HIGH ALTITUDE MEDICINE & BIOLOGY
Volume 14, Number 4, 2013
© Mary Ann Liebert, Inc.
DOI: 10.1089/ham.2013.1038

Static Biomechanics in Bone from Growing Rats Exposed Chronically to Simulated High Altitudes

Clarisa Bozzini, Graciela M. Champin, Rosa M. Alippi, and Carlos E. Bozzini

Abstract

Bozzini, Clarisa, Graciela M. Champin, Rosa M. Alippi, and Carlos E. Bozzini. Static biomechanics in bone from growing rats exposed chronically to simulated high altitudes. *High Alt Med Biol* 14:367–374, 2013.—Biomechanical behavior of bone is related to the amount (bone mass) and its architectural distribution, as well as the mechanical quality of bone material. This investigation reports the effects of exposure to different simulated high altitudes (SHA) (1850, 2900, 4100, and 5450 m) on femur biomechanical properties in female growing rats exposed to SHA (22–23 h/d) between the 32° and the 74° days of life. The ex vivo right femur was mechanically tested in three-point bending. The left femur was ashed at 600°C and the ash weight obtained. Final body weight and structural (loads at yielding and fracture, stiffness, and elastic energy absorption) and architectural (diaphyseal cross-sectional area, cortical area, and cross-sectional moment of inertia) were negatively affected in the animals exposed to the two highest SHA. Material properties of the mineralized tissue (Young's modulus and limit elastic stress) and the degree of mineralization were unaffected. In conclusion, hypoxic bone is weaker than normoxic one because of its smaller bone mass, which appear to have been negatively influenced by SHA in relation to its effects on overall body mass.

Introduction

Most of the bones that form the skeleton of vertebrates have a *support* function. 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 subjected (Ferretti et al., 1993).

Bones are made up of components that give them the capacity to *deform* under applied loads. The extent to which these components deform (and whether they will break) is governed by their *material properties. Biomechanics* is the branch of physics that analyzes the effects of forces to which a living structure is subjected. In the present study, the *static* aspect of biomechanics was analyzed, in which bones were considered as support organs and not as participants in either locomotion or postural reflexes. The support function of a bone is essentially a function of two mechanical properties: *stiffness* (or resistance to deformation) and *strength* (or resistance to fracture).

Several factors have been recognized to play an important physiological role in determining bone stiffness and strength. Both body weight and somatic muscles forces could 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 bones (Frost, 1987). However, other "nonmechanical factors" also exist that can modulate bone physiology, by either establishing or maintaining the mechanical competence of bones.

Body growth rate is one of them. Several reported studies (Boyer et al., 2005; Bozzini et al., 2012; Ferretti et al., 1991) suggest that the improvements of bone mechanical competence that occur in early life go *pari passu* with the increments in body mass as a consequence of body growth. Body growth rate is severely affected by exposure to hypobaric hypoxia (Alippi et al., 1983; Delaquierre et al., 1965; Morel et al., 2005; Timiras et al., 1957). Thus, one objective of the present investigation was to analyze the biomechanic behavior of the femur, a weight-bearing bone, in growing rats in response to chronic exposure to different simulated high altitudes. This treatment is known to alter body growth rate in proportion to the altitude level (Elia et al., 1985) and changes in bone mechanical properties could follow passively the alterations in body growth rate. Another objective was to separate the possible effect on bone quality associated with reduced body growth in hypoxic animals from another possible effect of hypoxia on this parameter, which does not appear to be as-

Department of Physiology, Faculty of Odontology, University of Buenos Aires, Argentina.

sociated to body growth. Studies conducted in our laboratory (Bozzini et al., 2009) have shown that long-term exposure to hypobaric hypoxia in adult rats, whose body growth rate was almost negligible, affected negatively the bone tissue material properties, probably in relation to the development of stress erythropoiesis in response to the hypoxic stimulation of erythropoietin secretion (Bozzini et al., 2008). This negative effect on bone material quality was offset by the improvement in diaphyseal cross-sectional design, thus allowing a normal biomechanical response to bending of the femur as a whole. In summary, the present study was thus performed to give an answer to the following question: Are the changes induced by chronic exposure of growing rats to SHA on bone biomechanical properties only associated to the negative effect of hypoxia on body growth rate or, in addition, does hypoxia induce alterations at the level of bone tissue properties?

