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Biphasic Dose-Response Curves of Cortisol Effects on Rat Diaphyseal Bone Biomechanics

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Summary. Doses of 8, 16 (low), 32, 48, 64 (medium), and 150 (high) mg/kg/day of cortisol were administered to groups of 8 growing rats each during 16 days, and their femurs were then submitted to 3-point bending tests at low strain rate. Low doses had no effect. Medium doses, previously shown to improve calcium (Ca) balance and weight gain in the species, augmented diaphyseal elastic and ultimate strength, stiffness, and plastic-to-elastic deformation ratio with respect to untreated controls. This effect was achieved either by enhancing bone mass (volume, sectional moment of inertia, wall/lumen ratio) without changes in material quality parameters (32 mg/kg/day) or, conversely, by increasing bone tissue mechanical properties (stress, modulus of elasticity) not affecting bone geometry (48 and 64 mg/kg/day). The highest dose, known to depress Ca balance and weight gain, impaired diaphyseal mechanical performance in controls by substantially reducing bone mass without major variation in bone material properties, that is, developing a true osteopenic state in mechanical terms. The energy elastically absorbed per unit volume (proportional to the risk of comminute fractures) was greater with the highest dose because of enhanced deformability and diminished bone mass. The biphasic dose-response curves obtained, grossly parallel to those previously demonstrated for metabolic actions of cortisol in the same species, showed that biomechanical repercussion of this treatment on bone depends on different, dose-dependent effects which vary independently in temporal course, intensity, and sign.

Key words: Cortisol – Cortical bone – Bone biomechanics – Rat – Femur.

It is known that glucocorticoids exert three independent actions on the skeleton: (1) depress bone formation directly; (2) interfere with some bone resorbing agents—including PTH—through direct effects on bone cell differentiation; and (3) stimulate PTH secretion as a consequence of a reduced Ca balance [1, 2]. The development of osteopenia in both trabecular and cortical bone [1, 3, 4] that leads to an increased propensity to bone fractures has been proposed as the most potentially disabling side effect of long-term glucocorticoid therapy [1].

However, the biomechanical repercussion of this state has been barely investigated. The few available studies show somewhat discrepant results from bending or compression tests of growing or adult rat femur diaphyses employing only moderate dose levels during different periods. Vogel [5] found that a 10-day treatment with 10 mg/kg/day of cortisol enhanced, whereas a 1–3 month treatment reduced diaphyseal bending strength and body weight gain with respect to normal controls. More recently, Ørtoft and Oxlund [6] reported that 1 mg/kg/day of methyl-prednisolone had no effect during 5, 10, or 30 days but reduced both diaphyseal strength and cortical bone stress in bending (not in compression) with respect to either normal or weight-paired (food-restricted) controls at 90 days. No data were available on other geometric and mechanical parameters such as the sectional moment of inertia and wall/lumen ratio of the diaphyses, the absolute and relative amounts of elastic and plastic components of diaphyseal deformation, the modulus of elasticity of cortical bone material, the tissue strain, or the energy absorbed by bones in elastic conditions.

The referred discrepancy may be explained by the well-demonstrated dose-dependence of glucocorticoid's effects on bone formation and resorption [7]. Biphasic dose-response curves of both Ca absorption and urinary excretion of bone metabolites were already shown by us in rats treated with 8–128 mg/kg/day of cortisol for 16 days [8]. It seems reasonable, therefore, to propose that biomechanical effects of cortisol on rat bone may also be described by biphasic curves if similar dose-range and period of treatment are assayed.

This paper shows the effects of a 16-day treatment with 8–150 mg/kg/day of cortisol on the mechanical integrity of rat bone as determined by testing fresh femurs in bending at a low strain rate [9–12]. Complete dose-response curves were obtained for every structural (whole bone) and material (bone tissue) mechanical properties assayable with the method. Our present results complete a preliminary communication [13], confirm the proposed hypothesis, and are discussed in the light of recent knowledge on basic bone biomechanics and corticoid effects on bone.

