# GROWTH INHIBITION IN RATS FED INADEQUATE AND INCOMPLETE PROTEINS: REPERCUSSION ON MANDIBULAR BIOMECHANICS

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**ABSTRACT**

*This study describes the effects of feeding growing rats with a diet containing inadequate and incomplete proteins on both the morphological and the biomechanical properties of the mandible. Female rats aged 30 d were fed freely with one of two diets, control (CD, 301 Cal/100g) and experimental (ED, 359 Cal/100g). CD was a standard laboratory diet, while ED was a synthetic diet containing cornflower supplemented with vitamins and minerals. Both diets had the same physical characteristics. Control (C) and experimental (E) animals were divided into 4 groups of 10 animals each. C40 and E40 rats were fed CD and ED, respectively, for 40 d; C105 were fed the CD for 105 d; and E105 were fed the ED for 40 d and then the CD for the remainder of the experimental period (65 d). Mandibular growth was estimated directly on excised and cleaned bones by taking measurements between anatomical points. Mechanical properties of the right hemimandible were estimated by using a 3-point bending test to estimate the structural properties of the bone. Geometric properties of both the entire bone and the cross-section were determined. Bone material properties were calculated from structural and geometric properties. The left hemimandibles were ashed and the ash weight obtained. Rats fed the ED failed to achieve normal body weight gain. Complete catch-up was observed at the end of nutritional rehabilitation. Mandibular weight and length were negatively affected by the ED, as were the cross-sectional area, the mineralized cortical area, and the cross-sectional moment of inertia. All of these parameters showed incomplete catch-up. The structural bone mechanical properties indicative of strength and stiffness were negatively affected. Intrinsic material properties, as assessed by the modulus of elasticity and maximal elastic stress, were within normal values. In summary, the experimental bone was weaker than the control and structurally incompetent. The bone considered was smaller than the control bone, showing a significant reduction in the cross-sectional area and the moment of inertia. However, material properties as well as the ash fraction and degree of mineralization were similar in E and C bones. Therefore, the E bone was weaker than the C bone because of its smaller bone mass, which appears to have been negatively influenced by the ED in relation to its effects on overall body mass.*

**Key words:** *Mandible, bone, biomechanics, nutrition.*

## INHIBICION DEL CRECIMIENTO EN RATAS ALIMENTADAS CON PROTEINAS INADECUADAS E INCOMPLETAS: REPERCUSION SOBRE LAS PROPIEDADES BIOMECANICAS DE LA MANDIBULA

**RESUMEN**

*El trabajo presente describe los efectos de la alimentación de ratas en crecimiento con una dieta, que contiene proteínas inadecuadas e incompletas, sobre las características morfológicas y las propiedades biomecánicas de la mandíbula. Ratas hembras jóvenes (30 d) fueron alimentadas ad lib con una de las siguientes dietas: Control (CD, 301 Cal/g) o Experimental (ED, 359 Cal/g). CD fue una dieta estándar de laboratorio, mientras que ED fue una dieta sintética conteniendo harina de maíz suplementada con vitaminas y minerales. Ambas dietas presentaron características físicas (dureza, humedad) similares. Los animales controles (C) y experimentales (E) fueron asignados a uno de cuatro grupos (n = 10/grupo). Las ratas de los grupos C40 y E40 recibieron, respectivamente, las dietas CD y ED durante 40 d. Los animales pertenecientes al grupo C105 fueron alimentados con CD durante 105 d, mientras que los del grupo E105 recibieron la dieta ED durante 40 d y la CD por el resto del período experimental (65 d). El crecimiento mandibular fue estimado directamente sobre el hueso (hemimandíbula) previamente despojado de las sustancias blandas, mediante mediciones entre puntos anatómicos. Las propiedades biomecánicas estructurales de la hemimandíbula derecha fueron estimadas mediante el test mecánico de flexión a 3 puntos. Las propiedades geométricas del hueso entero y de la sección transversal fueron también determinadas. Las propiedades del material óseo fueron calculadas a partir de las geométricas y las estructurales. La hemimandíbula izquierda de cada animal fue calcinada para la obtención de cenizas, que fueron pesadas. Las ratas alimentadas con dieta ED mostraron una tasa de crecimiento subnormal, observándose crecimiento compensador (catch-up) completo hacia el final del período de rehabilitación nutricional. El peso y la longitud mandibulares fueron negativamente afectados por ED, así como el área de sección transversal, el área de tejido mineralizado cortical y el momento de inercia de la sección. Todos estos parámetros mostraron crecimiento compensador incompleto durante la rehabilitación proteica.. Las propiedades óseas estructurales indicadoras de la resistencia y la rigidez mandibulares fueron afectadas negativamente. Las propiedades materiales intrínsecas, evaluadas a partir del módulo elástico de Young y el estrés elástico máximo mostraron valores normales. En resumen, el hueso considerado fue más pequeño que el normal, mostrando una reducción significativa a nivel del área de sección transversal y el momento de inercia. Sin embargo, las propiedades materiales, el contenido fraccional de cenizas y el grado de mineralización fueron similares en los huesos C y E. Por lo tanto, el hueso E fue menos resistente que el C debido a su menor masa ósea, la que pareciera estar negativamente influenciada por la ED en relación con sus efectos sobre la masa corporal general.*

