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Comparison of Trabecular Bone Microarchitecture and Remodeling in Glucocorticoid-Induced and Postmenopausal Osteoporosis

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ABSTRACT

Long-term treatment with glucocorticoids (GCs) leads to a rapid bone loss and to a greater risk of fractures. To evaluate the specific effects of this treatment on cancellous bone remodeling, structure, and microarchitecture, we compared 22 transiliac biopsy specimens taken in postmenopausal women (65 ± 6 years) receiving GCs (≥7.5 mg/day, for at least 6 months) and 22 biopsy specimens taken in age-matched women with postmenopausal osteoporosis (PMOP), all untreated and having either at least one vertebral fracture or a T score < −2.5 SD. On these biopsy specimens, we measured static and dynamic parameters reflecting trabecular bone formation and resorption. Also, we performed the strut analysis and evaluated the trabecular bone pattern factor (TBPf), Euler number/tissue volume (E/TV), interconnectivity index (ICI), and marrow star volume (MaSV). Glucocorticoid-induced osteoporosis (GIOP), when compared with PMOP, was characterized by lower bone volume (BV/TV), trabecular thickness (Tb.Th), wall thickness (W.Th), osteoid thickness (O.Th), bone formation rate/bone surface (BFR/BS), adjusted mineral apposition rate/bone surface (Aj.AR/BS), and higher ICI and resorption parameters. After adjustment for BV/TV, the W.Th remained significantly lower in GIOP ($p < 0.0001$). The active formation period [FP(a+)] was not different. Patients with GIOP were divided into two groups: high cumulative dose GCs (HGCs; 23.7 ± 9.7 g) and low cumulative dose GCs (LGCs; 2.7 ± 1.2 g). HGC when compared with LGC was characterized by lower W.Th ($p < 0.05$), BV/TV ($p < 0.001$), Tb.Th ($p < 0.05$), trabecular number (Tb.N; $p < 0.05$), FP(a+) ($p < 0.05$), and nodes ($p < 0.05$), and higher E/TV ($p < 0.05$), ICI ($p < 0.005$), and TBPf ($p < 0.05$). When HGC was compared with PMOP, the results were similar except for the MaSV, which was significantly higher ($p < 0.005$). In summary, GIOP was characterized by lower formation and higher resorption than in PMOP, already present after LGC. With HGCs, these changes were associated with a more dramatic bone loss caused by a major loss of trabecular connectivity. (J Bone Miner Res 2001;16:97–103)

Key words: glucocorticoids, histomorphometry, microarchitecture, postmenopausal osteoporosis, remodeling

INTRODUCTION

PROLONGED glucocorticoid (GC) therapy is well recognized as an independent risk factor of osteoporosis and fractures.[1–3] The overall incidence of osteoporosis in patients taking GCs for over 6 months is approximately 50%[4,5] and the incidence of fractures is about 30% after 5 years of treatment.[6] Nevertheless, this treatment represents one of the most important approaches in several chronic diseases such as rheumatoid arthritis or asthma and after

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organ transplantation. In these conditions, GC-induced osteoporosis (GIOP) represents a major problem.[7,8] Previous studies have indicated that bone mineral density (BMD) was decreased at different sites[9,10] and that the risk of fracture was high after GC treatment,[3,6,11] as a result of marked inhibition of osteoblastic activity, confirmed in animal studies.[12] This osteoblastic dysfunction has been associated with an increased osteoclastic bone resorption,[13] secondary to a decreased calcium absorption inducing a secondary hyperparathyroidism.[14–16] More recently, it also has been suggested that the osteoblastic dysfunction could be caused by the GC-mediated inhibition of osteoblastogenesis and promotion of apoptosis of osteoblastic cells.[17]

At the histological level, the histomorphometric analysis of iliac crest biopsy specimens taken from GC-treated patients has shown a decrease in the wall thickness (W.Th) of trabecular packets and as a consequence in the trabecular thickness (Tb.Th) and trabecular bone volume.[18–20] However, there are few published data about the trabecular microarchitecture in GIOP. In GC-treated men compared with age-matched controls, exponential relationships have been found between the bone volume and various connectivity parameters.[20] Furthermore, in women with postmenopausal osteoporosis (PMOP) compared with controls, histomorphometric studies[21] have shown a significant decrease of 40% in the bone volume, Tb.Th and trabecular number (Tb.N), W.Th, and active formation period [FP(a+)], but without any significant difference in bone formation at the tissue and bone mineral unit (BMU) levels. Only Aaron et al. have compared GIOP with primary osteoporosis in a group of men and women and observed in the primary osteoporosis group a decline in the Tb.N, associated with increased resorption, leading to plate perforations and to disruption of the three-dimensional (3D) trabecular architecture.[22]

Substantial advances have been accomplished in the methods now available for the analysis of cancellous bone structure on histological sections. These new evaluations provide precise information concerning connectivity of the trabecular network. Connectivity is a specific feature, which describes different types of connection between nodes (points at which three or more trabeculae joined), connection branches (struts), and terminal or free-end branches (termini).[23] In addition, other parameters have been validated to assess indirectly bone microarchitecture through the measurement of the marrow star volume (MaSV),[24] Euler number,[25] trabecular bone pattern factor (TBPf),[26] and interconnectivity index (ICI).[27] All these parameters represent a more precise and interesting approach to bone microarchitecture evaluation in 2D sections. In fact, high values of these parameters (or less negative in the case of Euler number) contribute to define a low degree of organization of trabecular network, showing a low connectivity between the trabeculae.

