space (I), the most posterior point of the angular process (II), and the most superior point of the coronoid process (III); (b) the *length of the base* of the jaw was estimated by the distance between the most anterior superior point of the interdental process (IV) and the most posterior point of the angular process (II) (gonion); (c) the *length of the mandible* was estimated by the distance between the most anterior superior point of the interdental space (IV) and the most posterior point of the angular process (II) (gonion); (d) the *mandibular height* corresponded to the distance between the most posterior point of the angular process (II) (gonion) and the most superior point of the coronoid process (III); (e) the *alveolar length* was the distance between two points on the alveolar process immedi-

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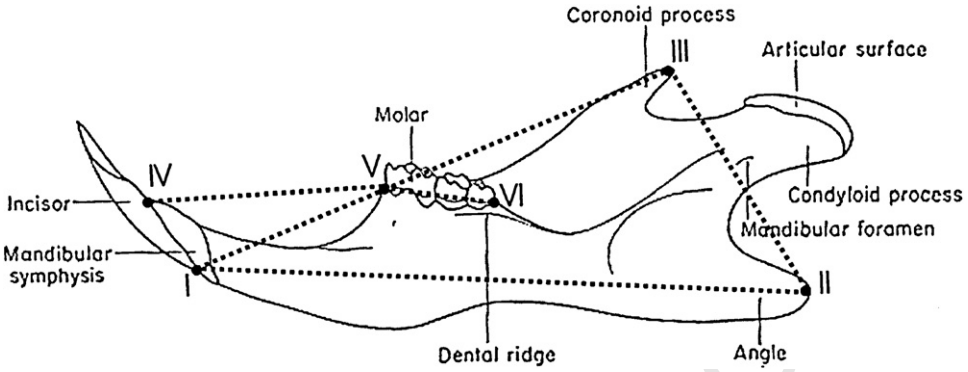

Fig. 1 – Medial aspects of the right hemimandible showing the bony points between which measurements were taken (see text).

ately anterior to the anterior surface of the first molar (V) and immediately posterior to the posterior surface of the third molar (VI). The *interdental length* (incisor alveolar process) was the distance from the most anterior superior bone point of the interdental spine (IV) to the anterior surface of the first molar (V). The *alveolar length* was the distance between two points on the alveolar process immediately anterior to the anterior surface of the first molar (V) and immediately posterior to the posterior surface of the third molar (VI). The *interdental length* (incisor alveolar process) was the distance from the most anterior superior bone point of the interdental spine (IV) to the anterior surface of the first molar (V). The mandibular length was divided into *anterior* and *posterior* 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 on the growth of the bone as a whole without considering its morphological units.[25]

Mechanical properties of the rat hemimandible were determined using a three-point bending mechanical test.[26] 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. For this biomechanical test, *load/deformation* (*W/d*) *curves* (Fig. 2) showing both the *elastic* (Hookean behaviour) and the *plastic* (non-Hookean behaviour) phases, separated by the *yielding point*, enabled graphic determination of the main *structural* mechanical properties of the bones which essentially measures the resistance to both deformation (*stiffness*) and fracture (*strength*). They are (A) *structural properties* (whole-bone properties, as derived from the slope of the *W/d* curve in the linear region of the elastic behaviour): (1) *maximal stress deflection* (yield deflection d_y, elastic limit, or load at the yielding point W_y) represents the end point of the elastic deformation (*yielding point*) and defines a threshold about which unrecoverable permanent deformation occurs, marking the initiation of damage accumulation with the first appearance of the first microcracks that occur on the periosteal surface of the bone; it is a measure of the bone strength; (2) *structural elastic stiffness* (load/deflection relationship, diaphyseal stiffness, bone rigidity, or slope of the linear phase of the *W/d* curve) represents the rigidity of the bone or the resistance to deformation; and (3) *structural strength* (whole-bone strength, maximal supported load, ultimate load, load at fracture W_f) 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. The estimated structural properties were based on the load-deformation curve. The deformation here was derived from the displacement as measured by the Instron rather by an independent extensiometer; thus the compliance of the machine and set up were not considered. This means that the results are relatively good for comparisons, but not should be made of them as absolute values. (B) *Geometric properties* (bone design characteristics). They are: (1) *bone length and diameters*; (2) *cross-sectional area* (CSA): 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, 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: $CSA = 3.14\ (VOD \times VID - HOD \times HID)/4$. (3) *second moment of inertia of cortical bone* (with reference to the anterior-posterior bending axis, xCSMI) as estimated by the equation: $xCSMI = (3.14\ [VOD^3 \times HOD - VID^3 \times HID/64])$. CSMI captures both bone mass and distribution on the cross section. The larger the CSMI, the further the disposition of bone cortical mass from a given reference axis. (C) *Bone material properties* (*intrinsic properties of the mineralised tissue*) as calculated from structural and geometric properties. Thus, bone material properties were not directly determined by mechanical means: (1) *Young's modulus of elasticity* (Bone material stiffness, intrinsic stiffness, strain–stress relationship) calculated by the formula: $E = W_y L^3/48 dy\ I_x$ (W_y = load at the yielding point, L = distance between supports, dy = maximal elastic deflection, I_x = second moment of inertia of the cross-section in relation to the horizontal axis); and (2) *maximal elastic stress*, which expresses the reacting force opposed by the deformed bone to the deforming load. It was calculated by the formula:

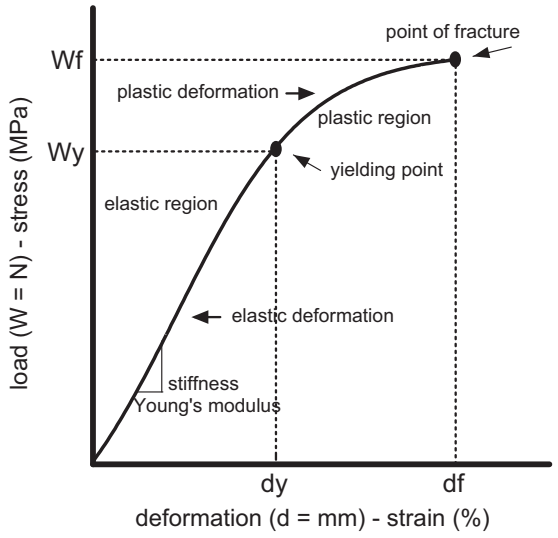

Fig. 2 – The mechanical test generates a "*load/deformation*" (*W/d*) *curve* from which several parameters can be measured. These parameters can be normalised after adjusting for the sample size (cross-sectional area or moment of inertia), allowing load conversion to *stress* and deformation to *strain*, and obtaining the *stress/strain curve*. The first, linear portion of the curve is known as the *elastic region*, where there is a proportional deformation with increasing load (stress) exerted. When the load is removed, bone returns to the original shape. After the *yielding point*, increasing load causes permanent damage to the bone structure: relative small increments of load causes relative large increments of deformation (*plastic region*). The *point of fracture* corresponds to the maximum load (stress) that bone can sustain without breaking. The *slope* of the curve within the elastic region is a measure of the *stiffness* of the whole bone (structural property) when obtained from the *W/d* curve. When obtained from the *S/S* curve, it is called *Young's modulus of elasticity, and* is an index of the stiffness of the bone material (material property). *Strength*, the other important bone property, can be defined by the load at fracture or by the load at yield. W_f = load at fracture, W_y = load at yield, d_f = deformation at the fracture point, d_y = deformation at the yielding point.

$\delta = LBW_y/8I_x$ (*B* = vertical outer diameter of the regularised fracture section).

The left hemimandible of each animal was ashed at 600 °C in a muffle furnace for 18 h and the ash weight obtained. The bone ash was dissolved in 2-NHCl and calcium content determined by atomic energy absorption spectrometry.[27] The *degree of mineralisation* (α) was estimated as the ratio between ash mass and dry bone mass.

Results were summarised as means ± SEM and were considered statistically significant at the level of $P < 0.05$. Comparisons between parameters were performed by one-way analysis of variance (ANOVA) and test of Student–Newman–Keuls by using GraphPad Prism Software (GraphPad Software Inc., San Diego, CA, USA). Apparent dose–response effects were analysed by linear regression by using the same cited software. Correlation coefficient (r), determination coefficient (r^2), P value to test the null hypothesis that the slope is 0, and test for linearity are given for each graph.

The experiment was conducted in accordance with the principles outlined in the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes, and approved by the University of Buenos Aires Ethics Committee.