**Palabras clave:** *mandíbula – biomecánica ósea - nutrición*

## INTRODUCTION

Most of the bones that form the skeleton have a *support* function, thus playing a critical functional role in bearing functional loads (body weight, mastication, somatic muscle tension). The criterion for adequate support function is the formation and maintenance of sufficient *quantity* and *quality* of bone tissue adequately distributed in individual bones to support the body throughout life and to withstand ordinary stresses to which skeletal components are subject[1]. Bone is considered as a composite material, thus formed of several components that give it the capacity to *deform* under applied loads. *Biomechanics* is the branch of physics that analyzes the effects of forces to which a living structure is subject. In this study, the *static* aspect of biomechanics will be analyzed, in which bones are considered as support organs and not as participants in either locomotion or postural reflexes (in the mandible, as participant of mastication).

Both body weight and regional somatic muscle contraction 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. Moreover, it arises from a different embryonic germ layer (neuroectoderm) than the bones of the axial or appendicular skeleton, 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[2,3].

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 to the weight-bearing bones from the mechanical point of view.

It is assumed that the *"load-carrying behavior of bone"* or *"mechanical properties"* of bones integrated as organs *(structural properties)* are directly related to both the amount *(bone mass)* and the architectural distribution of the mineralized 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 mineralized tissue* (Young's modulus of elasticity) and the *maximum elastic stress.* These properties are determined by matrix mineralization as well as by other mineralization-unrelated, microstructural factors, such as crystal size and packing and disposition of collagen fibers[4]. The structural stiffness, and indirectly the strength of bones, is thought to be controlled by a *"bone mechanostat"* [5]. This is a feedback mechanism that optimizes the bone design through permanent re-distribution of the mineralized tissue. Mechanical factors are thus the primary factors determining bone strength However, there are also other "non-mechanical factors" 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[6-10] the results of experiments conducted to establish the effects of dietary protein concentration and quality on bone biomechanical properties (femoral shaft and mandible) in growing rats. The general conclusion was that dietary protein affects bone quality through its effects on bone mass without affecting either bone architecture or the quality of the bone mineralized material. The effects on bone mass were associated with the general effects on body growth rate. Only when protein was absent from the diet was the reduction of bone mass associated with deterioration of the stiffness of the bone tissue[11].

Cornflour or cornstarch is ground from the white endosperm at the heart of corn kernels. It is a yellow flour made of dried and ground corn. In maize, the ethanol-soluble or prolamin fraction, the *zeins*, constitutes as much as half of the total protein in the endosperm[12]. Zein is thus the main protein of maize, being deficient in lysine and tryptophan, two of the twenty essential amino acids that allow normal growth in monogastric animals. Proteins lacking an essential amino acid are *inadequate* proteins. According to its adequacy, zein is considered as an *incomplete* protein because it is incapable of either maintaining life or supporting growth. In fact, experimental animals fed zein as their sole protein lose weight. If tryptophan is added to the zein, weight is maintained but growth does not occur. Only after lysine and tryptophan are added can normal growth take place. It has been suggested that all of the essential amino acids in zein are utilized poorly and that depression of appetite may account for the effect of corn in depressing growth[13].

The present study was designed to explore the mechanical behavior of the rat mandible in post-weaning female rats stunted by being fed cornstarch supplemented by vitamins and minerals. The possibility of catch-up growth after the end of the nutritional insult was also investigated.

## MATERIALS AND METHODS

Forty female Sprague-Dawley rats aged 30 d and weighing 47.75 ± 4.23 g at the start of the experiment were housed in stainless-steel cages under natural light-dark photoperiod in a temperature-controlled (23ºC) room. Rats were fed freely with one of two diets, control (CD, 301 Cal/100g) and experimental (ED, 359 Cal/100g). The CD was prepared as follows: 30 g of gelatin dissolved in 800 ml of distilled water was added to 1.0 kg of crushed pellets of the standard rat laboratory diet and mixed thoroughly. The homogeneous paste obtained was treated as explained below. The ED was prepared as follows: 1.0 kg of cornstarch, 10 g of vitamins (AIN Vitamin Mixture 76, MP Biomedicals, Ohio, USA), 35 g of minerals (AIN Mineral Mixture 76), 80 ml of soy oil and 1.78 mg of choline were mixed thoroughly. Thirty g of gelatin were dissolved in 800 ml of distilled water and added to the first preparation. The homogeneous paste obtained was maintained in a refrigerator until its consistency was similar to the standard pellet. It was then cut in small portions to be offered to the experimental animals in the usual food containers. Gelatin was added to both diets because of its agglutinating effect. Its constituent proteins have low biological value because they lack many essential amino acids, and thus do not meet protein demands or represent complete nutritional value. Analytical determination of a sample of the ED provided the following results (g/100g): Humidity: 11.6; Ashes: 0.5; Carbohydrates: 80.2; Proteins: 6.2; Lipids: 1.5; and Ca: 0.8 mg.