Materials and Methods

Animals and experimental design

Female Sprague-Dawley rats aged 32 days and weighing 91.86 ± 9.86 g were used as experimental subjects. They were housed in stainless steel, wire-bottomed cages under a natural light-dark in a temperature-controlled (22°–24°C) room. The rats were divided into five groups of 7 animals each. Statistical power of the test was calculated and the null hypothesis can be accepted with confidence in all cases with the sample size employed. Hypobaric hypoxia was induced by placing animals into simulated altitude chambers in which the desired air pressure was maintained using vacuum pumps and adjustable inflow valves. Exposure was intermittent (minimum 22–23 h/day) with a daily interruption to replace food and water, clean animal cages, and perform experimental maneuvers when necessary, and lasted 42 days. During weekends, exposure was continuous. The different animal groups were exposed to air pressures of 608, 532, 456, and 380 mmHg, which correspond to 1850, 2900, 4100, and 5450 m of simulated altitude, respectively. Control rats were maintained at 760 mmHg (0 m). During the experimental period, body weight was registered periodically. At its end, final body weight was established. Rats were then sacrificed by ether overdose. The femurs were dissected and 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).

Biomechanical testing

On the day of testing, each bone was thawed at room temperature before analysis. To assess cortical bone mechanical properties, the right femur was tested in three-point bending (Hogan et al., 1999; Turner and Burr, 1993), which combines compression and tension. Each bone was placed horizontally with the anterior side facing down on two transverse supports and central along its length. The assayed bone portion was always within two-fifths of the total bone length. This condition rendered the method suitable for comparative purposes (Ferretti et al, 1993). Load was applied perpendicularly to the long axis of the bone until fracture. The test machine (Instron model 4442, Instron Corp., Canton, MA, USA) was operated in stroke control at a constant rate of 5 mm/min, which is useful for describing the static properties of the bone structure.

For this biomechanical test, load/deformation curves (W/d) showing both the elastic (Hookean behavior) and the plastic (non-Hookean behavior) phases separated by the yielding point (Fig. 1), enable graphic determination of the main structural mechanical properties of bone shafts as beams (Turner and Burr, 1993), which essentially measures the resistance to both deformation (stiffness) and fracture (strength) and the ability to absorb energy by deforming. The geometric properties were determined as follows: (a) *Bone length and diameters*: the femur length was measured directly using a stereomicroscope (Stenu DV4 Stereo microscope Carl Zeiss Microimaging, Gottinge, Germany) with an accuracy of ± 100 μm; (b) *Mid-diaphyseal cross-sectional area, CSA:* using a Isomet low-speed diamond saw (Buehler, Lake Bluff, IL, USA), a 2-mm cross-section slide was cut from the fracture section to perform regularized micromorphometrical determinations of the vertical (load direction) and horizontal (right angle to load direction) outer (HB) and inner (hb) diameters of the elliptic-shape fracture sections. Measurements were taken with a digital caliper with the aid of a magnifier 40×. CSA was calculated by applying the equation described in the appendix. (c) *Second moment of inertia of cortical bone (with reference to the anterior-posterior bending axis, xCSMI)* was estimated by the equation shown in the appendix. 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. As bones were tested in anterior-posterior bending, the selected reference axis was the "horizontal" diameters of the bone cross-section; and (d) *Bone volume between supports,* was estimated as shown in the appendix. Bone material properties (intrinsic properties of the mineralized tissue) were calculated from structural and geo-

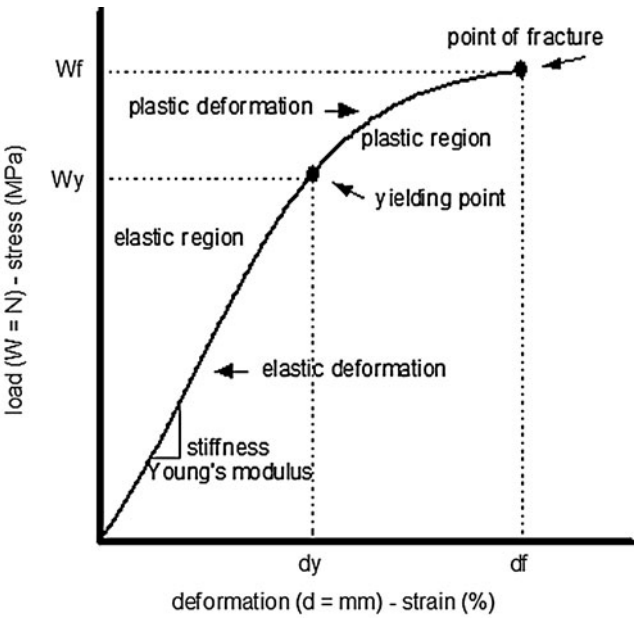

FIG. 1. The mechanical test generates a "*load/deformation*" (W/d) curve from which several parameters can be measured. These parameters can be normalized after adjusting for the sample size (cross-sectional area or moment of inertia), allowing load conversion to *stress* and deformation to *strain,* and obtaining the "*stress/strain*" curve. For more information, see appendix.

metric properties and not directly determined by mechanical means: they were the elastic modulus and the maximum elastic stress (see appendix).