3. Results

Results are presented graphically for easier interpretation.

Both body weight and body length was affected by dietary protein concentration and quality (Fig. 3A and B). Both parameters were highest in rats fed the diet containing 20% casein (control diet) and significantly less in animals fed gluten at every level of protein concentration. It was thus evident that none of the G-fed rats could achieve a normal growth performance as compared to the C-fed group: final body weight was below 80 g for all the former groups compared to almost 250 g for the latter. The groups fed G at the three highest concentrations showed a slow but continuous growth, whereas those fed the three lowest ones lose body weight continuously throughout the experimental period. However, the high correlation ($r = 0.9825$) found between body length and body weight for all animals together in the same graph suggests that body growth was harmonic and not influenced by the protein content of the diet. Like body size, mandibular weight, length, height, and area (an index of mandibular size) were significantly lower in all groups of G-fed rats than in the control one at the end of the experimental period (Fig. 4A–D). However, the four parameters were positively influenced by the G concentration in the diet. Both

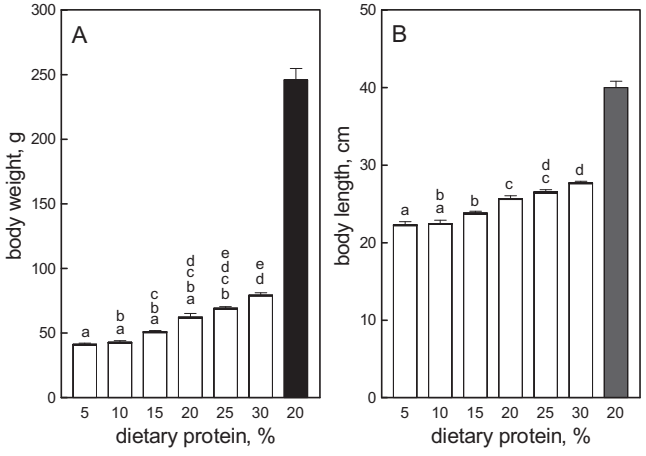

Fig. 3 – Final body weight (A) and body length (B) in female rats fed *ad lib.* diets containing wheat gluten (white bars) or casein (controls, black bars) as unique protein source between the 30th and 90th days of postnatal life, Each bar represents the mean ± SD for 7 rats; equal letters on top of bars indicate $P > 0.05$. Dose–response effects were derived from linear regression. A = r (0.9853), r^2 = 0.9708, P (0.0003), linearity (P 0.4000); B = r (0.9866), r^2 = 0.9734, P (0.0003), linearity (P 0.9000).

