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ORIGINAL ARTICLE

Biomechanical properties of the mid-shaft femur in middle-aged hypophysectomized rats as assessed by bending test

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Abstract Both stiffness and strength of bones are thought to be controlled by the "bone mechanostat". Its natural stimuli would be the strains of bone tissue (sensed by osteocytes) that are induced by both gravitational forces (body weight) and contraction of regional muscles. Body weight and muscle mass increase with age. Biomechanical performance of load-bearing bones must adapt to these growth-induced changes. Hypophysectomy in the rat slows the rate of body growth. With time, a great difference in body size is established between a hypophysectomized rat and its age-matched control, which makes it difficult to establish the real effect of pituitary ablation on bone biomechanics. The purpose of the present investigation was to compare mid-shaft femoral mechanical properties between hypophysectomized and weight-matched normal rats, which will show similar sizes and thus will be exposed to similar habitual loads. Two groups of 10 female rats each (H and C) were established. H rats were 12-month-old that had been hypophysectomized 11 months before. C rats were 2.5-month-old normals. Right femur mechanical properties were tested in 3-point bending. *Structural* (load-bearing capacity and stiffness), *geometric* (cross-sectional area, cortical sectional area, and moment of inertia), and *material* (modulus of elasticity and maximum elastic stress) *properties* were evaluated. The left femur was ashed for calcium content. Comparisons between parameters were performed by the Student's *t* test. Average body weight, body length, femur weight, femur length, and gastrocnemius weight were not significantly different between H and C rats. Calcium content in ashes was significantly higher in H than in C rats. Cross-sectional area, medullary area, and cross-sectional moment of inertia were higher in C rats, whereas cortical area did not differ between groups. Structural properties (diaphyseal stiffness, elastic limit, and load at fracture) were about four times higher in hypophysectomized rats, as were the bone material stiffness or Young's modulus and the maximal elastic stress (about 7×). The femur obtained from a middle-aged H rat was stronger and stiffer than the femur obtained from a young-adult C rat, both specimens showing similar size and bone mass and almost equal geometric properties. The higher than normal structural properties shown by the hypophysectomized femur were entirely due to changes in the intrinsic properties of the bone; it was thus stronger at the tissue level. The change of the femoral bone tissue was associated with a high mineral content and an unusual high modulus of elasticity and was probably due to a diminished bone and collagen turnover.

Keywords Bone biomechanics · Hypophisis · Rat femur · Bone quality

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Introduction

The effect of hypophysectomy on several physiological variables in the rat has been extensively studied. In relation to bone, adenohypophyseal hormones are important for normal growth of bone as well as maintenance of skeletal mass. Hypophysectomy in young rats slopes the rate of growth with diminution of longitudinal and radial bone growth [1–5], loss of cancellous bone [4], and diminution

of cortical bone gain [3, 5]. The causes of skeletal alterations in hypophysectomized rats are associated with decreases in growth- and modeling-dependent bone gain and bone turnover [3, 5]. Additional biological changes associated with hypophysectomy are reduction in body weight gain and lean body mass [6, 7] and development of permanent physiological anemia [8].

It is assumed that the mechanical properties of long bones integrated as organs are directly related to the amount (*bone mass*), the architectural disposition of bone material, and the mechanical quality of bone material [9, 10]. The structural stiffness (measurable as a load/deformation ratio) is usually kept high enough to withstand the every day bone deformation to avoid damage and hence fracture. The structural stiffness, and indirectly the strength of bones, is thought to be controlled by the "bone mechanostat" [11]. This is a feedback mechanism, which optimizes the bone's design through a permanent re-distribution of the mineralized tissue.

Both body mass and regional muscles mass increase with age in a normal rat. Therefore, a weight-bearing bone, like the femur, will increase its mass and adapt its mechanical properties with time in order to satisfy the mechanical demands imposed by growth. Hypophysectomy is characterized by growth failure, which will be responsible, from a theoretical point of view, for the lack of the age and growth-related increment in both body and bone mass. As a consequence, the latter parameters will be significantly lower in a pituitary ablated rat when compared to an age-matched normal rat. The difference will increase in relation to the time after surgery until the normal rat attains its peak of growth. The different growth patterns between normal and hypophysectomized rats create a great difficulty in analyzing the real effect of hypophysectomy on mechanical bone quality in the rat. In general, bones of different sizes should not be compared. One way to minimize the different structural properties that are observed between bones with different mass is the standardization of those properties by relating them to some allometric variables (the body weight of the animals, the mid-diaphyseal cross-sectional area, the bone ash content, etc.). This has been the procedure occasionally employed to compare results from rats of different body size.