Material and Methods

Seven groups of 8 IIM [14] inbred male rats weighing 80 ± 8 g were housed in individual metabolic cages under natural light cycle and controlled (23°C) temperature, fed a semisynthetic diet (total wheat flour 87%, casein 10%, salts mixture [15] 3%, Ca/P content = 1.0/0.8%) *ad lib*, and given daily s.c. injections of 8, 16, 32, 48, 64, or 150 mg/kg of cortisol hemisuccinate dissolved in isotonic glucose, or solvent alone (controls) during a 16-day period.

At the end of the experiment, the animals were sacrificed by ether overdose and both femurs, dissected to avoid periosteal lesion, were immediately submitted to 3-point bending tests [9–11]. The bones, laying with their anterior aspect facing down on supports separated by a constant distance L = 13 mm, were centrally loaded at a rate of 10 N/min in order to obtain the load (W)/deformation (d)

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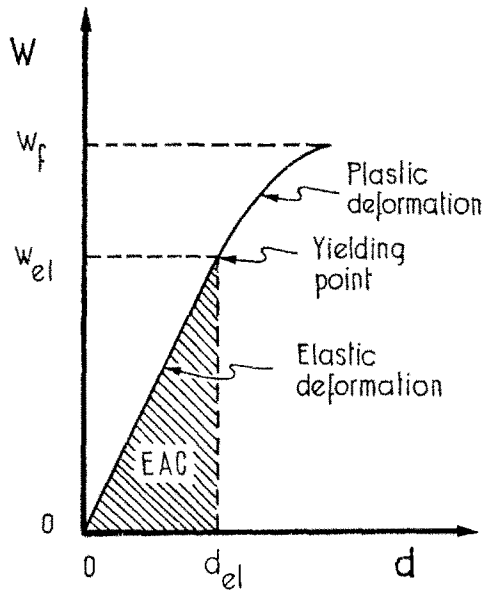

Fig. 1. Typical load (W)/deformation (d) curve for the bending tests employed, showing the elastic (linear) and plastic (nonlinear) components separated by the yielding point (W_{el}; d_{el}), the fracture load (W_f), and the energy elastically absorbed EAC (represented by the dashed area). The slope of the linear portion of the curve (W_{el}/d_{el}) represents diaphyseal stiffness.

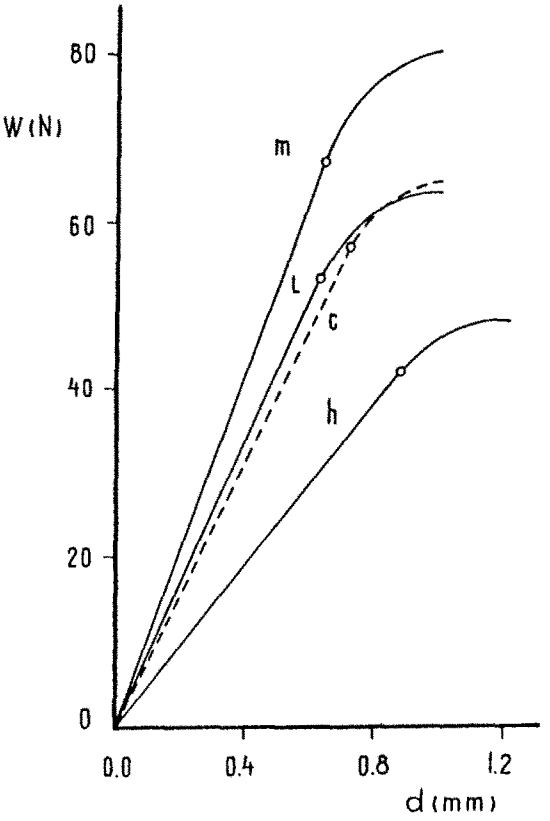

Fig. 2. Representative load (W)/deformation (d) curves for low (L), medium (m), and high (h) doses of cortisol and for control group (c), calculated from respective mean W_{el}, W_f, and d_{el} values.

curves (Fig. 1) showing both the elastic (linear) and plastic resistance components until fracture, separated by the "yielding point" (departure from linearity). Micromorphometrical determination of the horizontal, vertical, external (H, B), and internal (h, b) diameters of the elliptic crown-shaped fracture sections enabled the following: calculation of the volume (vol) of bone between supports = $L\pi(HB - hb)$, the wall-lumen-ratio (WLR) of the central part of bone shafts = $\frac{1}{2}[(H - h)/h + (B - b)/b]$, and the moment of inertia (Ix) of the fracture sections in relation to the horizontal axis = $\pi(B^3H - b^3h)/64$ (geometrical parameters).