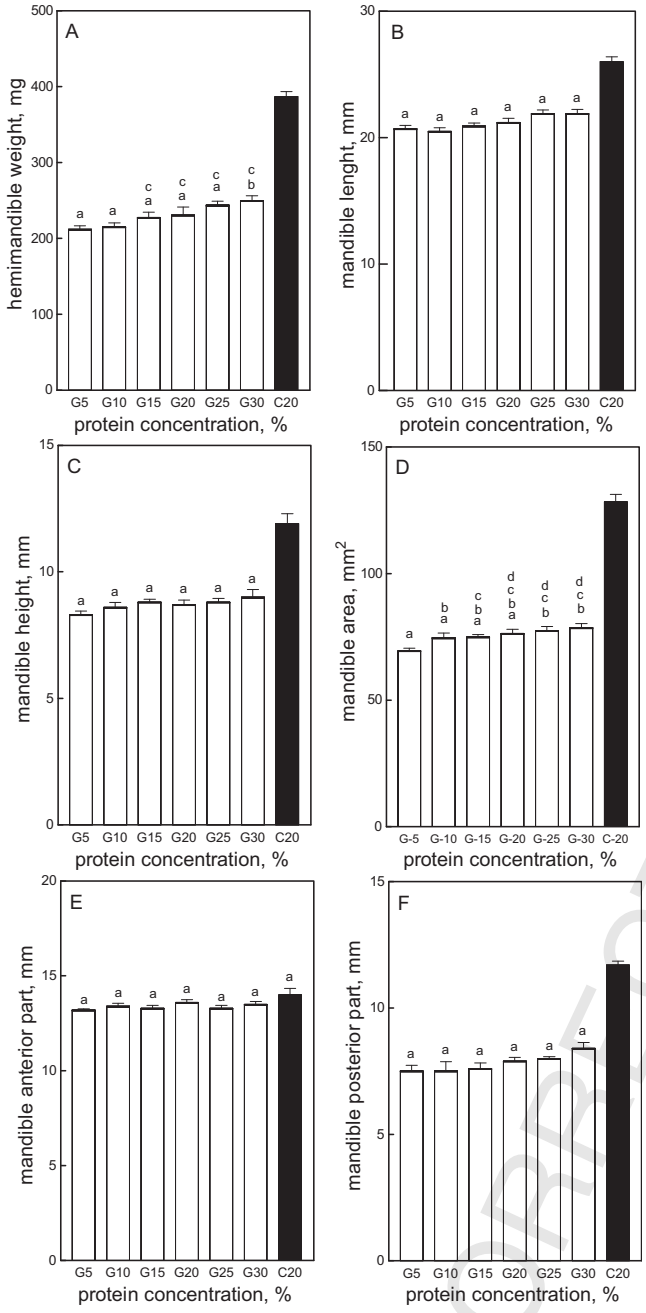

Fig. 4 – Mandibular morphometric properties in female rats treated as explained in Fig. 3. A = *r* (0.9402), r^2 (0.8939), *P* (0.0053), linearity (*P* 0.9999); B = *r* (0.9333), r^2 (0.8710); *P* (0.0065); linearity (*P* 0.8000); C = *r* (0.9035), r^2 (0.8163), *P* (0.0135), linearity (*P* 0.7000); D = *r* (0.9706), r^2 (0.9421), *P* (0.0013), linearity (*P* 0.8000); E = *r* (0.5447), r^2 (0.2967), *P* (0.2638), linearity (*P* 0.9999); F = *r* (0.8862), r^2 (0.7854), *P* (0.0187), linearity (*P* 0.8000).

alveolar and incisors alveolar process lengths were not influenced by either quality or concentration of the used dietary proteins. 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 reduced in the G-fed groups although its size was positively influenced by the G concentration in the diet (Fig. 4E and F). The analysis of the regularised fracture section indicated that both horizontal and vertical diameters in the G-fed animals were significantly less than in the C-fed ones, as were the cross-sectional area (CSA) (Fig. 5A) and the cross-sectional moment of inertia (xCSMI) (Fig. 5B). All values were not positively affected by the G concentration in the diet. Structural properties, as derived from the slope of the load/deformation curve in the linear region of the elastic behaviour, are shown in Fig. 6. The values for the elastic limit (Fig. 6A), the load at fracture (Fig. 6B) and the structural stiffness (Fig. 6C) were also significantly less in G-fed than in C-fed rats and positively correlated to the concentration of G in the diet. The yielding load/fracture load ratio did not differ significantly among experimental and control groups, indicating that the elastic and plastic components of the load/deformation curve was not altered neither by the concentration nor the quality of proteins in the diet. The bone material quality indicators, the elastic modulus (Fig. 5C) and the maximum elastic stress (Fig. 5D) did not differ significantly among all studied groups, as were the calcium concentration in ashes (Fig. 5E) and the degree of mineralisation (Fig. 5F).

4. Discussion

Infant and young animals can be seen as evolving metabolic systems as they go through a series of critical periods during the process of growth and maturation.[28] This process, which is governed by major determinants, can be influenced by several factors. Among them, the effects of dietary protein quality and concentration on both the dimensions and structural and material biomechanical properties of the rat mandible are relevant to the present discussion.

Modifications of the protein content and quality of the diet may be imposed at any phase of the growth of the organism. Specific effects in each period may or may not be similar and/or reversible. The results of this study provide details of how the concentration in the diet of a protein with a low biological value (wheat gluten) affects the mechanical properties of the mandible in young rats, as derived from determinations performed in early adulthood. Healthy bones at this stage of life are dependent on the development of a healthy structure and adequate bone mass during the growth period.