In spite of the diminution of linear growth that follows hypophysectomy in the juvenile rat, we have observed that the biomechanical quality of the femoral shaft changes with time: the juvenile femur is transformed in a type of adult bone that is characterized by a high mineral density and an unusual large modulus of elasticity [12]. These findings suggest that the femur of the hypophysectomized rat is "overdesigned" in relation to the mechanical loads acting on it. A similar "stiffening" response was also evident in the mandible, which is not a weight-bearing bone but subjected to the loads created during mastication (load-bearing bone) [13]. The increased elastic modulus of cortical bone in 75-day-old hypophysectomized rats has been also observed by Feldman et al. [14].

In order to determine the real effects of hypophysectomy on femoral bone biomechanics, it would be desirable to compare bones obtained from normal and hypophysectomized rats both having similar body weights and regional muscular mass. Under these conditions, bones should be exposed to similar load-induced strains and the effects of the absence of adenohypophyseal hormones on bone quality could thus be estimated, by assuming that hypophysectomy does not change the skeletal response to mechanical loading. This brings on to another situation: femora obtained from pituitary-ablated and control rats sharing similar body and muscle masses should have been obligatory obtained from rats of different ages. In relation to age, rats can be considered as young adults (3-month old), mature adults (6-month-old), middle-aged adults (12-month-old), and aged adults (24-month-old). In general, the elastic deformation of aged rats was significantly impaired both at the tissue and the organ levels with increasing age, probably associated with increased mineralization, crystallinity, and type-B carbonate substitution [15]. However, the maximum breaking force required to fracture femurs at mid-shaft did not change with age because architectural compensations, even though the normalized tissue strength decreased with age [16]. The above changes with age were observed in aged adult animals and were not apparent neither in young adults nor in mature adults. In the experiments reported here, we used normal rats aged 2.5 months (young adults) and hypophysectomized rats aged 12 months (middle-aged adults). The ages examined in the present study avoid the large amount of bone modeling and skeletal growth characteristic of very young rats (<2-month-old) and ensure the achievement of peak bone mass in older rats, which occurs at $\sim$10 months in female rats. Concerning these particular ages, it has been demonstrated [15, 16] that (1) periosteal and endosteal diameters were not significant different between young and middle-aged rats; (2) that the cross-sectional area of the mid-femoral diaphysis significantly increased with age, being about 20% higher in middle-aged rats than in young rats; (3) moment of inertia about the bending axis of middle-aged rats was about 25% higher than in young rats; and (4) that structural stiffness, yield stress, resilience, and bending modulus were not different between young rats and middle-aged rats Thus, by mainly considering the findings mentioned in the last paragraph**,** it is possible to assume that any difference in bone quality found between both types of animals should not be related to age.

The present investigation was designed to estimate the mechanical quality of two bones showing similar sizes and

exposed to similar habitual loads. One of them was obtained from a normal young-adult rat, the other bone from a middle-aged hypophysectomized rat. The purpose of the study was to increase our knowledge in an attempt to explain the reasons that transform a normal bone into an "overdesigned" bone after hypophysectomy.

Materials and methods

Experimental design

Two groups of 10 female Sprague–Dawley rats each (hypophysectomized [H] and control [C]) were established. H rats were 12-month old (middle-aged adults) and had been hypophysectomized 11 months before by the standard parapharyngeal approach. They were purchased from Charles River Laboratory International Inc., Wilmington, USA. C rats were 2.5-month-old (young adults). H rats weighed 138.4 ± 7.06 g, and C rats weighed 139.0 ± 7.97 g ($P > 0.05$) at the time of autopsy. C rats were thus weight-matched with H ones, but not age-matched. During the pre-autopsy period (10 months for the H rats, 2.5 months for the C rats), all animals were allowed free access to water and to a standard pelleted chow diet that has been shown to meet all necessary requirements to allow normal growth and development of rats [17]. Animals were maintained under local vivarium conditions (temperature 22–23°C, 12-h on/off light cycle). At the time of autopsy, both 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 femurs were dissected, cleaned of adhering soft tissue, weighed in a Mettler scale and their lengths measured. They were then stored at −20°C wrapped in gauze soaked with Ringer's solution in sealed plastic bags, in accordance with Turner and Burr [18]. Gastrocnemius muscles were dissected and weighed immediately.