Graphic analysis [16] of the W/d curves determined the following:

Whole bone (structural) mechanical variables (which allow estimation of diaphyseal mechanical performance):

- Limit elastic strength (W_{el}, load at the yielding point)
- Ultimate strength (W_f, load at fracture)
- Plastic/elastic behavior ratio = $(W_f - W_{el})/W_f$
- Deformation (arrow of the arch formed by the bending bone) at the end of the elastic period (d_{el})
- Stiffness (W_{el}/d_{el} ratio, slope of the W/d curve during elastic behavior)
- Energy absorbing capacity (EAC) in elastic conditions = $\frac{1}{2}W_{el} \cdot d_{el}$.

Bone tissue (material) mechanical properties (intrinsic parameters which allow comparison of bones of different size and/or shape):

- Strength, or limit elastic stress $S_{el} = L.B.W_{el}/8Ix$
- Stiffness, or modulus of elasticity $E = W_{el} \cdot L^3/48d_{el} \cdot Ix$
- Limit elastic strain $\epsilon = 6d_{el} \cdot B/L^3$
- Energy-absorbing capacity/unit volume = EAC/vol.

The femurs were then defatted in chloroform-methanol (1:1), dried at 100°C, calcinated in a muffle furnace at 600°C for 6 h and dissolved in 2 ml of 50% (v/v) HCl in a boiling water bath. The resulting solution was taken to 10 ml and aliquots were used to measure the Ca content [17] referred to dry bone weight.

Values of geometric and structural variables, previously shown to linearly correlate with bw [10], were considered either crude or statistically adjusted to 150 g (the bw point of convergence for each variable as previously determined in rats from different strains). This procedure was preferred to the use of weight-paired, food-restricted controls [6] because it was previously shown by us [11, 12] that dietary protein and/or calorie restrictions alter geometric, structural, and material properties of growing rat bones. For S_{el}, WLR, Ix, EAC, and E, standard deviations were estimated taking into account their particular error propagation. Data were averaged for each animal and group. Standard statistical analyses [18] were carried out after having achieved normality in every case.

Results

Low cortisol doses (8 and 16 mg/kg/day) provoked no evident bone biomechanical or morphometric effects (Figs. 2–4).

Medium doses (32, 48, and 64 mg/kg/day) augmented diaphyseal resistance to elastic deformation and fracture (W_{el}, W_{el}/d_{el} ratio, W_f) and the relative participation of plastic deformation (plastic/elastic ratio) in the load-deformation curves (Figs. 2 and 3). At 32 mg/kg/day, an increase in diaphyseal bone mass was observed (vol, Ix, WLR) (Fig. 3) with no change in mechanical quality of bone material (limit elastic stress S_{el}, modulus of elasticity E). Conversely, with 48, and more clearly with 64 mg/kg/day, increments were found not in bone mass but in material properties (S_{el}, E).

The highest (toxic [5]) dose (150 mg/kg/day) diminished diaphyseal resistance to deformation and fracture (increase in strain ϵ and decrease of W_{el}, W_{el}/d_{el}, and W_f and plastic/elastic ratio) (Figs. 2 and 3) and dramatically reduced diaphyseal bone mass (vol, Ix, WLR) (Fig. 3) with respect to controls but did not alter material properties S_{el} or E. Elas-