Ashing of the specimens

The left femur 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 N HCl and calcium content determined by atomic energy absorption spectrophotometry (Willis, 1961). The *tissue degree of mineralization* (α), which expresses the percentage of mineral substance in the dried bone, was calculated as the ratio ash weight/dry bone weight.

Statistical analysis

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 test of Student-Newman-Keuls by using GraphPad Software (GraphPad Software Inc., San Diego, CA, USA).

The experiment was conducted in accordance with the principles outlined in the National Institute of Health Guide for the Care and Management of Laboratory Animals, and approved by the University of Buenos Aires Ethics Committee.

Results

Pre-experimental body weight did not differ between groups. As expected, body growth rate, as assessed by daily body weight gain, was negatively affected in rats exposed to SHA in close relation to the level of the exposure (Fig. 2A). While control rats grew at a rate of 3.45 ± 0.21 g/day, body weight gain was 3.16 ± 0.14 g/day in animals exposed to 1850 m ($p > 0.05$), 2.96 ± 0.04 g/day when exposure was 2900 m ($p > 0.05$), and 2.24 ± 0.09 g/day ($p < 0.01$) and 1.98 ± 0.15 g/day ($p < 0.01$) when the level of exposure was 4100 and 5450 m, respectively (Fig. 2B). The coefficient of

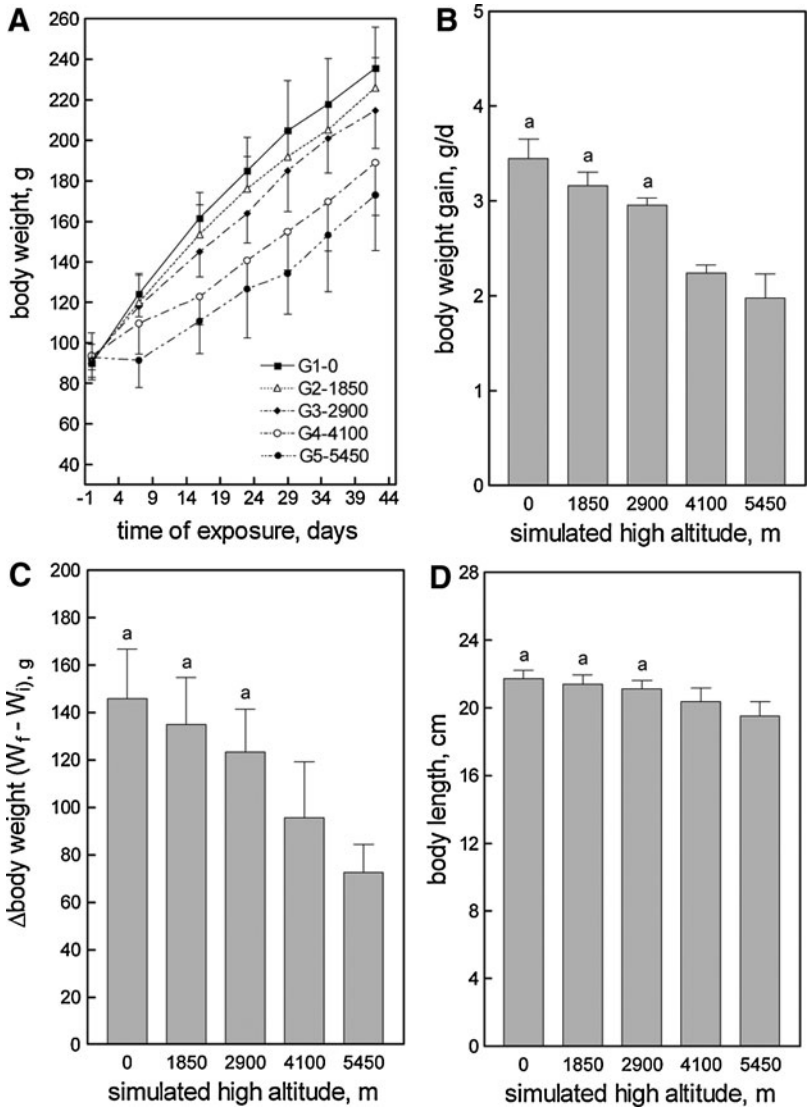

FIG. 2. Body weight as function of time of exposure to different simulated high altitudes **(A)**, and daily body weight gain **(B)**, difference between initial and final body weight **(C)**, and body length **(D)** as function of the level of the exposure to simulated high altitudes. Values are Mean ± SD. *Equal letters on the top of bars* indicate $p > 0.05$.

determination (r^2) between the slopes of the regression curves for body weight gain and SHA level was 0.9344 ($p = 0.0073$). As a consequence of the differences in daily body weight gain observed among groups in relation to the level of the altitude, final body weight was reduced by 5%, 9%, 20%, and 28% in increasingly hypoxic animals when compared to controls. Figure 2C shows that a difference was evident between initial and final body weights in exposed and non-exposed animals. The difference between both groups was only significant in the groups exposed to the two highest levels of SHA. The body length of the treated rats was similarly affected (Fig. 2D).