Control (C) and Experimental (E) animals were divided into 4 groups of 10 animals each. $C_{40}$ and $E_{40}$ rats were fed CD and ED, respectively, for 40 d; $C_{105}$ were fed CD for 105 d; and $E_{105}$ were fed ED for 40 d and then CD for the remainder of the experimental period (65 d). During this period, body weight and food intake were recorded periodically. At the end of the period, final body weight and length were determined. The rats were then killed by anesthesia overdose. The hemimandibles were dissected and cleaned of adhering soft tissue, weighed in a Mettler scale and stored at -20ºC wrapped in gauze soaked with Ringer's solution in sealed plastic bags, following Turner and Burr[14]. 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) with digital calipers, following Eratalay et al.[15] with some modifications[6]. 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

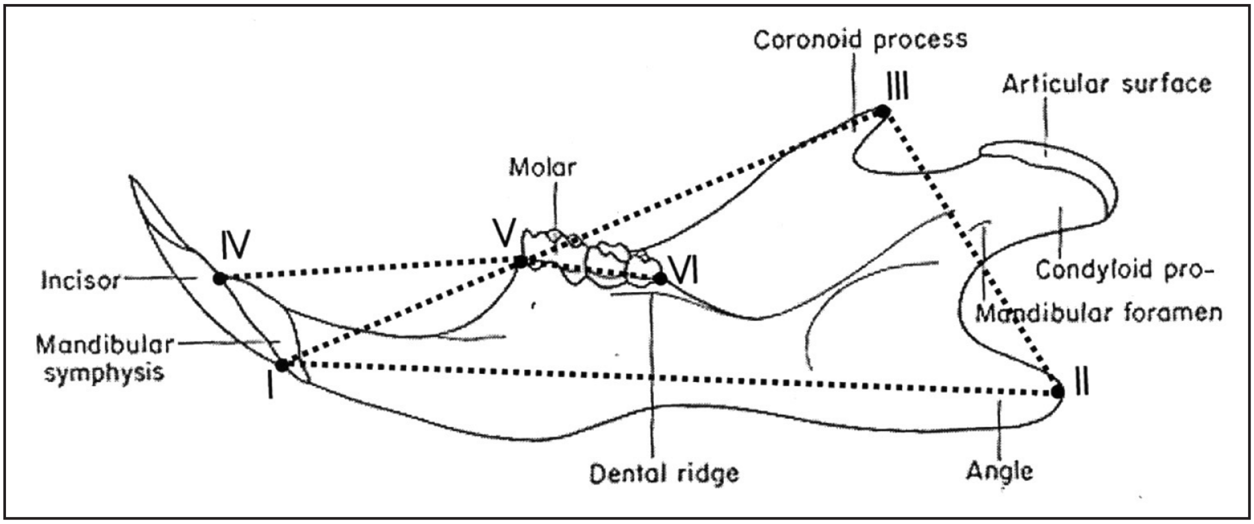

*Fig. 1: Medial aspects of the right hemimandible showing the bony points between which measurements were taken (see text).*

*length of the base* of the jaw was estimated by the distance I-II ; 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); d) the *mandibular height* corresponded to the distance between the most posterior point of the angular process (II) and the most superior point of the coronoid process (III); e) 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); f) 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 provide information on the growth of the bone as a whole without considering its morphological units[16].

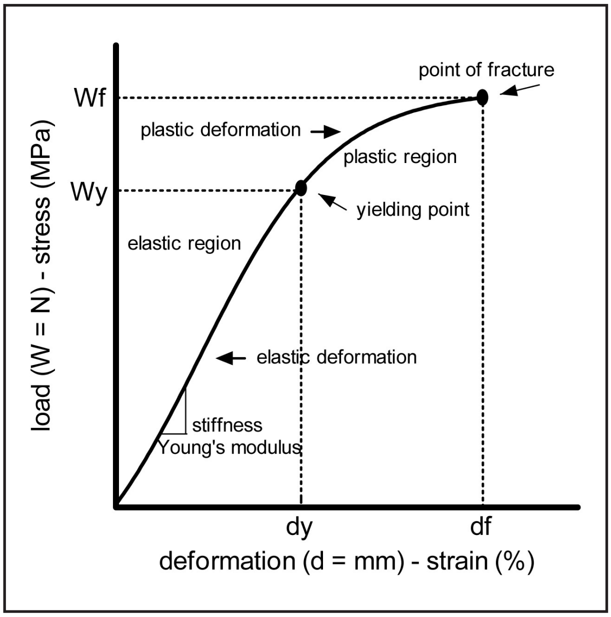

*Fig. 2: The mechanical test generates a "load / deformation" curve (W/d) from which several parameters can be measured. These parameters can be normalized after adjusting for the sample size (cross sectional area or moment of inertia), allowing load conversion to "stress" and deformation to "strain", and obtaining the "stress / strain" curve (S/S). The first linear portion of the curve is known as the "elastic region", where there is a proportional deformation (or strain) with increasing load (or stress) exerted; when the load is removed, bone returns to its original shape. After the "yielding point", increasing load causes permanent damage to the bone structure: relatively small increments of load cause relatively large increments of deformation (plastic region). The "point of fracture" corresponds to the maximum load the bone can sustain without breaking. The slope of the curve within the elastic region is a measure of the "stiffness" of the whole bone (extrinsic bone 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 (intrinsic bone property). "Strength", the other important bone property, can be defined either by the point of fracture or by the load at yield. Wf = load at fracture; Wy = load at the yielding point; df = deformation at the fracture point; dy = deformation at the yielding point.*