Mechanical testing of femurs

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 3-point bending [19]. Each bone was placed horizontally with the anterior side facing down on two transverse support ($L = 13$ mm span) and central along its length. Load was applied perpendicularly to the long axis on 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* (*W/d*) *curves* (Fig. 1) showing both the *elastic* (Hookean behavior) and the *plastic* (non-Hookean behavior) phases separated by the *yielding point*, enabled graphic assessment of the main *structural* mechanical properties of the bone shafts as beams [18] which essentially measures the resistance to both deformation (*stiffness*) and fracture (*strength*) and the ability to absorb energy by deforming. 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 behavior): (1) *maximal stress deflection* (yield deflection d_y, elastic limit or load at the yielding point W_y) represents the endpoint of elastic deformation (*yielding point)* and defines a threshold above which unrecoverable permanent deformation occur, marking the initiation of damage accumulation with the appearance of the first microcracks

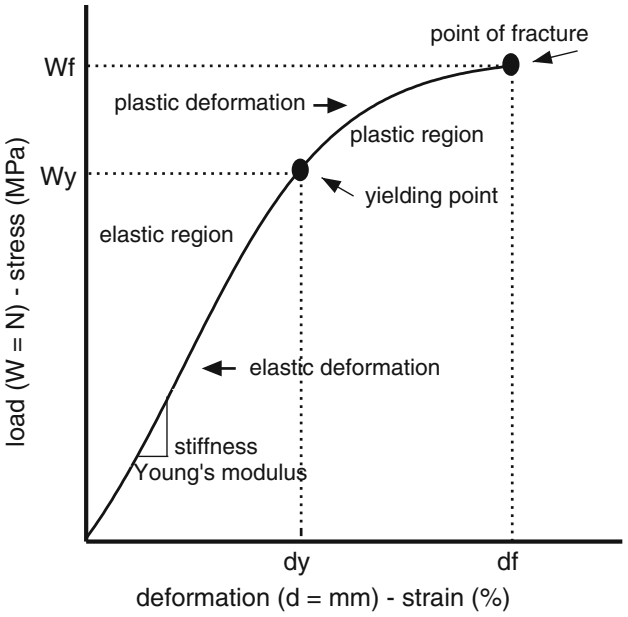
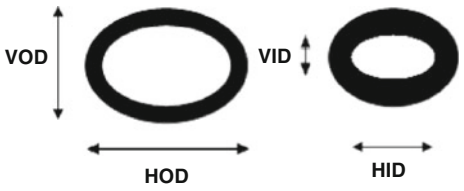


Fig. 1 The mechanical test generates a "load/deformation" (*W/d*) curve from which several parameters can be measured. These parameters can be normalized after adjusting for the sample size (cross-sectional area or moment of inertia), allowing load conversion to *stress* and deformation to *strain*, and obtaining the "stress/strain" curve. 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 (stress) is removed, bone returns to its original shape. After the yielding point, increasing load (stress) causes permanent damage to the bone structure: relative small increments of load (stress) causes relative large increments of deformation (strain) (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 (extrinsic property) when obtained from the *W/d* curve. When obtained from the *S/S* curve, it is called *Young's modulus* and is an index of the stiffness of the bone material (intrinsic property). *Strength*, the other important bone property, can be defined either by the ultimate load at fracture or by the load at yield. W_f load at fracture, W_y load at the yielding point, d_f deformation at the fracture point, d_y deformation at the yielding point

Table 1 Femur diaphyseal geometric properties



Parameter	C	H	*P*
Horizontal OD (mm)	3.47 ± 0.20	3.02 ± 0.28	<0.001
Horizontal ID (mm)	2.48 ± 0.26	1.58 ± 0.25	<0.0001
Vertical OD (mm)	2.48 ± 0.15	2.13 ± 0.09	<0.0001
Vertical ID(mm)	1.64 ± 0.17	0.94 ± 0.18	<0.0001
CSA (mm^2)	6.76 ± 0.65	5.05 ± 0.52	<0.0001
CtA (mm^2)	3.53 ± 0.48	3.86 ± 0.37	>0.05
MA (mm^2)	3.23 ± 0.68	1.19 ± 0.34	<0.0001
WLR	0.46 ± 0.10	1.15 ± 0.39	<0.0001
xCSMI (mm^4)	2.058 ± 0.38	1.36 ± 0.21	<0.0002
VOL (mm^3)	45.90 ± 6.30	50.2 ± 4.80	>0.05