Changes in femoral weight and length, as well as in cross-sectional geometry of the femur diaphysis, are summarized in Figure 3. All parameters, with the exception of the medullary area that was increased, were significantly reduced in the rats exposed to 4100 and 5450 m of simulated altitude. The structural properties of the femoral diaphysis are shown in Figure 4. The load at yielding, the load at fracture, the diaphyseal stiffness, and the elastic energy absorption followed a similar behavior to that observed in the geometrical femoral properties: all were significantly and negatively affected by exposure to the two highest altitudes tested. It is noteworthy that a high correlation ($r = 0.8528$) between the load at fracture and body weight was observed (Fig. 4E) when data from all animals, normoxic and hypoxic, were included in the graph. The plastic/elastic ratio (Fig. 4F) did not differ significantly among groups. Bone material quality indicators, or pre-yielding bending stiffness (elastic modulus E) (Fig. 5C) and maximal elastic stress (Fig. 5D) were not affected by exposure to altitude, as was the concentration of calcium in ashes (Fig. 5A). However, the femoral calcium mass (Fig. 5B) was affected by exposure to the two highest altitudes in direct relation to their effects on bone size.

Discussion

Bone can be studied at several organization levels. The present investigation estimated mechanical properties of the entire bone as a structure, which incorporates the properties of the materials that compose the whole bone, as well as its internal and external geometry. The mechanical behavior of whole-bone specimens approximates the behavior of these structures *in vivo*. The study examined the effects of intermittent exposure to different simulated high altitudes on skeletal growth and appendicular bone biomechanics in female rats during days 32° and 74° of postnatal life. This period of life is characterized by a high body growth rate. The femur, a weight-bearing bone, was chosen as representative of the peripheral skeleton. The femoral mid-diaphysis in the rat is primarily composed of cortical tissue, whose primary function is strength and support. Therefore, this study really evaluated the effects of treatments on cortical bone biomechanics.

As expected, chronic exposure of growing rats to SHA depressed body growth rate as assessed from measurements of body weight gain, which is a gross indication of the overall