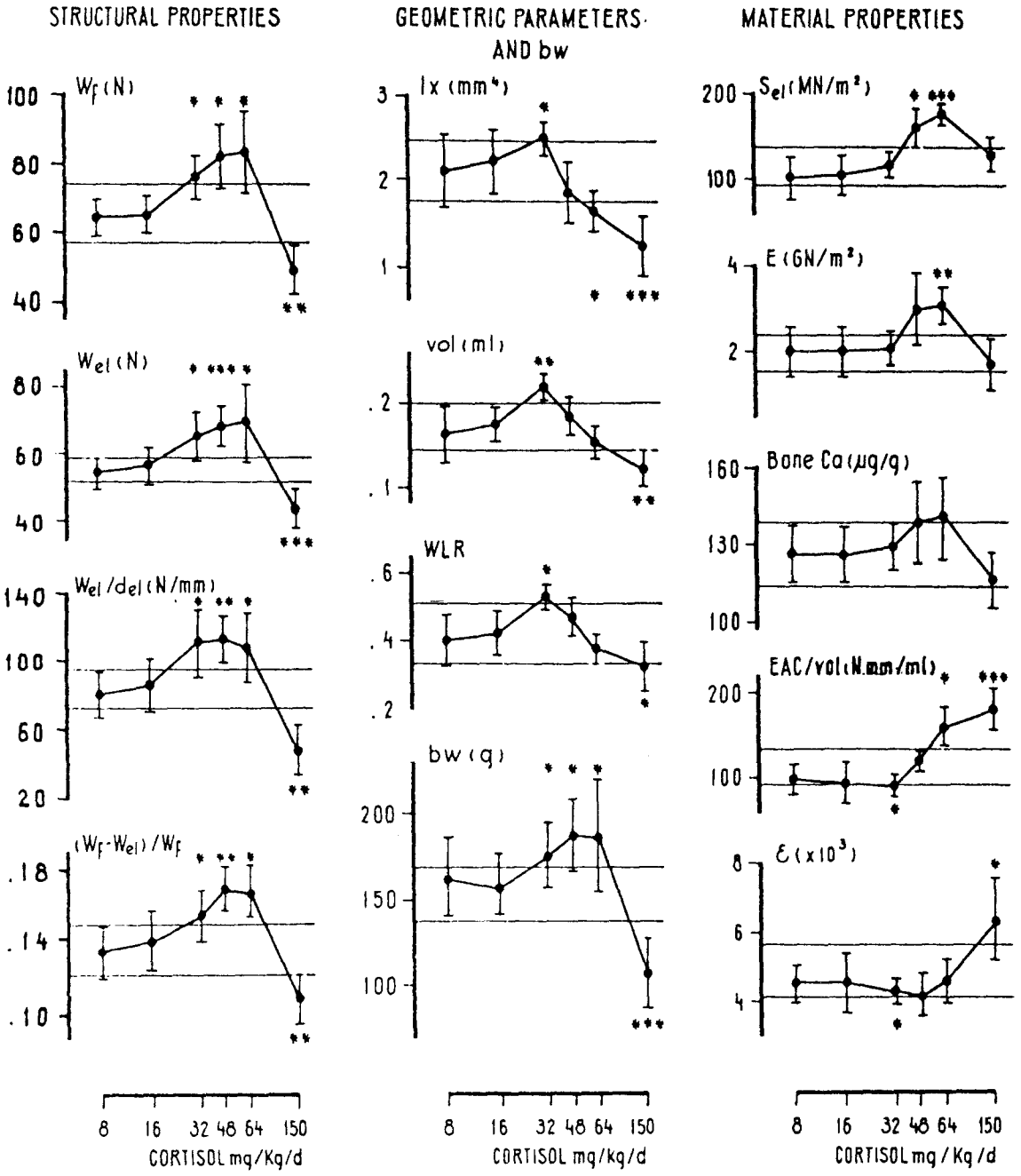

Fig. 3. Means ± SD of structural (fracture load W_f, limit elastic strength W_{el}, stiffness W_{el}/d_{el}, and plastic/elastic resistance ratio [$W_f - W_{el}]/W_f$, left), geometric [sectional moment of inertia Ix, volume between supports (vol), wall/lumen ratio (WLR), center], and material properties [limit elastic stress S_{el}, modulus of elasticity E, Ca content per mass unit, energy-absorbing capacity per volume unit (EAC/vol), limit elastic strain (ϵ) of femur diaphyses], and final bw (center, bottom) of every studied group. Control values ($\bar{x}$ ± SD) are represented by the two horizontal lines in each graph. *, **, *** indicate 0.05, 0.01, and 0.001, respectively, significance levels of differences with respect to controls.

tically absorbed energy/vol unit (EAC/vol) (Fig. 3) was enhanced by this treatment.

Changes in bone Ca content per dry weight (Fig. 3) were parallel to those of material elastic stress and stiffness, but showed no significant differences between groups because of their great variance.