All data are expressed as mean ± SD

CSA cross-sectional area, *CtA* cortical area, *MA* medullary area, *WLR* wall/lumen rati, *xCSMI* cross-sectional moment of inertia, *VOL* volume between supports (see text for explanation of the upper diagram)

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 beam' rigidity, or slope of the linear phase of the W/d curve) represents the rigidity of the beam or the resistance to deformation; (3) *elastic absorption of energy by the whole bone* (the total energy absorbed by the specimen up to the yielding point) represents the energy necessary to initiate the first microcracks and permanently affect bone structure, and (4) *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 behaviors. (b) *geometric properties* (bone design characteristics). They are: (1) *bone length and diameters*: the bone length was measured directly using a digital caliper (Digginess, Geneva, Switzerland) with an accuracy of ±100 μm; (2) *mid-diaphyseal cross-sectional area*, *CSA*: using an Isomet low-speed diamond saw (Buheler, Lake Bluff, IL, USA) the fracture section was regularized to perform micro-morphometrical determinations of the *vertical* (load direction) and *horizontal* (right angle to load direction) *outer* (VOD, HOD) *and inner* (VID, HID) *diameters* of the elliptic-shaped fracture sections (Table 1). Measurements were taken directly using a stereomicroscope (Stenu DV4, Carl Zeiss Microimagen, Gottingen, Germany) with an accuracy of ±0.01 mm. CSA was calculated by applying the equation: $CSA = 3.14(VOD \cdot VID - HOD \cdot HID)/4$; (3) *second moment of inertia of cortical bone (with reference to the anterior–posterior bending axis*, *xCSMI*) as estimating by the equation: $xCSMI = (3.14 [VOD^3 \cdot HOD - VID^3 \cdot HID/64])$. 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. As bones were tested in anterior-posterior bending, the selected reference axis was the "horizontal" diameter of the bone cross section, and (4) *bone volume between supports:* $(L\pi [HOD - HID])$; and (4) *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. They are: (1) *Young's modulus of elasticity* (*bone material stiffness*, *intrinsic stiffness*, *strain–stress relationship*) calculated by the formula: $E = W_y \cdot L^3/48 \cdot d_y \cdot I_x$ (W_y = load at the yielding point, L = distance between supports, d_y = maximal elastic deflection, I_x = second moment of inertia in relation to the horizontal axis); (2) *maximal elastic stress*, which expresses the reacting force opposed by the deformed bone to the deforming load (estimation of tissue strength). It was calculated by the formula: $\sigma = L \cdot VOD \cdot W_y/8I_x$; and (3) *energy absorbing capacity* (EAC, expressed per unit of bone tissue volume, EAC/vol). It should be pointed out that estimating intrinsic material properties using 3-point bending and beam theory is not without limitations [18]. Notable sources of artifact include displacement due to indenting at the contact points and nonuniformity of the cross-sectional geometry in the loaded portion of the diaphysis. Although these undoubtedly

diminish the accuracy of the derived quantities as true material properties in an absolute term, they are still deemed useful and meaningful for comparative purposes.

Bone ash determination

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 absorption spectrophotometry [20]. Femoral calcium content corresponds to the amount of calcium in ashes.

Statistics

Results were summarized as mean ± SD and differences were considered statistically significant at the level of $P < 0.05$. Comparisons between parameters were performed by the Student's t test by using GraphPad Prism 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

At the time of autopsy, average body weight was 139.0 ± 7.97 g in C rats and 138.4 ± 7.06 g in H rats ($P > 0.05$). Body length (C = 30.35 ± 0.70 cm; H = 30.85 ± 0.34 cm), femur weight (C = 501.3 ± 30.2 mg; H = 528.9 ± 36.2 mg), and femur length (C = 24.51 ± 0.52 mm; H = 25.1 ± 0.34 mm) were not significantly different between C and H rats. Femoral calcium content was significantly greater ($P < 0.0003$) in H than in C rats (137.9 ± 36.2 vs. 62.6 ± 16.6 mg, respectively). The weight of the gastrocnemius was 249.2 ± 30.3 mg and 225.4 ± 32.2 mg in C and H rats, respectively ($P > 0.05$).