Mechanical properties of the rat right hemimandible were determined using a three-point bending mechanical test[14]. Each bone was placed on two lower supports (11 mm span) with the lateral aspect facing down and centered 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 behavior) and the *plastic* (non-Hookean behavior) phases, separated by the *yielding point,* enabled graphic determination of the main *structural* mechanical properties of the bones, which essentially measure the resistance to both deformation (*stiffness*) and fracture *(strength)*. They are the following: (A) *structural properties* (whole-bone properties, as derived from the slope of the W/d curve in the linear region of the elastic behavior): (1) *maximal stress deflection* (yield deflection *dy*, elastic limit , or load at the yielding point *Wy*) 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 Wf) 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 behaviors. (B) *geometric properties* (bone design characteristics): (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 regularized 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: $CSA = 3.14\ (VOD\ .\ VID - HOD\ .\ HID)\ /4$. (3) *second moment of inertia of cortical bone* (with reference to the anterior-posterior bending axis, xCSMI) as estimated by the equation: $xCSMI = (3.14\ [VOD^3\ .\ HOD - VID^3\ .\ HID\ /\ 64])$. 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 mineralized 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: $E = WyL^3\ /\ 48\ dy.Ix$ (Wy = load at the yielding point, L = distance between supports, dy = maximal elastic deflection, Ix = 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: $\delta = LBWy\ /\ 8Ix$ (B = vertical outer diameter of the regularized fracture section.

The left hemimandible of each animal was ashed at 600ºC in a muffle furnace for 18 h and the ash weight obtained. The *degree of mineralization* (α) was estimated as the ratio between ash mass and dry bone mass.

Results were summarized as means ± SD 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 Student-Newman-Keuls test by using GraphPad Prism Software (GraphPad Software Inc., San Diego, CA, USA). Correlation was analyzed by using the same software. 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.

## RESULTS

Rats fed the ED did not attain the same weight as the age-matched C rats (Fig. 3-A, C40 vs. E40). In fact, body weight gain in E animals fed the ED for 40 d was undistinguishable from that of the same rats at the beginning of the experimental period (Fig.3, INI vs. E40). During this period, C rats grew at a rate of 3.91 ± 0.2 g/d, while E rats grew

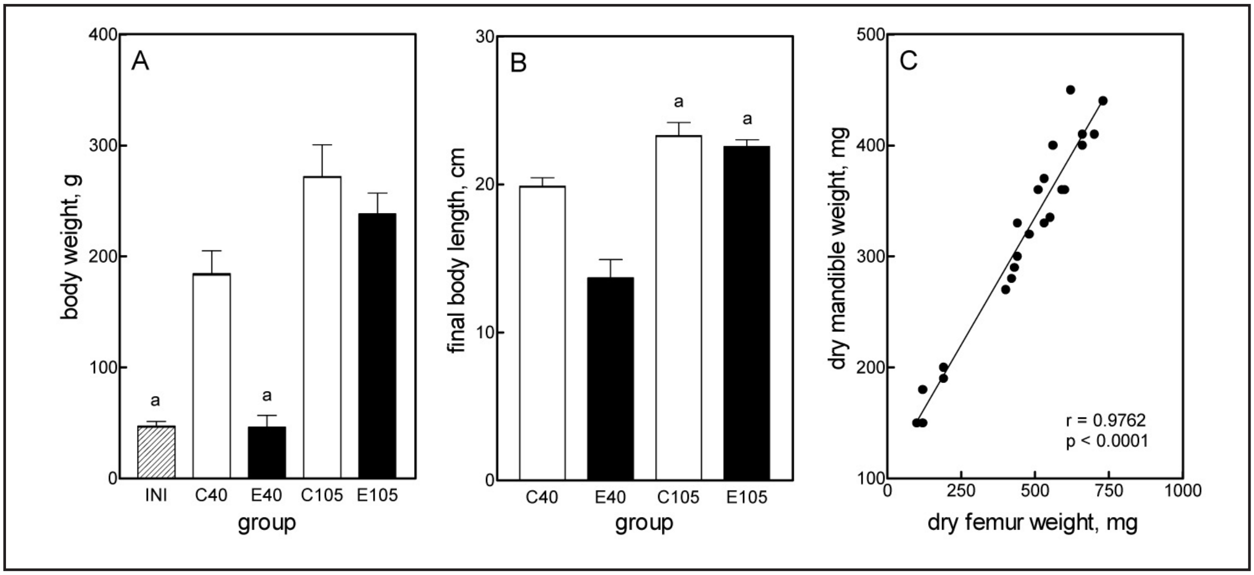

*Fig. 3: Body weight (A), body length (B), and correlation between mandible and femur weights in rats treated as described in Materials and Methods. Bars represent Mean ± SD of 7 rats. Equal letters on top of bars indicate p > 0.05.*

