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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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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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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-