Values of cross-sectional geometry of the femur mid-diaphysis are shown in Table 1 that includes a schematic diagram showing an approximate representation of the cross-sectional area in C and H rats. The shaded region of each diagram roughly represents the thickness of the cortical mineralized area (CtA), which was higher (44%) in H than in C rats. Both horizontal and vertical diameters were significantly higher in the C than in the H groups, as were the cross-sectional area (CSA) and the medullary area (MA). The cortical area (CtA) did not significantly differ between both groups and thus explains the higher cortical wall thickness and the wall/lumen ratio observed in the H animals. The cross-sectional moment of inertia (xCSMI), which was calculated by considering the four diameters measured at the regularized cross-section, was higher in C than in H rats. Structural properties, as derived from the slope of the load/deformation curve in the linear region of the elastic behavior, are shown in Table 2. The values for diaphyseal stiffness, the elastic limit, and the load at fracture were 4.48, 4.44 and 4.00 times higher, respectively, in H than in C rats. The elastic absorption of energy by the whole bone was also significantly higher in the former than in the latter (2.08 times). Bone material or intrinsic properties of the mineralized tissue, as derived from structural and geometric properties, are also shown in Table 2. Bone material stiffness or Young's modulus of elasticity E and the maximal elastic stress were 7.55 and 7.89 times higher, respectively, in H than in C rats. The elastic energy absorption related to bone tissue volume, was also higher in the former than in the latter (2.08 times).

Table 2 Femur diaphyseal mechanical properties in normal and hypophysectomized rats

Parameter	C	H	*P*
Extrinsic			
Stiffness (N/mm)	63.89 ± 25.60	286.39 ± 37.87	<0.0001
Elastic limit (N)	17.40 ± 4.29	77.36 ± 10.82	<0.0001
Fracture load (N)	23.73 ± 5.74	95.01 ± 12.60	<0.0001
EEA (N/mm)	3.05 ± 2.01	9.80 ± 1.78	<0.0001
Intrinsic			
E (N/mm^2)	1348.0 ± 356.0	10182.0 ± 413.0	<0.0001
S_{el} (N m^2)	1.94 ± 0.73	15.31 ± 1.99	<0.0001
EEA/vol (N/mm/mm^3)	0.085 ± 0.051	0.177 ± 0.062	<0.01

All data are expressed as mean ± SD

EEA elastic energy absorption, E elastic modulus, S_{el} limit elastic stress

Discussion

During the course of the current investigation, we evaluated the biomechanical properties of the femoral mid-diaphysis of bones obtained from rats sharing similar body weights but different ages and endocrine-metabolic status: one group of animals was formed by normal young adult rats, the other being integrated by middle-aged adult animals that had been hypophysectomized 11 months before. Based on previously reported studies [15, 16], we have assumed that the differences found between both groups in relation to their femoral biomechanical quality were independent of the age of the animals. This assumption is further enhanced by data shown in our previously published work of the subject: the elastic modulus E was 2.5 times greater in rats after 5 months of hypophysectomy

than in age-matched normal rats. Body weight in the latter group was 4.18 times higher than that in the hypophysectomized group [12]. However, it should be remembered that for a similar body weight, the body composition of the hypophysectomized rat differs from that of a normal rat, the increment of fat and the diminution of protein contents being the main observations [6, 7]. It is not known whether these differences could be responsible, at least partially, for the changes induced by hypophysectomy on bone biomechanics. Finally, bone size and strength are usually related not only to body weight but also to regional muscle mass and strength (customary strain stimulus) [10, 21]. The weight of the gastrocnemius, one of the regional muscles involucrated, did not differ significantly between C and H rats in this study. These considerations should indicate that the chosen experimental model was suitable for the purposes of the current investigation.

For a similar body weight and regional muscle mass, the femoral diaphysis of the middle-aged hypophysectomized rat was stronger than that of a young-adult normal rat. The indicators of whole-bone quality (assimilable to resistance to fracture) were significantly higher in the former than in the latter. Bones fracture when external stresses exceed the local capacity of the material to withstand them [22].