The bw gain did not vary at low doses but was greater than that of controls at medium doses and notably smaller at the highest dose (Fig. 3). Statistical adjustment of diaphyseal resistance to deformation and fracture (W_{el}, W_{el}/d_{el}, W_f) to a common 150 g bw only partially reduced the above-mentioned differences (Fig. 4). Adjusted morphometrical variables, instead, became similar to those of controls for the highest dose, whereas the resulting vol and Ix were lower than normal with the 48 and 64 mg/kg/day doses.

Discussion

General Effects on Structural Properties

In agreement with the proposed hypothesis, biphasic dose-

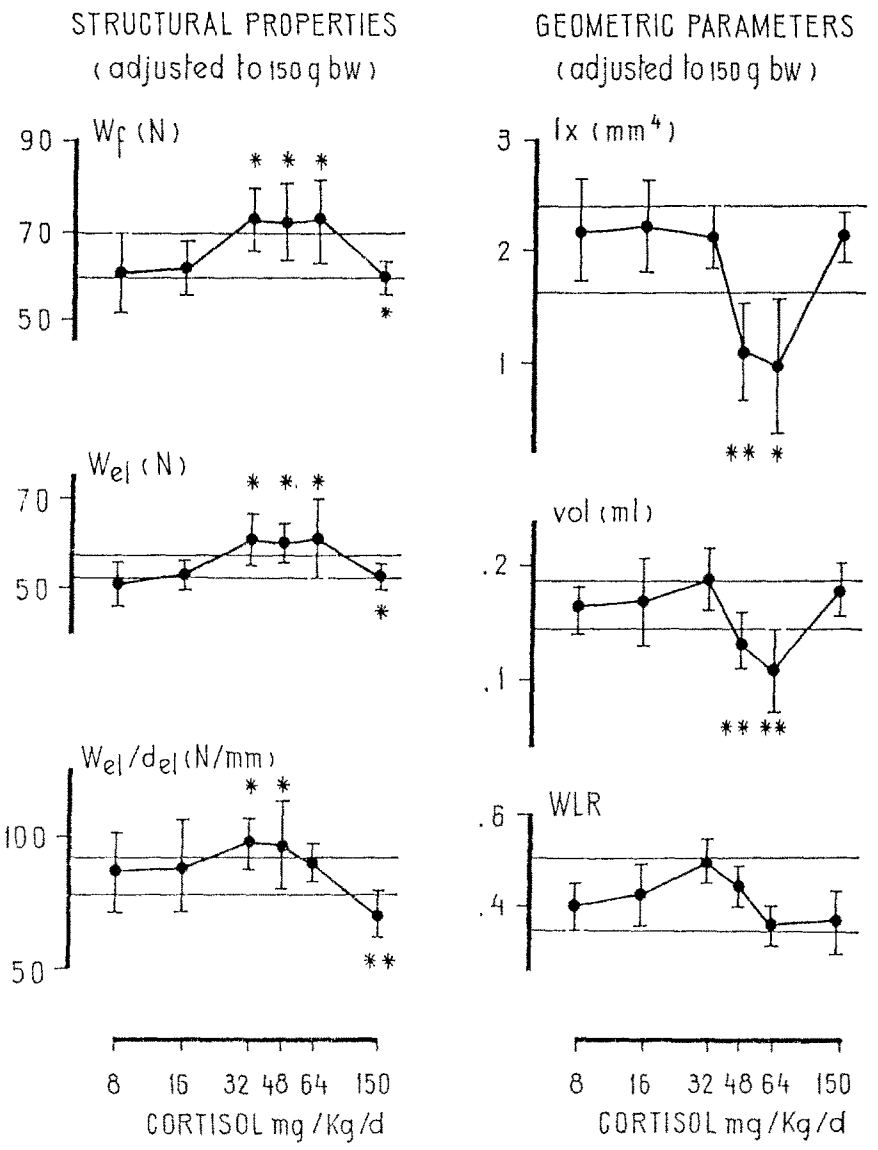

Fig. 4. Means ± SD of structural (fracture load W_f, limit elastic strength W_{el}, stiffness W_{el}/d_{el}, left) and geometric properties [sectional moment of inertia (Ix), volume between supports (vol), and wall/lumen ratio (WLR), right] of femur diaphyses from every studied group, statistically adjusted to 150 g bw. Control values are represented by the two horizontal lines in each graph. *, ** indicate 0.05 and 0.01, respectively, significance levels of differences with respect to controls.

response curves were described for structural properties (Figs. 3 and 4). These results resemble those obtained by Vogel [5] by varying the duration of treatment with a fixed, 10 mg/kg/day dose in rats and with doses of 0.2–100 mg/kg/day during a fixed, 14-day period in chicks.