at a rate of 0.06 ± 0.06 g/d. During the recovery period, E rats grew at a rate of 4.09 ± 0.31 g/d while C rats did so at a rate of 1.39 ± 0.18 g/d (days 40 to 75 of the experimental period (data not shown). Growth rate was 0.70 ± 0.19 g/d and 1.44 ± 0.18 g/d in C and E rats, respectively, from day 75 to 105. Final body weight reached a value that was about 90% of the C value by the end of the recovery period (Fig. 3, C105 vs. E 105, $p > 0.05$). The lower body weight found in E than C animals was accompanied by a significant 31.3% reduction in body length (Fig. 3-B). The parameter recovered totally by the end of the recovery period. Like body size, mandibular dry weight as well as mandibular length, height and area (Fig. 4, A, B, C and D, respectively), which collectively represent the size of the bone, were significantly lower in E40 than in C40 rats. With the exception of mandibular height, which reached normal values by the end of the recovery period (day 105), the other three parameters in the E105 group were about 10% ($p < 0.01$) lower than in the C105 group. It is worth noting that a high correlation ($r = 0.9762$) was found between mandible and femur weights (Fig. 3-C) when data from animals were put in the same graph. When the length of the bone was divided into an anterior and posterior part by a line

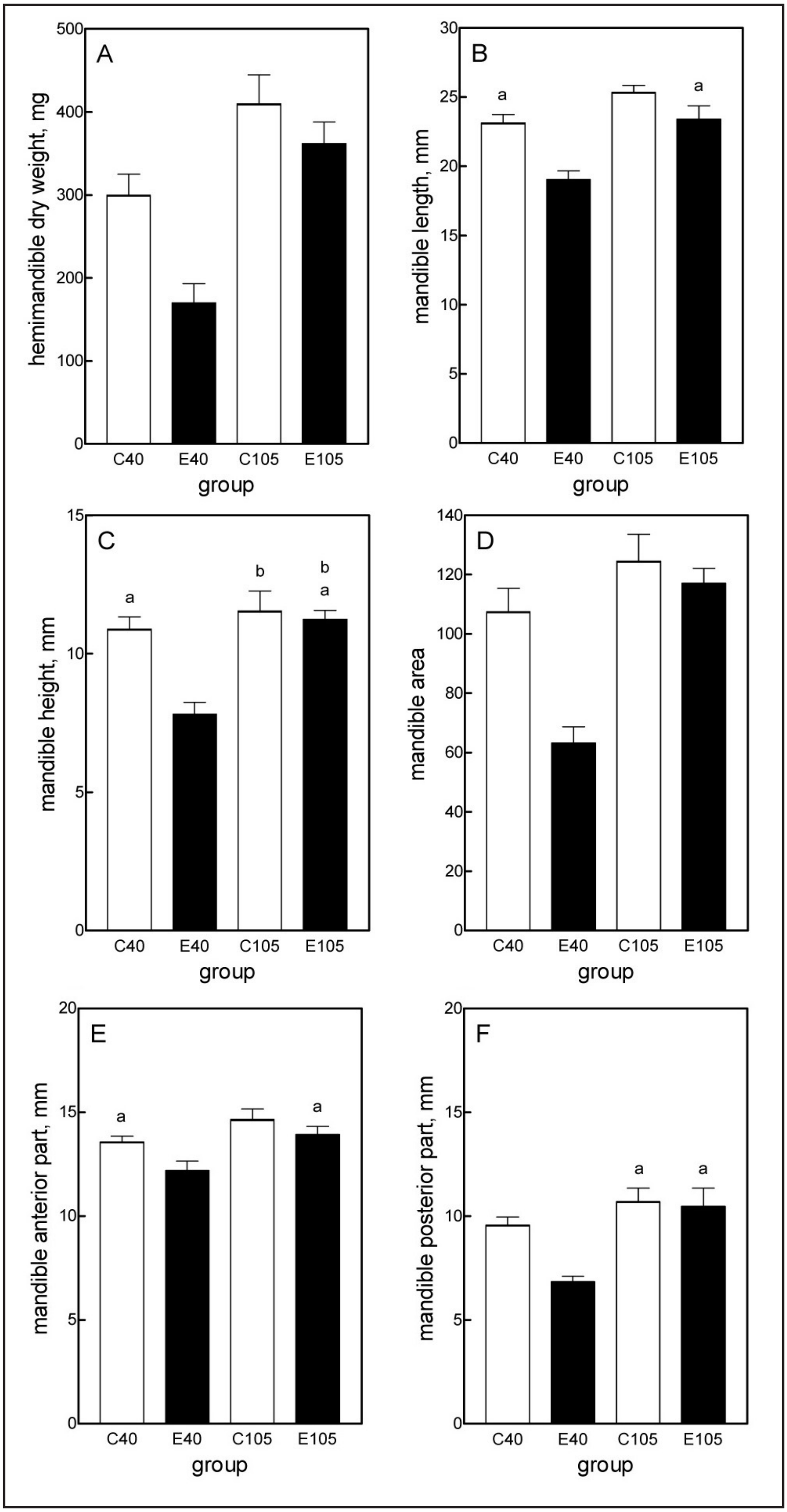

*Fig. 4: Morphometric properties of the rat mandible. For explanation of groups see Materials and Methods. Bars represent Mean ± SD of 7 rats. Equal letters on top of bars indicate $p > 0.05$.*

drawn immediately posterior to the posterior surface of the third molar, it was remarkable that in E40 rats the anterior part was significantly reduced by 9% while the posterior part was significantly reduced by 33% (Fig. 4-E and 4-F). The analysis of the regularized fracture section indicated that the cross-sectional area (CSA) and the cross-sectional moment of inertia (xCSMI) (Figs. 6-A and 6-B, respectively) were markedly reduced in E40 rats and the recovery was incomplete for both parameters in the E105 animals. Structural properties (Fig. 5), as derived from the slope of the load/deformation curve in the linear region of the elastic behavior, were drastically affected by treatment. The values for both the yielding and the fracture loads and the structural stiffness were less than 50% in the E40 rats than in C40 rats. These structural properties recovered totally by the end of the