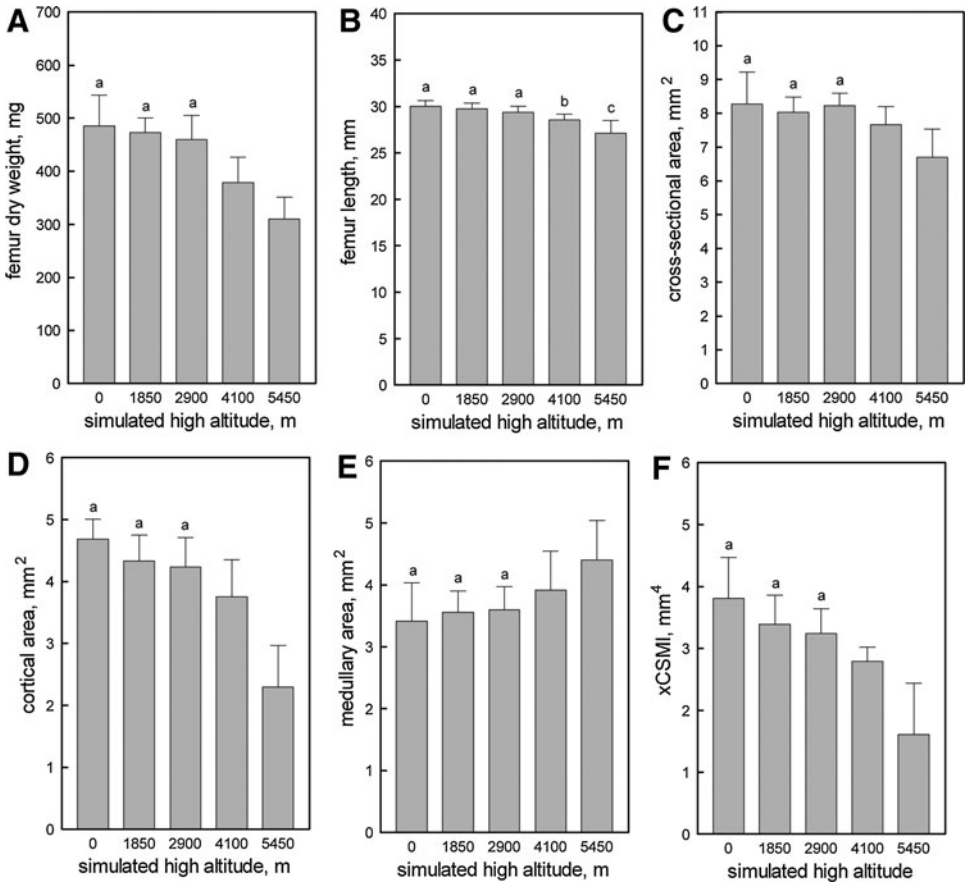

FIG. 3. Morphometric and geometric properties of the right femur in control (0) and experimental rats exposed to different levels of simulated high altitude. Values are Mean ± SD. *Equal letters on the top of bars* indicate $p > 0.05$.

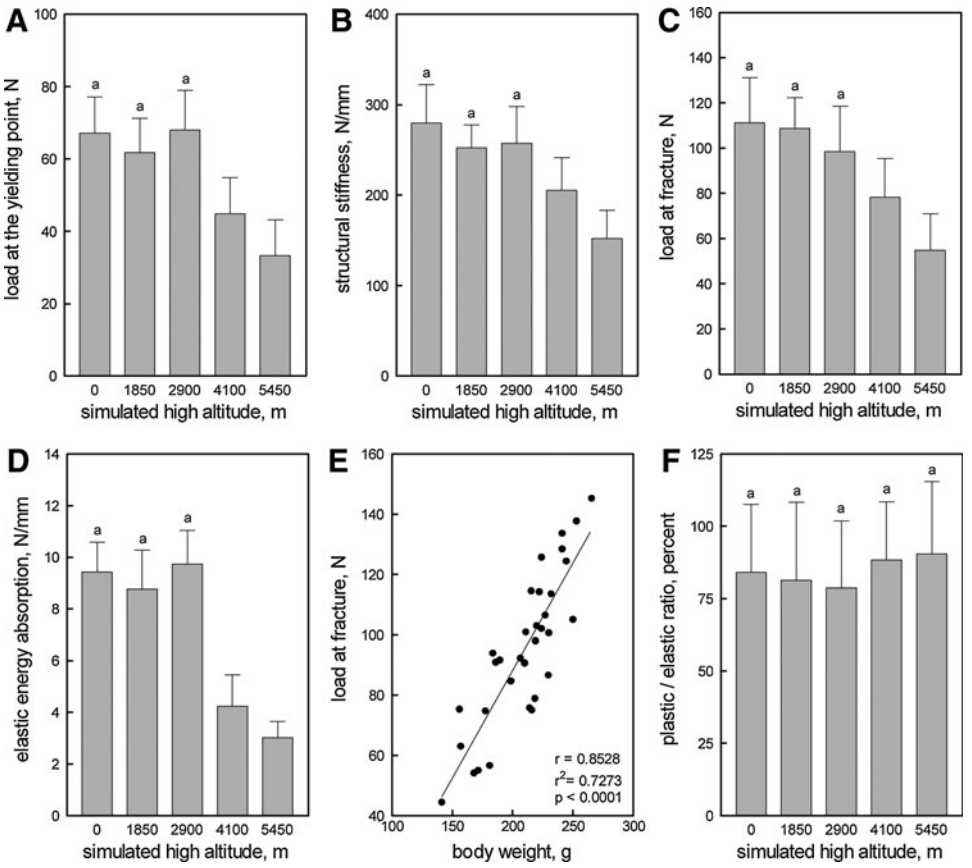

FIG. 4. Femoral structural properties in control (0) and experimental rats exposed to different levels of simulated high altitude. Values are Mean ± SD. *Equal letters on the top of bar* indicate $p > 0.05$.

growth of the animals. The longitudinal growth of the skeleton was also affected by altitude. Both types of growth were related to the level of the SHA, with significant depressions at simulated altitudes of 4100 m and 5450 m. The two other altitude levels tested (1850 m and 2900 m) also depressed growth, although values did not reach significance. When the slopes of the regression curves for daily body growth rates were related to the levels of the SHA, it was observed that exposed animals were unable to gain as much as 0.29 ± 0.04 g/day for every 1000 m of altitude ($r^2 = 0.9344$, $p = 0.0073$).