Multiple factors contribute to bone strength. For the present discussion, it is pertinent to briefly consider only those factors that can make a bone stronger that, according to Turner [23], are the increment of the bone mass, its more efficient distribution, and the improvement of the bone material properties (stronger at the tissue level). Thus, it seems necessary to consider the changes, if any, induced on those factors by hypophysectomy in an attempt to give a plausible explanation for the bone effects described here.

Bone size is recognized as an important component of bone strength [24]. Structural dimensions or geometry determine the stresses that bones can handle under loading conditions [22]. From a mechanical standpoint, increasing the external diameter of a cylinder increases resistance to flexion [18]. When loads act on a long bone in flexion or traction, the efficiency of the sectional design depends on the endosteal and periosteal diameters, the absolute and relative (wall/lumen ratio) thickness of the cortical mineralized tissue, and the cross-sectional moments of inertia. Neither the femur size (weight and length) nor the mineralized cortical area (mm^2) measured at the mid-shaft cross-section significantly differed between H and C rats. However, both the endosteal and periosteal diameters and the cross-sectional moment of inertia were higher in C than in H rats, whereas the thickness of the cortical mineralized tissue and the wall/lumen ratio were higher in H than in C animals. According to Frost [25], the "mass" factor (amount of bone in a bone's cross-section) and the "architectural" factor (cross-sectional and longitudinal shapes and size of a bone and the distribution of its compacta) affect its strength. Therefore, from the analysis of both factors in the bones of C and H rats, it would be possible, from the geometrical point of view, that the femoral mid-shafts of the C rats should be stronger than that of the H rats because of the higher cross-sectional area and the cross-sectional moment of inertia. The latter appears to be the most important of the geometric factors in the determination of the resistance to deformation under elastic conditions. However, if one analyze the equation for the calculation of deformation ($d = WL^3/(48EI)$ (W = load, L = distance between supports, E = elastic modulus, I = moment of inertia) one will observe that E is in the denominator, whose value is extremely high in the H rat and thus will cancel the importance of the CSMI as the main geometric determinant of the resistance to deformation.

Although it seems that the "architectural" factor may apparently favor the bending strength of the femoral diaphysis in the C rats, the measured strength in the bending test indicated that the "whole bone" stiffness and strength were far superior in H than in C rats. From the analysis of the load/deformation curve, the H femur showed an increased stiffness in elastic conditions, an increased maximal stress deflection, an increased elastic absorption of energy, and an increased structural strength.

These indicators of the increased whole-bone strength in the H rats in relation to C rats have to be primarily related to changes occurring in the bone material properties after hypophysectomy since they do not appear to be greatly associated to the architectural properties described above.

Focusing on the intrinsic material properties, the differences found between H and C rats were dramatic. The Young's modulus of elasticity, the maximal elastic stress, and the EAC expressed per unit of bone tissue volume were significantly higher in H than in C rats.

Bone tissue is a two-phase porous composite material comprised primarily of inorganic bone apatite crystals that mineralize an organic type I collagen matrix, which together provide its mechanical properties [26]. An increase in tissue mineral density increases the stiffness but sacrifices flexibility [27]. Mineralization of bone matrix is a 2-fold process: (1) mineralization of new collagen matrix that starts ~5–10 days after deposition in the resorption site (primary mineralization); and (2) a much slower process of secondary mineralization that begins after completion of the bone remodeling unit, which progressively adds ~50–60% of mineral content on bone matrix over the ensuing months [22]. Changes in the bone remodeling rate can influence the degree of mineralization of bone [28]. During rapid remodeling, full mineralization cannot occur because resorption resumes before the process is complete. A similar phenomenon occurs during the modelation phase

of the growth period in which the median degree of mineralization is low. In the adult, the median degree of mineralization depends on the remodeling rate, or bone turnover [29]. When it is low, there is more time for secondary mineralization to proceed, whereas in high turnover rates, recently formed bone is removed before there is time for prolonged secondary mineralization. Reduced bone turnover increases mean tissue age [26]. In the present experiments, the young adult rats used as controls had not terminated their growth period and thus their modeling rate may be high. In contrast, Martínez et al. [3] have suggested that bone turnover is lowered in hypophysectomized rats and proved that the total mineral density is thus larger in the cortical bone, suggesting an increase in the normal maturation of the mineralized matrix. The "remodelatory space" should thus be decreased and the time for secondary mineralization increased. The difference in bone turnover rate between C and H rats in the present study could explain the finding of the significantly higher (2.2×) amount of Ca in the ashes of the whole femur of the H rat in relation to the C rat. The high mineralization of the H bone could be an important determination of its high rigidity.