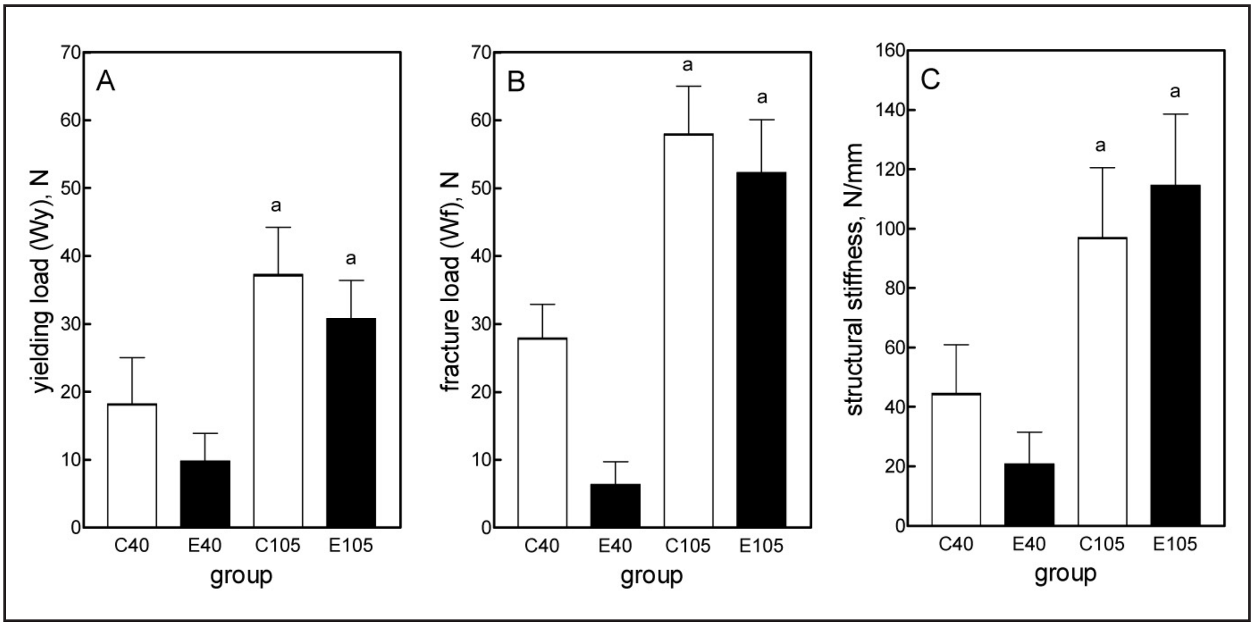

*Fig. 5: Mandibular structural properties in female rats, as derived from the slope of the load / deformation curve in the linear region of the elastic behavior. For explanation of groups, see Materials and Methods. Bars represent Mean ± SD of 7 rats. Equal letters on top of bars indicate $p > 0.05$.*