The present study began with very young animals and the effects of six diets containing different concentrations of G as the unique protein source on mandible morphometrics and bone biomechanics were assessed in early adulthood (90 d of age) by comparison to control rats fed a standard diet (C-20%).[21] We have previously shown[29] that the rat mandible attains its adult size, bone calcium mass and bone biomechanical competence at some point between 90 and 120 d of postnatal life. Since the different diets were offered to rats between the 30th and 90th days of life, it became evident that the animals were in a period of active growth during the treatment period. Therefore, the very well known effects of dietary proteins on body growth should be separated from their possible direct effect on bone mechanical properties.

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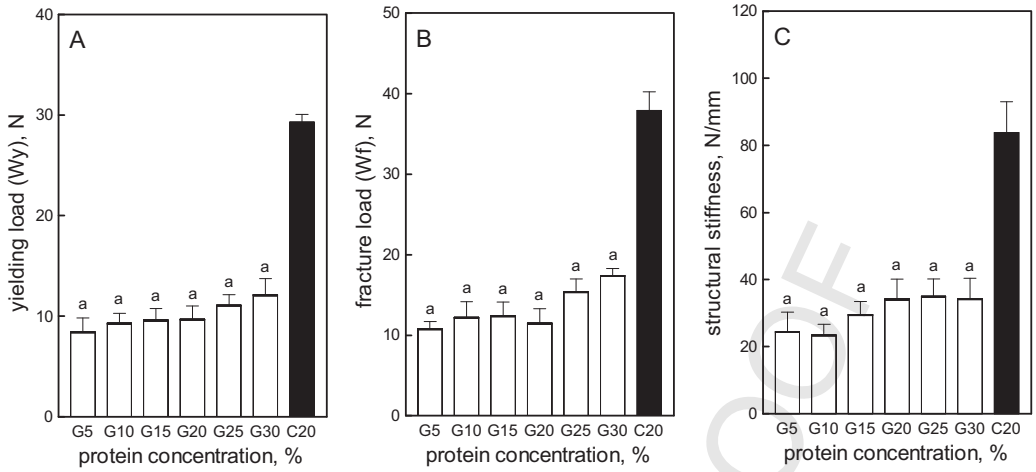

Fig. 5 – Cross sectional area (CSA, A), cross sectional moment of inertia (xCSMI, B), Young's modulus of elasticity (E, C), maximal elastic stress (D), calcium concentration in ashes (E), and degree of mineralisation (F) in female rats treated as explained in Fig. 3. A = *r* (0.7611), r^2 (0.5793), *P* (0.0788), linearity (*P* 0.3000); B = *r* (0.6839), r^2 (0.4677), *P* (0.1341), linearity (*P* 0.3000); C = *r* (0.2693), r_2 (0.0725), *P* (0.6059), linearity (*P* 0.9000); D = *r* (0.3070), r^2 (0.0942), *P* (0.5539), linearity (*P* 0.4000); E = *r* (0.2844), r^2 (0.0834), *P* (0.7022), linearity (*P* 0.6000); F = *r* (0.3022), r^2 (0.0878), *P* (0.6954), linearity (*P* 0.5000).

The marked negative effect of G on body growth was evident: none of the G-fed rats could achieve a normal growth performance as compared to the C-fed group. Concentrations of 5, 10, and 15% in otherwise normal diets impeded body growth, while concentrations of the protein of 20, 25, and 30% only induced a very slow continuous growth. These growth characteristics may explain the differences in final body weight and length that were evident among groups, and especially with the control one. However, previous studies from this laboratory have pointed out that neither the quantity nor the quality of dietary proteins affects the harmony of growth,[20] as evidenced by the high correlation found between body length and body weight in the treated animals.

Growth retardation associated with protein undernutrition has been previously reported.[10–19] Both the final mandibular weight and the mandible general morphometry in the present study were undoubtedly affected by growth retardation. This is clearly evidenced by the positive correlation (r^2 = 0.9022) (Fig. 7-1) between mandibular weight and body weight. The differences in cross-sectional area (CSA) and cross-sectional moment of inertia (xCSMI) indicate that the size of the bone, in terms of the cross section, was significantly affected by subnormal body growth. The rat mandible can be arbitrary partitioned into an anterior and posterior part. The former comprises the alveolar and the symphyseal regions, while the condyloid, the coronoid and the angular process compose the latter. In the weaning rat, the length of the posterior part of the mandible is about one-half of the anterior part.[29] From this time on, the relative increase of the posterior part of the bone is more than two times higher than that of the anterior part, because the condyle, the growth cartilage of the mandible, is situated posteriorly. The difference in the rates of growth between the anterior and posterior parts of the bone is responsible for the observation that both portions show almost equal lengths in adulthood.[29] In the present study, rats started their alimentary regimen when the growth of the anterior part of the mandible was almost finished. Therefore, no significant differences were encountered between rats maintained on the different diets (Fig. 4E) during the studied period. In relation to the posterior part, feeding animals with G produced a depression of growth, as evidenced by their lower value found in G-fed than in control rats (Fig. 4F). Therefore, the *anterior part/posterior part ratio* in G-fed animals (1.71) was different from that found in C-fed ones (1.22), which indicates that G-containing diets induced some deformation of the mandible relative to age.