The aim of this study was: (1) to assess GC effects on microarchitecture and remodeling of cancellous iliac bone in women by using the most recent 2D histomorphometric approach; (2) to compare the microarchitectural characteristics in two types of osteoporosis, GIOP and PMOP, in untreated postmenopausal women; and (3) in GIOP, to evaluate the influence of the cumulative dose of GC on the histomorphometric parameters.

MATERIALS AND METHODS

Patients

Twenty-two transiliac biopsy specimens taken in untreated postmenopausal women aged 65 $\pm$ 6 years who received GC therapy (at least 7.5 mg/day of prednisone for at least 6 months) were compared with 22 biopsy specimens taken in women aged 66 $\pm$ 11 years with PMOP. In both groups osteoporosis was confirmed by the presence of at least one vertebral fracture or by a T score < -2.5 after dual-energy X-ray absorptiometry (DXA) evaluation at the proximal femur. Ten patients in each group had one or more vertebral fractures. None of the patients had ever been treated for their bone condition and none presented with any other disorders that could have affected bone metabolism. Forty-two of the 44 patients were doubly labeled with tetracycline (Lederlé, Oullins, France) before biopsy (demethylchlortetracycline, 600 mg per day, 2 days on, 12 days off, and 2 days on). The patients who received GC therapy were treated for rheumatic diseases, asthma, dermatologic diseases, or intestinal diseases.

Histomorphometric analysis

In these undecalcified bone biopsy specimens taken with a 7.5-mm inner-diameter Bordier-Meunier trephine (Lepine, Lyon, France), we evaluated the histomorphometric parameters with either a semiautomatic (Ibas 1; Leica, Wetzlar, Germany) or an automatic (Visiolab 1000; Biocom, Paris, France) analyzer. The measurements of indirect parameters of microarchitecture were carried out with modules developed at our institution. All parameters were expressed according to the recommended American Society for Bone and Mineral Research (ASBMR) nomenclature.[28] All measurements of thickness were adjusted for obliquity of sections, by multiplying by $\pi/4$.[28]

Bone structure: We evaluated the W.Th (μm) of cancellous packets, which represents the end product of the osteoblastic activity exerted in a remodeling site, under polarized light on Solochrome cyanin–stained sections. We also measured the cancellous bone volume/tissue volume (BV/TV; %) on Goldner-stained sections.

Assessment of bone remodeling: To evaluate the remodeling, we measured the following parameters in cancellous bone on Goldner-stained sections: osteoid surface/bone surface (OS/BS; %), osteoid thickness (O.Th; μm), eroded surface/bone surface (ES/BS; %), and osteoclast number/bone surface (N.Oc/BS; /mm). Dynamic parameters also were evaluated on unstained sections measured under UV light: mineral apposition rate (MAR; μm/day) and mineralizing surface/bone surface (MS/BS; %). Bone formation rate/bone surface (BFR/BS) was calculated as (MS/BS) $\times$ MAR ($\mu m^3/\mu m^2$ day), and adjusted apposition rate/bone surface (Aj.AR/BS), that is, the BFR adjusted for osteoid, was expressed as micrometers per day. FP(a+) was calculated as W.Th/MAR and expressed in days.

TABLE 1. REMODELING PARAMETERS OF ILIAC TRABECULAR BONE FROM 22 WOMEN WITH PMOP AND 22 WOMEN WITH GIOP

Parameters	*PMOP*	*GIOP*	p
Mean age (years)	66 ± 11	65 ± 6	NS
OS/BS (%)	5.77 ± 3.36	8.34 ± 9.55	NS
O.Th (μm)[a]	8.88 ± 1.84	7.28 ± 1.46	<0.003
MAR (μm/day)	0.59 ± 0.13	0.53 ± 0.13	NS
MS/BS (%)	6.2 ± 2.3	2.2 ± 2.6	<0.0001
BFR/BS ($\mu m^3/\mu m^2$ per day)	0.037 ± 0.016	0.013 ± 0.016	<0.0005
Aj.AR/BS (μm/day)	0.83 ± 0.73	0.22 ± 0.16	<0.005
FP(a+) (days)	53 ± 11	47 ± 13	NS
ES/BS (%)	2.18 ± 1.24	4.06 ± 2.45	<0.005
N.Oc/BS (/mm)	0.06 ± 0.10	0.12 ± 0.04	<0.05

[a] Adjusted for obliquity.