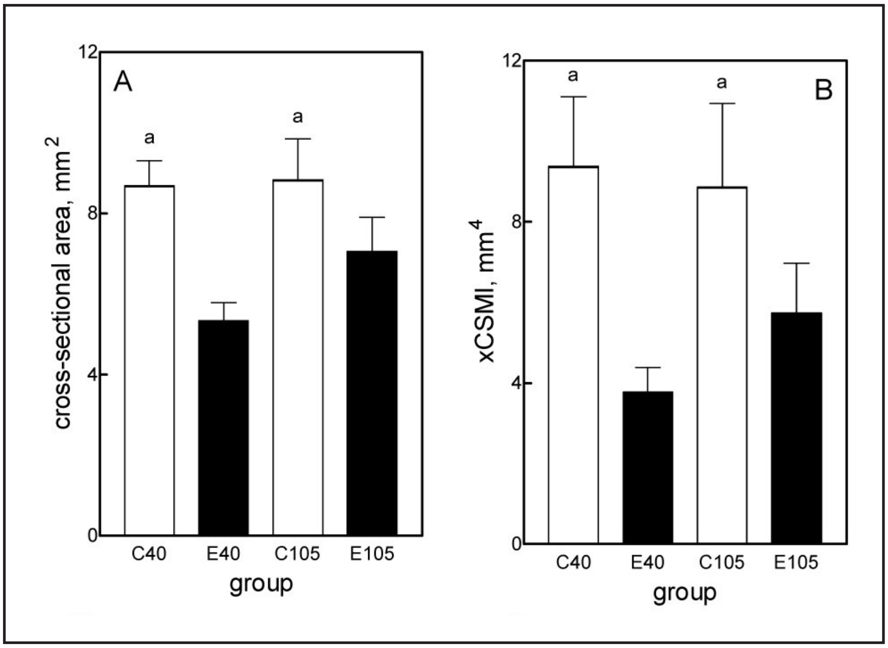

*Fig.6: Characterization of the cross-section (A, B).*

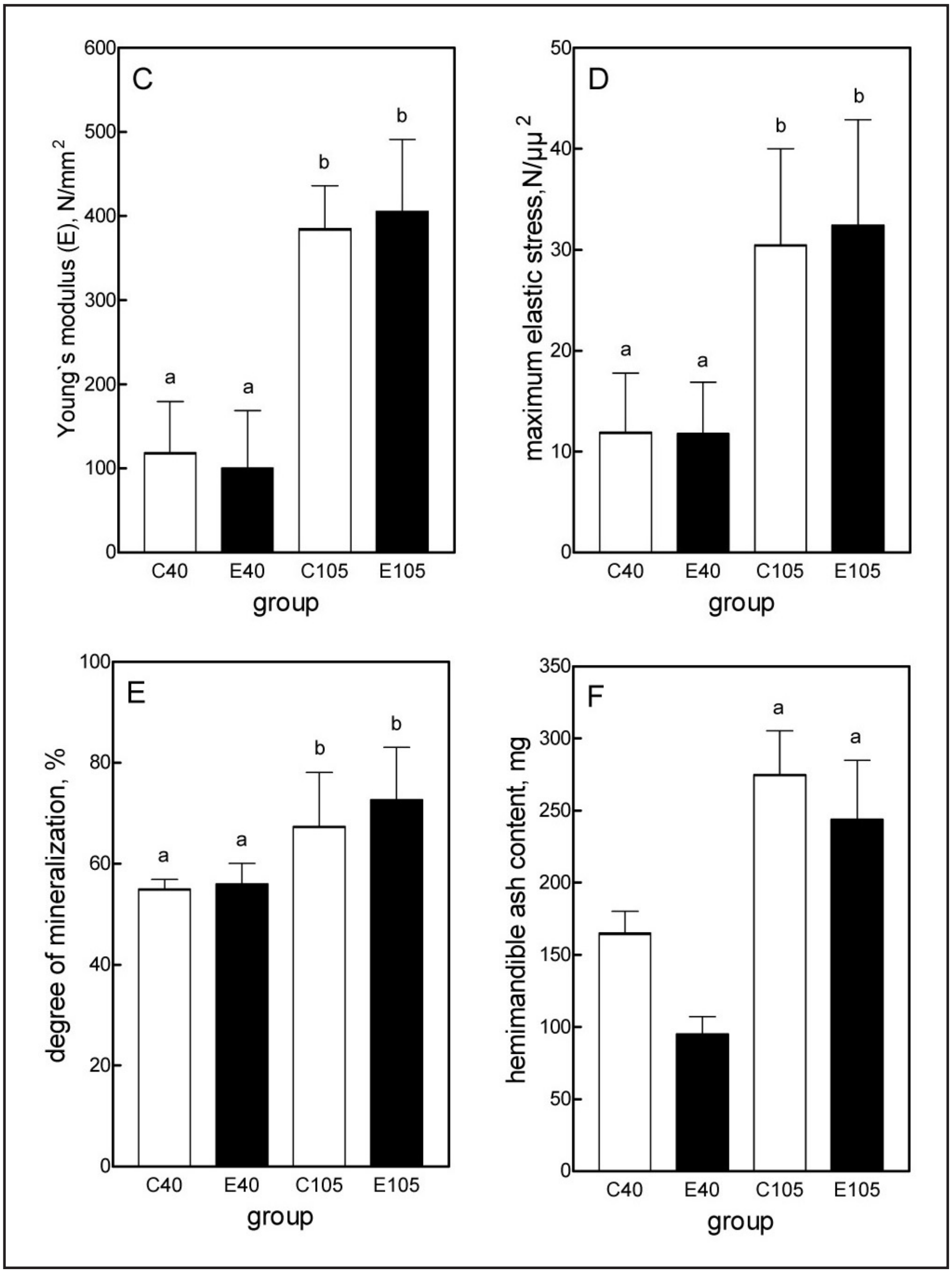

*Fig.6 cont.: Material intrinsic properties (C, D) and degree of mineralization (E) and hemimandible ash content (D). For explanation of groups, see Materials and Methods. Bars represent Mean ± SD of 7 rats. Equal letters on top of bars indicate Mean ± SD of 7 rats. Equal letters on top of bars indicate* $p > 0.05$.

recovery period (E105 vs. C105). Bone material quality indicators, or pre-yielding bending stiffness (elastic modulus, E) (Fig. 6-C) and maximal elastic stress (Fig. 6-D) did not differ significantly between C and E rats, as was the degree of mineralization (Fig. 6-E), during the first and the second phases of the experiment. The hemimandible ash content, which represents the mineral mass of the bone, was highly diminished in E40 rats, recovering totally in E105 rats.