The high percentage of plastic deformation observed at medium doses is thought to be a crack-blunting element because it implies a significant fall in local stress that makes it difficult for a preexisting crack in the material to spread [19]. Values lower than normal for this variable were obtained, however, at the highest dose (Figs. 2 and 3). This suggests that the expansion of the largest microcracks eventually produced prior to the yielding point by local "stress risers" [19, 20] should have been prevented [19] and favored [21, 22] at medium and high doses, respectively. This fact may help to explain the parallel variations of diaphyseal ultimate strength at the corresponding doses from a mechanical point of view. The following analysis of effects on geometric and material properties offers a reasonable explanation for the referred biphasism.

Effects on Geometric and Material Properties at Medium Doses

The finding of some positive effects of certain doses of cortisol on skeletal performance and even on biomass in the species studied, absent in Ørtoft and Oxlund's [6] report studying older animals and taken as "unexpected" by Vogel [5], contrasts with the impairment described in our highest-dose group or in the referred long-term studies [5, 6]. This should not be surprising, however. We have already demonstrated that (1) dose-response curves for a similar range of cortisol doses on Ca absorption and balance and urinary excretion of bone catabolites per unit bw of the same type of animals were also biphasic, showing high Ca balance values and low catabolite excretion rate for medium doses; and (2) this striking increment in Ca absorption (later corroborated by others studying different intestinal segments [23–27]) was pharmacologically coincident with slight increases of growth rate and food conversion efficiency [8]. Vogel [5] also showed that connective tissue strength was enhanced by cortisol, diminished after adrenalectomy and normalized by compensatory doses of corticoids. This points out that the integrated action of corticotherapy on the skeleton at either a given dose or moment of the treatment is not easily predictable, because it results from a sum of transient or cumulative effects that vary independently in temporal course, intensity, and sign [28, 29].

In fact, our data show that the improvement in structural

properties obtained with medium doses could be explained either (1) by an increase in bone mass (vol, Ix, WLR) without any change in material properties as observed with 32 mg/kg/day; or (2) conversely, by an improvement in material quality (S_{el}, E) with no variation of bone geometry (even with certain reduction detected in Ix) as found with 48 and 64 mg/kg/day. In the first case, the changes should be attributed to an increased bone balance [8], in consonance with the known ability of glucocorticoids to protect bone against parathyroid hormone, prostaglandins, and other osteolytic agents [28–33] and to promote certain aspects of bone formation [34] *in vitro*. In the second, a qualitative improvement of bone material should be proposed. This effect on material properties is congruent with (1) the positive effect on Ca balance previously obtained with similar doses [8]; (2) the occasional enhancement of bone density or ash, insoluble hydroxyproline, and mucopolysaccharide content provoked by glucocorticoids on bones and cartilages from non-Ca-P-restricted rats, rabbits, and chicks *in vivo* [3, 5, 35, 36]; and (3) the relative increase in collagen mineralization occasionally observed by others in different types of osteoporosis [21, 37]. This may suggest a high degree of calcification to be the cause [38], as the parallel improvement in bone stress and Ca content found by Vogel [5] seems to indicate. It must be noted, however, that no significant differences were observed by us nor by Ørtoft and Oxlund [6] in Ca content of bones per mass unit in any group. Nevertheless, the relatively great variance shown by our data (Fig. 3) may have blunted some small, individual differences with respect to the pretreatment values which could be relatively important. It was shown that little changes in mineralization usually correlate with substantial variations in bone strength or stiffness [38]. Dilucidation of this matter would have required noninvasive bone mineral content determinations prior to treatment which were not done in this study, so that it remains an open question. Besides, our data does not allow for ruling out some qualitative effects on microstructural arrangement of tissue crystals and/or fibers [5, 21] which could also be postulated.