These alterations were paralleled by weakening of bone strength (Fig. 6A and B) and structural stiffness (Fig. 6C), which were highly dependent on the quality of the dietary protein. The body weight or mass of the animals is one of the most important factors which influence bone ability to develop or resist stress in weight-bearing bones. A positive linear correlation (r^2 = 0.8921) (Fig. 7-2) between the load at fracture of the mandible and the mandible area suggests that the dependence of bone strength to bone mass is also evident in a load-bearing bone, as the mandible. Therefore, it appears that mandible mass, and consequently the structural mandible strength, grew up following the normal proportionality with body mass in all animals. In other words, growth retardation induced by the low quality of wheat gluten as source of dietary protein made animals to have smaller bones. Therefore, the load at fracture normalised by body mass was not different from that similarly sized control rats.

The above discussion suggests that the impaired mechanical performance of the mandibular bone induced by the low quality of the dietary protein tested is the result of changes in the amount of cortical bone mass (Fig. 5A), although the spatial distribution of this cortical bone (Fig. 5B) could be an additional factor. However, the high positive correlation between the strength of the bone and its size (Fig. 7-2) suggests that the main affected variable was the mandible mass. The lower values of xCSMI (which captures both, bone mass and distribution) may only reflect the much lesser amount of bone mass in the cross-sections, and not necessarily the

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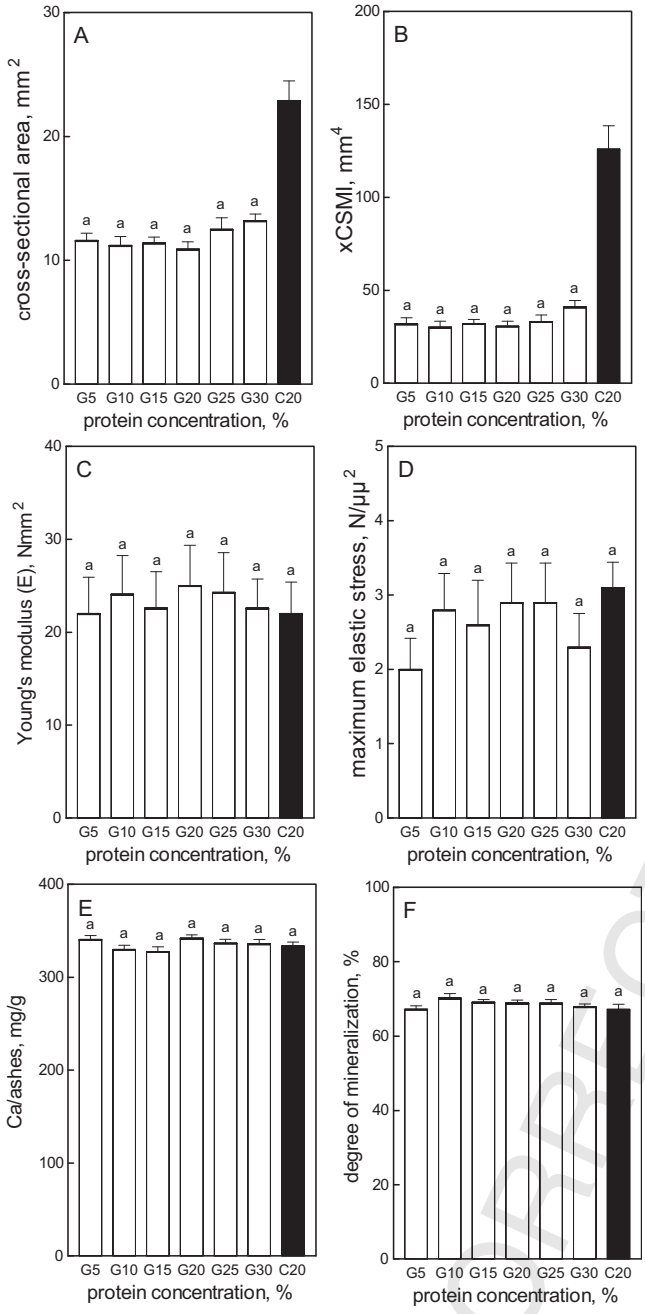