The skeletal functional integrity in both normoxic and hypoxic animals was assessed in the present experiment by the three-point bending test, which is a structural strength test that measures how well the whole bone can bears loads. Both the *structural stiffness*, which is a measure of the resistance to deformation under the applied load, and the *structural strength*, which represents the load required to fail the whole bone, were determined. These two whole bone measurements are structural properties and are influenced by both the material from which the structure is composed (tissue material properties), as well as how and where the material is distributed (the geometric form of the tissue).

The contribution of structural, geometric, and material analysis to the changes in bone quality observed in the animals exposed to SHA is pertinent to the present discussion. It has to be stated that the changes in bone quality was observed at all levels of exposure, although they were statistically significant only in animals exposed either to 4100 or 5450 m. Therefore, the discussion of the effects of altitude on bone quality will be only referred to these two latter groups of exposed animals.

Both the final weight and the length of the femur were undoubtedly affected by body growth retardation, as was the bone volume. The differences in cross-sectional area and cross-sectional moment of inertia indicate that the size of the bone, in terms of the mid-diaphysis cross-sections, was significantly affected.

These alterations were paralleled by a weakening of bone beams, shown by the differences in diaphyseal ultimate strength and stiffness. The other mechanical properties were also adversely affected in exposed rats. Based on this analysis alone, one would conclude that the hypoxic bone was weaker than the normoxic one and structurally incompetent. The body weight of animals is one of the most important factors that influences bone ability to develop or to resist stress. A positive linear correlation between the load at fracture of the femur and the body weight of all animals (normoxic + hypoxic) was established (Fig. 4E). Therefore, it appears that bone mass, and consequently the structural bone strength, grew up following the normal proportionality with body weight support in hypoxic rats. The "*plastic/elastic*" ratio, which represents the percent fraction of the fracture load that is supported in plastic conditions, was not significantly different between normoxic and hypoxic rats. Thus, the amount of bone resistance to load while undergoing microcracks in hypoxic animals was normal, as apparently was the state of the microstructural determinants of the bone material properties.

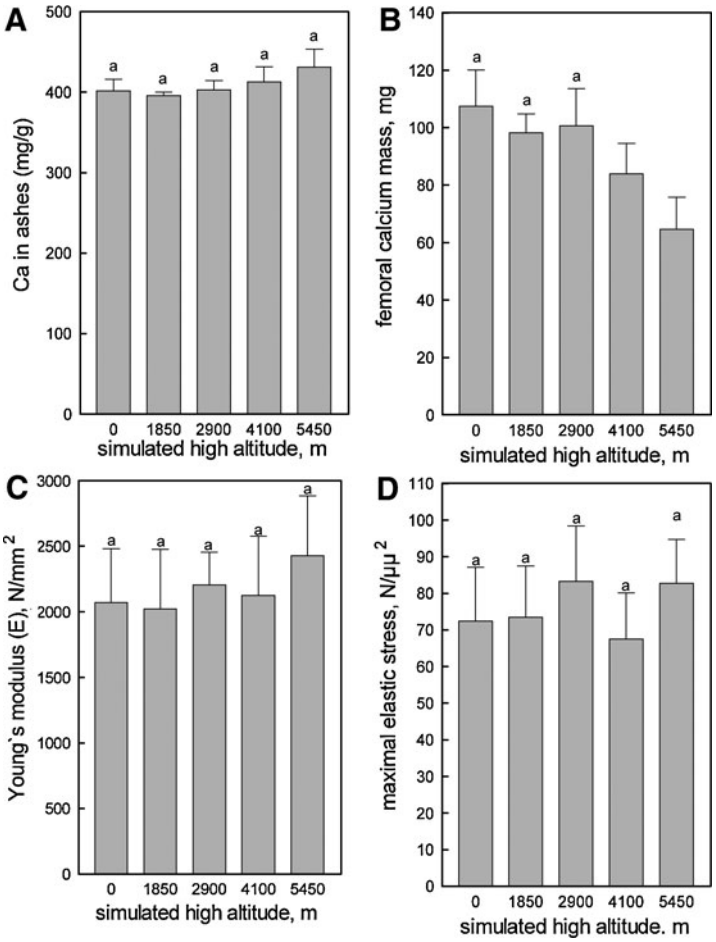

FIG. 5. Calcium concentration in ashes **(A)**, femoral calcium mass **(B)**, and intrinsic material properties of the mineralized tissue **(C + D)** in control (0) and experimental rats exposed to different levels of simulated high altitude. Values are Mean ± DS. *Equal letters on top of bars* indicate $p > 0.05$.