## DISCUSSION

This study estimated mechanical properties of the entire hemimandible as a structure, which approaches the behavior of the mandible *in vivo*. The study examined the effect of a diet (ED) containing maize proteins as a sole source of proteins on body and mandibular growth and mandible biomechanics in female rats from days 30 to 70 of postnatal life. A high body growth rate occurs during this period. Mechanical testing at the chosen level of bone organization measures properties of the entire bone as a structure, which incorporates the properties of the materials that compose the bone, as well as its internal and external geometry[17]. These properties were measured at the level of a line drawn perpendicularly to the bone behind the posterior part of the third molar. The rat mandible has the capacity to achieve complete catch up during protein recovery at the end of a short period of dietary protein restriction[18]. This study thus also analyzed whether body and mandibular growth accelerated during nutritional rehabilitation after the imposition of the experimental diet and evaluated the possible recovery of mandible biomechanical behavior.

The ED freely offered to the experimental rats during the first 40-day period of the study contained 6.2% of proteins from maize which, taken together, are considered both inadequate (deficient in essential aminoacids) and incomplete (unable to support body growth and/or maintain life). The diet, therefore, did not contain adequate and complete proteins, which were also present in a concentration that does not allow normal growth in the rat. In fact, it has been demonstrated that 5% concentration of high quality protein is necessary to maintain body weight in an immature rat, although it does not permit growth[19, 20]. These characteristics of the ED explain the absolute lack of overall body growth (as assessed by body weight) and body length (an index of longitudinal skeletal growth) in the treated animals.

Catch-up growth has been defined as growth at a velocity above the statistical limits of normality for age during a defined period that follows a period of impaired growth[21]. Such an increase in growth rate may or may not allow an animal to attain its normal adult size. If it does, catch-up is said to be complete and if it does not, incomplete. In this study, the severe growth restriction seen in the rats fed on ED was immediately reversed when the diet was changed to CD. During the first 35 days of nutritional rehabilitation, E rats grew at a rate that was 2.94 times greater than that of the C rats at the same age, which fulfills the requisites for catch-up growth. Body growth rate in E rats decreased over the next 30 days, although was still 2.06 times higher than the rate for the C rats. These changes in body growth rate enabled E rats to approach a final body weight (mature weight) that was not significantly different from that of C rats and give reasons for the conclusion that catch-up growth was complete after the end of the recovery period.

Both final mandibular weight and mandible general morphology in this study were undoubtedly affected by growth retardation. This is clearly evidenced by the positive correlation ($r = 09789$, $r^2 = 0.9582$, $p < 0.0001$) found between mandibular weight and body weight. A highly significant positive correlation ($r = 0.9762$) was also found between mandible (a non weight-bearing bone) weight and femur (a weight-bearing bone) weight, which suggests that the rate of body growth may be more important than the types of loads acting on the bones in the determination of their individual growth.

Mandibular weight and area, both taken as indexes of bone size, caught up in E rats during nutritional rehabilitation. However, final values differed slightly thoug significantly from the C values, which indicates that catch-up was incomplete. It is suggested that bone would have caught up completely if the rehabilitation period had been longer. 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. Here again, catch-up was incomplete during protein recovery. The rat mandible can be arbitrarily dividedinto anterior and posterior parts. The former comprises the alveolar and symphyseal regions, while the latter comprises the condyloid, the coronoid and the angular process. In the weaning rat, the length of the posterior part of the mandible is about one-half of the anterior part[22]. From this time on, the relative increase of the posterior part of the bone is more than twicw as high as that of the anterior part, because the condyle, the growth cartilage of the mandible, is situated in the posterior part. The difference in growth rates between the anterior and posterior parts of the bone is responsible for the observation that both portions have almost equal lengths at adulthood. The deleterious effect of ED on mandibular growth was more accentuated on the posterior part of the bone. Therefore, the *"anterior/posterior ratio"* differed in E (1.76 ± 0.09)) and C rats (1.43 ± 0.10) ($p < 0.01$), which indicates that ED induced some deformation of the mandible relative to age.

These alterations were paralleled by weakening of bone strength (Fig. 5A and B) and structural stiffness (Fig. 5C). The body weight or mass of animals is one of the most important factors influencing the ability of weight-bearing bones to develop or resist stress. A positive linear correlation ($r = 0.8648$, $r^2 = 0.7480$, $p < 0.0001$) 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 non weight-bearing bone such as the mandible. Therefore, it appears that mandible mass, and consequently the structural mandible strength, increased following the normal proportionality with body mass in all animals. In other words, growth retardation induced by maize proteins made animals have smaller bones. Therefore, the load at fracture, normalized by bone mass, was not different from that of 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, although the spatial distribution of this cortical bone could be an additional factor. However, the high positive correlation between the strength of the bone and its size suggests that the main affected variable was the mandible mass. The lower value 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 distribution of those small amounts in the E animals.

The large differences in mandibular strength between groups fed on the ED or C diets contrasted with the maintenance of normality of the elastic modulus and the maximum elastic stress, both indicative of the intrinsic properties of bone material, which depends on the constitution but not on its amount or spatial distribution, suggesting that the adverse effects caused by treatment may have been only quantitative in nature. The lack of effects of ED on both calcium concentration in ashes and the degree of mineralization could explain the normal mandibular bone rigidity.

In conclusion, we have described a number of alterations in both morphological and biomechanical variables in the rat mandible resulting from feeding growing rats for 40 days on a diet containing maize proteins. The clear differences in strength and stiffness of the bone between treated and control rats 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 mineralized material. Protein recovery in treated animals caused catch-up growth, which was complete or incomplete depending on the variable examined.

**ACKNOWLEDGMENTS**

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

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