Fig. 6 – Mandibular structural properties (yielding load, A), fracture load (B), and structural stiffness (C), as derived from the slope of the load/deformation curve in the linear region of the elastic behaviour, in female rats treated as explained in Fig. 3. A = *r* (0.8694), r^2 (0.7558), *P* (0.0245), linearity (*P* 3000); B = *r* (0.9521), r^2 (0.9065), *P* (0.0034), linearity (*P* 0.7000); C = *r* (0.9310), r^2 (0.8668), *P* (0.0070), linearity (*P* 0.8000).

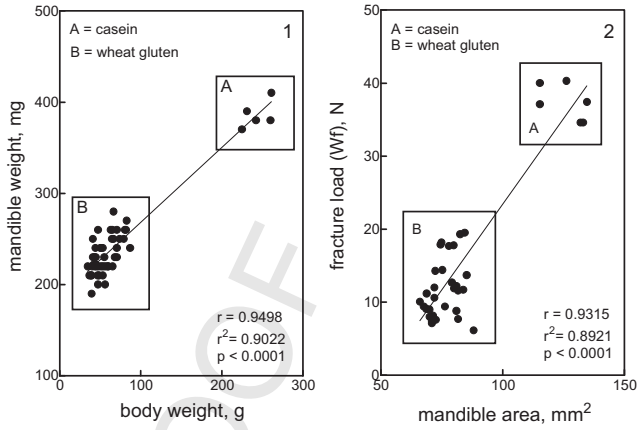

Fig. 7 – Correlation between mandible weight and body weight (1) and fracture load and mandible area (2) in female rats treated as explained in Fig. 3.

distribution of those small amounts of mass in the experimental animals.

The large differences in mandibular strength between groups maintained on diets containing G or C contrasted with the maintenance of normality of the elastic modulus (Fig. 5C) and the maximum elastic stress (Fig. 5D), both indicative of intrinsic properties of bone material, which depends on its constitution but not on its amount or spatial distribution, which suggests that the adverse effects evoked by treatment may have been only quantitative in nature. The lack of effects of G on both calcium concentrations in ashes (Fig. 5E) and the degree of mineralisation (Fig. 5F) could explain the normal rigidity of the mandibular bone material. Material properties of bone tissue are usually thought to depend on many factors, calcium content being one of the main determinants.[30] It is norworthy that the effect of the nutritional alteration imposed to rats in the present study affected the biomechanical performance of the mandible as affected that of the femur (20) in spite of the fact that the femur is a ''weight-bearing bone'' and the mandible is not.

In conclusion, we have described a number of alterations in both morphological and biomechanical variables in the rat mandible resulting from feeding growing rats from weaning to early adulthood with diets containing different concentrations of wheat gluten, a low quality protein. The clear differences in strength and stiffness of the bone between treated rats and controls (fed a 20%-casein diet) seemed to be the result of an induced loss of gain in bone structural properties as a consequence of a correlative loss of gain in bone growth and mass, in the absence of changes in the quality of the bone mineralised material. These effects could be ascribed to the low quality of the tested protein, the depression of food intake,[20] and other effects such as toxicity.[31] The latter has been described in humans but not in rodents.

Acknowledgments

This work was supported by Research Grants from the University of Buenos Aires (UBACYT 20020100100389 and 20020100100067). RMA and CEB are Career Investigators from National Research Council (CONICET).

Funding: The research was supported by grants from the University of Buenos Aires, Argentina (UBACYT 20020100100389 and 20020100100067).

Competing interests: None declared.

Ethical approval: Ethical Approval was given by the Ethical Committee FOUBA UBACYT 2011–2014 No. 1.

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