The above discussion suggests that the impaired performance of diaphyseal shafts induced by exposure to SHA is the result of changes in the amount of cortical Ca bone mass and cortical bone volume. However, the spatial distribution of the cortical bone could be an additional factor (Fig. 3F). This possibility is supported by the high positive correlation found between the strength of bone beams and their sectional moment of inertia ($r = 0.7591$, $p < 0.0001$). However, the high correlation coefficient ($r = 0.8023$, $p < 0.0001$) between the xCSMI and bone Ca content for all groups together in the same graph (data not shown) suggests that there were not direct effects of exposure to SHA on the distribution of bone mineralized tissue within the diaphyseal cross-sections. Therefore, the affected variable was only bone mass (normally related to body weight), not bone mass distribution. 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-section, and not necessarily the distribution of those small amounts of mass in hypoxic animals.

The significant differences in diaphyseal strength between normoxic and hypoxic rats contrasted with the maintenance of normality of the elastic modulus (Fig. 5C) and the maximal elastic strain (Fig. 5D), both indicative of intrinsic properties of bone material, which depends on the constitution but not on its amount or spatial distribution. This further suggests that the adverse effects evoked by treatment may have been only quantitative in nature. The natural stimuli for the bone mechanostat would be the strain of bone tissue, sensed by osteocytes that are induced by both gravitational forces and tensions developed by regional muscles activity (Frost, 1966). Therefore, in a weight-bearing bone as the femur, different body weights will produce different loads and strains.

Exposure to SHA induced significant changes in the femoral Ca mass (Fig. 5B), although did not alter Ca concentration in ashes (Fig. 5A). The proportion of mineralized tissue in the whole bone was not affected by exposure. Therefore, it seemed that the cortical femoral shafts in the treated animals were not different from normal with respect to the relative content of mineralized mass and its Ca content.

In summary, we could conclude that the bone in the rats exposed to 4100 and 5450 m of SHA was weaker than that of animals either exposed to lower altitudes or not exposed and, therefore, structurally incompetent. Alter analyzing its geometry, we learned that the considered bone was smaller than that of the latter groups, showing a significant reduction in the cross-sectional area and the cross-sectional moment of inertia. However, the estimated material properties, the elastic modulus, and the maximal elastic stress, as well as the ash fraction and Ca concentration, were similar in all groups. Therefore, based on the structural, geometrical, and material properties

of the studied bones, we might conclude than the bone in the groups exposed to the highest levels of SHA is weaker than that of the groups exposed to the lowest levels or not exposed to altitude because of its smaller bone mass, which appear to have been significantly influenced by hypoxia in relation to its effects on overall bone mass.

It has to be noted that exposure to SHA in this investigation was relatively short. Therefore, the study does not provide more evidence that prolonged sojourns at high altitude may increase fracture risk. It is remarkable that exposure of adult rats to SHA (Bozzini et al., 2009) affected the bone tissue material properties negatively, an effect that was offset by the improvement in diaphyseal cross-sectional design, thus allowing a normal biomechanical response to bending of the femur as a whole. This study in adult rats, as well as the present one in growing rats, may suggest that exposure to SHA does not increase fracture risk, at least in rats. In order to get more information on the problem, a study performed on rats born at SHA and followed for 6 months in the altitude is currently under investigation in this laboratory.

Acknowledgments

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

Author Disclosure Statement

Authors declare no institutional or commercial affiliations that might pose a conflict of interest regarding the publication of the manuscript.

References

Alippi RM, Barceló AC, Río ME, and Bozzini CE. (1983). Growth retardation in the early developing rat exposed to continuous hypobaric hypoxia. Acta Physiol Latinoam 33:1–5.

Boyer PM, Campagnucci GE, Olivera MI, Bozzini C, Roig MC, Campagnucci CV, and Alippi RM. (2005). Bone status in an animal model of chronic sub-optimal nutrition: A morphometric, densitometric and mechanical study. Brit J Nutr 93:663–669.

Bozzini CE, Olivera MI, Conti MI, Martínez MP, Guglielmotti MB, Bozzini C, and Alippi RM. (2008). Decreased femoral diaphyseal mechanical strength mainly due to qualitative impairment of cortical tissue in growing rats with stress erythropoiesis. Comp Clin Pathol. 17:1722.

Bozzini C, Olivera MI, Huygens P, Alippi RM, and Bozzini CE. (2009). Long-term exposure to hypobaric hypoxia in rats affects femur cross-sectional geometry and bone tissue material. Ann Anat 191:212–217.

Bozzini C, Bozzini CE, and Alippi RM. (2012). Biomechanical properties of femoral shafts and bone tissue in protein-malnourished rats from weaning to adulthood. Comp Clin Pathol 21:1159–1165.

Delaquierre-Richardson L, Forbes S, and Valdivia E. (1965). Effect of simulated high altitude on the growth rate of albino guinea pigs. J Appl Physiol 20:1022–1025.