Data is sufficient to discard any negative effect of medium doses on the mechanism that physiologically adequates quantity and spatial distribution of bone tissue to material quality in relation to the biomass to be supported [10, 39]. Subnormal values of vol and Ix after adjustment to 150 g bw at 48–64 mg/kg/day (Fig. 4) were associated with overnormal material and structural properties (Figs. 2 and 3). In this regard, it should be noted that bw values were better paralleled by the structural properties of the diaphyses than by their sectional geometry.

Effects on Geometrical and Material Properties at the Highest Dose

The impairment of diaphyseal strength and stiffness evoked by the highest dose (d_{el}, ϵ, W_{el}/d_{el}, and W_f data, either crude or adjusted to bw) (Figs. 2–4), was undoubtedly the consequence of a reduction in bone mass not compensated by an improvement of its intrinsic mechanical quality. This condition is mechanically similar to that reported by Vogel [5] and Ørtoft and Oxlund [6] through bending tests after 3-month treatments and to that described by us in age-paired rats forced to extreme protein restriction over a 20-day period [11]. A direct reduction in bone formation and/or an increase in bone resorption derived from a secondary hyperparathyroidism evoked by an exaggerated fecal Ca loss [1, 8] can be proposed as causal factors. As no changes in bone Ca content per mass unit were detected, this state is congruent with the physiopathologic concept of osteopenia and seems to be the only described feature corresponding to a "corticoid-induced osteoporosis." Long-term treatments [5, 6] induced, besides, a slight reduction in one of the material properties (bending stress) that was circumstantially not reproduced in this group.

Complete normalization of vol, Ix, and WLR data for this dose after adjustment to a common bw (Fig. 4) suggests the expression of the known general, antianabolic effects of cortisol. This points out that, although crude values were much lower than those of controls, diaphyseal morphometry could be considered acceptable for animals bearing such a small biomass. However, the structural properties (diaphyseal strength and stiffness) of this group did not become completely normal after adjustment to bw, indicating an inadequate mechanical adaptation. The previously observed neutralization of the main metabolic effects of this dose by means of dietary Ca supplementation [8] suggests a hyperparathyroid status [10] to be at least partially a causal factor for this inadequacy.

The positive effect of this dose on elastic energy absorption by mass unit (EAC/vol, a variable directly proportional to both limit elastic strength and deformation of the diaphyses) must be also taken as deletereous. In fact, it was not associated in this case to an increased, but rather to an impaired load resistance previous to fracture (W_{el} and W_f values, crude or adjusted) and to high strain values (Figs. 3 and 4). As a significant reduction of bone volume was also observed, the high EAC/vol values in this group should be thought of as merely derived from both an increase of bone deformability and a reduction of bone mass. In addition, it is known that the increased amount of potential energy elastically accumulated per mass unit may be released at failure, allowing for the production of more new surfaces [11, 40], that is, enhancing the probability of the occurrence of comminute fractures.

In conclusion, our data show that the dose-dependent cortisol effects on geometric, structural, and material properties of rat femur seem to derive from complex interactions, the complete description of which will require further investigation in fields other than biomechanics. Nevertheless, this study reveals that, after a 16-day cortisol treatment in rats, (1) the dose-response curves for structural and geometric properties, not completely explained by the concomitant effects on bw, were biphasic, grossly parallelling those previously obtained for Ca absorption or balance and bone metabolism [8]; (2) doses of 32–64 mg/kg/day produced positive effects on structural variables, mediated by improvements of either geometric or material properties; (3) a dose of 150 mg/kg/day provoked negative effects on structural variables and an undesirable increment in bone capacity to elastically absorb energy (mainly as a result of an impairment of geometric properties), and affected the integrity of the mechanism that adequates bone mass or spatial distribution to material properties in relation to animal weight; and (4) typical, distinct patterns of variation were shown for each group of structural, geometrical, and material variables.

Although not directly assimilable to human bone [19, 41], these findings may improve our basic understanding of bone biomechanics and allow for a proper pathogenetic interpretation of the different changes in bone strength induced by hypercorticism.

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