The contribution of collagen content and bone mineral crystals structure to bone strength is not yet well defined [22]. The hydroxyapatite crystals of bone mineral contribute to the strength and rigidity of the collagen matrix. Collagen contributes to its flexibility, which allows it to absorb energy on loading [26]. The role of collagen may be related either to the amount of collagen or its molecular stability and cross-linking. It has been proposed that the interfibrillar pyrrole of bone cross-links have a greater influence on the bending strength than the intrafibrillar pyridoline cross-links. The reduction in collagen cross-links in rats severely affects the biomechanical properties of cortical bone. The deflection capacity, the bending strength and elastic stiffness are reduced [30]. In contrast, when numerous intermolecular cross-links are formed, they produce a stable, porous structure from which the bone, in part, derives its ultimate yield strength [31]. It could be argued that the intermolecular collagen cross-links provide spacing for HAP mineral crystal nucleation and mineral enlargement. Martinez et al. [3] have found a greater amount of the stable nonreducible HP cross-links in the femur of pituitary-deficient male rats which was attributed to a decrease of collagen turnover in cortical bone that was accompanied by a greater amount of the stable nonreducible HP cross-links. The authors attributed these findings to the down-regulation of bone collagen turnover in response to hypophysectomy. These changes in the collagen matrix should be added to the high mineralization of the studied bone in the genesis of its increased stiffness and strength. Other factors are usually thought to affect bone material properties. They are tissue composition, the presence of microdamage, mineral composition, particle size, and distribution. Their participation in the considerable increase in stiffness and strength found in the femoral cortical bone of the hypophysectomized rat is not known at present.

The observed changes in femoral material properties as the result of hypophysectomy could have been derived from the absence of one of all pituitary hormones. It is well established that growth hormone is a major regulator of bone growth and turnover and that administration of growth hormone to hypophysectomized rats can restore normal bone growth and increase turnover. Feldman et al. [14] have shown that despite reduction in the cortical vBMD, hypophysectomy enhanced as much as 70% the intrinsic bending stiffness (E) of cortical bone (in age-matched rats) and that treatment of hypophysectomized rats with increasing doses of rhGH improved the observed reduction of vBMD but did not affect the abnormally enhancement of E. Previously reported studies from this laboratory [32] might suggest that secondary hypothyroidism induced by the lack of TSH could be at least partially responsible for the unusually large modulus of elasticity found in our hypophysectomized rats. In these studies, performed in young rats made hypothyroid by propylthiouracil, it was observed that the intrinsic mechanical quality of the hypothyroid bone tissue, estimated from the modulus of elasticity, was significantly higher than the control bone 30 d after treatment. This was accompanied by a higher Ca content in femoral ashes. Thus, the femoral shafts of hypophysectomized rats and hypothyroid rats have in common an unusual increment of the intrinsic stiffness and elevated calcium content. In contrast, lack of other adenohypophyseal hormones has not reproduced the bone responses to hypophysectomy [33–35]. The increased material stiffness found by Feldman et al. [14] in the femoral shaft of hypophysectomized rats occurred in animals that had been daily injected with 500 μg of hydrocortisone, thus suggesting that the absence of ACTH in the hypophysectomized rat might not be responsible for the stiffening of its bone material.

In summary, the femur obtained from a middle-aged hypophysectomized rat was stronger and stiffer than the femur obtained from a young-adult normal rat, both specimens showing similar size and bone mass and almost equal geometric properties. The higher than normal structural properties shown by the hypophysectomized femur were entirely due to the changes found in the intrinsic properties of the bone; the bone was thus stronger at the tissue level. This effect would not be the result of a failure of a bone regulatory mechanism operating under the pituitary control but the consequence of the lowering rate of physiological aspects of bone physiology. The change of the femoral bone tissue was associated with a high mineral

content and an unusual high modulus of elasticity and was probably due to a diminished bone and collagen turnover.

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Conflict of interest The authors declare that they have no conflict of interest.

Ethical standards Authors declare that the experiments comply with the current laws of the country in which they were performed.

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