Elia R, Elgoyhen AB, Bugallo G, Rio ME, and Bozzini CE. (1985). Effect of exposure to reduced atmospheric pressure on body weight, food intake and body composition of growing rats. Acta Physiol Latinoam 35:311–318.

Ferretti JL, Capozza RF, Mondelo M, and Zanchetta JR. (1993). Interrelationship between densitometric, geometric and mechanical properties of rat femur. Inferences concerning mechanical regulation of bone modeling. J Bone Min Res 8:1389–1395.

Ferretti JL, Capozza R, Cointry G, Bozzini C, Alippi RM, and Bozzini CE. (1991). Additive effect of dietary protein and energy deficiencies on diaphysis and bone tissue of rat femurs as determined by bending tests. Acta Physiol Pharmacol Toxicol Latinoam 41:253–262.

Frost HM. (1966). Bone mass and the "mechanostat". Anat Rev 219:1–9.

Frost HM. (1987). Strain and other mechanical influences of bone strength and maintenance. Curr Opin Orthop 8:60–70.

Hogan HA, Groves JA, and Simpson HW. (1999). Long-term alcohol consumption in the rat affects cross-sectional geometry and bone tissue material properties. Alcohol Clin Exp Res 23:1825–1833.

Morel OE, Aubert R, Richalet JP, and Chapelot D. (2005). Simulated high altitude decreases protein intake and lean body mass gain in rats. Physiol Behav 86:145–153.

Timiras PS, Krum AA, and Pace N. (1957). Body and organ weights of rats during acclimatization to an altitude of 12470 feet. Am J Physiol 191:598–604.

Turner CH, and Burr DB. (1993). Basic biomechanical measurements of bone: A tutorial. Bone 14:595–608.

Willis JB. (1961). Determination of calcium and magnesium in urine by atomic absorption spectroscopy. Anat Chem 33:556–559.

Address correspondence to:
Professor Carlos E. Bozzini
Department of Physiology
Faculty of Odontology
University of Buenos Aires
Marcelo T. de Alvear 2142
Buenos Aires 1122
Argentina

E-mail: cebozi@fisio.odon.uba.ar

Received March 25, 2013;
accepted in final form, June 26, 2013.

(*Appendix follows* →)

Appendix

Figure 1: 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 o 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: relative small increments of load causes relative 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. Df, deformation at the fracture point; dy, deformation at the yielding point; Wf, load at fracture; Wy, load at the yielding point.

Bone biomechanics: It is assumed that the "mechanical properties" of bones integrated as organs (whole-bone level = structural properties) are directly related to both the "amount" (bone mass) and the "architectural distribution" of the mineralized tissue (geometric properties), and the mechanical "quality" of bone material (material properties). **(A)** STRUCTURAL PROPERTIES are: (1) *load at yielding (Wy)* (maximal elastic deflection, elastic limit, yield deflection) represents the value of the load at the upper extent of the linear region (yielding point) and indicated the appearance of the first microcracks that occur on the periosteal surface of the bone; (2) *structural stiffness* (load/deflection relationship, diaphyseal stiffness, bone beam's rigidity, or slope of the linear phase of the W/d curve) represents the rigidity of the beam or the resistance to deformation; (3) *load at fracture (Wf)* (structural strength, whole-bone strength, maximal supported load, ultimate load) represents the value of the load at fracture and express directly the resistance of the whole bone to fracture, incorporating both the elastic and the plastic behaviors; and (4) *elastic energy absorption* represents the energy necessary to initiate the first microcracks. **(B)** GEOMETRIC PROPERTIES (bone design characteristics): (1) *bone length and diameters;* (2) *mid-diaphyseal cross-sectional area (CSA)* was calculated by applying the equation: CSA = 3.14 (HB – hb) 4 mm^2; (3) *second moment of inertia of cortical bone* (with reference to the anterior-posterior bending axis, xCSMI) was estimated by the equation xCSMI = (3.14 [$B^3H - b^3h/64$]. B = vertical outer diameter, H = horizontal outer diameter, b = vertical inner diameter, h = horizontal inner diameter); and (4) *bone volume between supports* ($L\pi$ [HB – hb]); and **(C)** BONE MATERIAL PROPERTIES (intrinsic properties of the mineralized tissue), as calculated from structural and geometric properties: (1) *Young's modulus of elasticity* (bone material stiffness, intrinsic stiffness, stress-strain relationship) calculated by the formula: $WyL^3/48\,dy.Ix$, where dy = maximal elastic deflection, L = distance between supports, Ix = second moment of inertia of the cross-section in relation to the horizontal axis, Wy = load at the yielding point; 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: $\sigma = LBWy/8Ix$. B = vertical outer diameter of the regularized fracture section.