Assessment of trabecular microarchitecture: Trabecular number (Tb.N; mm), trabecular separation (Tb.Sp; μm), and Tb.Th (μm) were calculated according to Parfitt formula.[29] In addition, Tb.Th was evaluated by direct measurement using a grid, as orthogonal intercepts from intersections of the grid lines with trabecular boundaries.[30]

After skeletonization, we evaluated trabecular network in terms of number of nodes (N.Nd/TV) and number of node-to-node branches (N.Nd-Nd/TV), expressed as percent of tissue volume, node-to-node length (L.Nd-Nd/TSL), expressed as percent of total skeletonized structure length), and nodes/termini ratio (Nd/Tm).

The following indirect parameters reflect the microarchitecture that also were assessed: (1) The MaSV, that is, the mean volume of all the parts of an object that can be unobscured in all the directions from a point inside the object. This parameter, by the analysis of marrow space distribution, gives an indirect evaluation of the trabecular network organization[24]; for its automatic measure, the method developed by Levitz was used.[31] (2) The Euler number, expressed per tissue volume (E/TV), that is, the number of holes minus the number of connected components, which can be interpreted as the maximum number of branches that could be removed without breaking the network into different parts.[25] (3) The TBPf, that is, the relation between convex and concave elements, considering a concave element as expression of a well-connected structure.[26] (4) The ICI, after skeletonization of the bone marrow, defined as $(N \times NN)/[T \times (NF + 1)]$, where N is the number of nodes, NN is the number of node-to-node branches, NF is the number of node-to-terminus branches, and T is the number of "trees," a tree being an independent portion of the medullary space totally enclosed by a trabecular structure.[27] All these parameters, as well as the strut analysis, contribute to describe the connectivity of the trabecular network.

Statistical analysis

All the results were expressed as mean ± SD. For all the variables having a Gaussian distribution, multiple group comparisons were made by analysis of variance (ANOVA). The Newman–Keuls test was performed as a post hoc test for the difference between two groups. Simple regression analysis was performed to evaluate the relationship between two variables. All statistical analyses were performed with Statistica software (Statsoft, Tulsa, OK, USA).

RESULTS

Bone remodeling

The values of remodeling parameters of the two groups are shown in Table 1. When compared with PMOP, GIOP showed a significantly lower bone formation because the parameters reflecting bone formation at the tissue (BFR/BS) and at the BMU levels (Aj.AR/BS) were decreased in GIOP. FP(a+) was similar in the two groups. In contrast, the GIOP group was characterized by a significant increase in resorption parameters, with higher eroded surface and osteoclast number.

Bone structure and microarchitecture

The calculated versus the direct measurement of Tb.Th showed a high significant correlation ($r = 0.95$; $p < 0.0001$). For this study, the calculated one was used.

Table 2 shows the values of main structural and microarchitectural parameters of the two groups. GIOP, when compared with PMOP, was characterized by lower wall and Tb.Th, and as a consequence, lower BV/TV. In addition, the ICI was also higher in GIOP with higher Tb.Sp. After adjustment for BV/TV, only the W.Th was still lower in GIOP with respect to PMOP (29.8 ± 2.5 μm vs. 23.3 ± 3.1 μm; $p < 0.0001$).

Biopsy specimens from patients with GIOP were divided into two groups with respect to the cumulative dose of GCs: high cumulative dose glucocorticoid (HGC; higher than 10 g of prednisone; mean, 23.7 ± 9.7 g) and low cumulative dose glucocorticoid (LGC; lower than 10 g of prednisone; mean, 2.7 ± 1.2 g). The same number of subjects composed each group. HGC when compared with LGC showed significant lower W.Th, BV/TV, Tb.Th, Tb.N, N.Nd/TV, N. Nd-Nd/TV, L.Nd-Nd/TSL,

TABLE 2. PARAMETERS OF TRABECULAR STRUCTURE AND MICROARCHITECTURE IN PMOP AND GIOP

Parameters	*PMOP (22)*	*GIOP (22)*	p
W.Th (μm)[a]	30.3 ± 2.5	22.8 ± 3.1	<0.0001
BV/TV (%)	16.4 ± 5.3	12.1 ± 4.8	<0.01
Tb.N (/mm)	1.17 ± 0.20	1.03 ± 0.32	NS
Tb.Sp (μm)	746 ± 180	965 ± 439	<0.05
Tb.Th (μm) calculated[a]	109 ± 27	90 ± 21	<0.05
Tb.Th (μm) measured[a]	110 ± 25	91 ± 21	<0.05
N.Nd/TV (/mm^3)	0.64 ± 0.38	0.51 ± 0.42	NS
N.Nd-Nd/TV (/mm^3)	0.47 ± 0.40	0.36 ± 0.47	NS
L.Nd-Nd/TSL (%)	0.26 ± 0.19	0.16 ± 0.17	NS
Nd.Tm	0.57 ± 0.47	0.38 ± 0.35	NS
E/TV (/mm^3)	−0.22 ± 0.15	−0.29 ± 0.24	NS
TBPf	2.26 ± 1.62	3.37 ± 2.24	NS
ICI	13.64 ± 19.64	46.51 ± 53.20	<0.01
MaSV (mm^3)	0.59 ± 0.13	0.53 ± 0.13	NS

[a] Adjusted for obliquity.

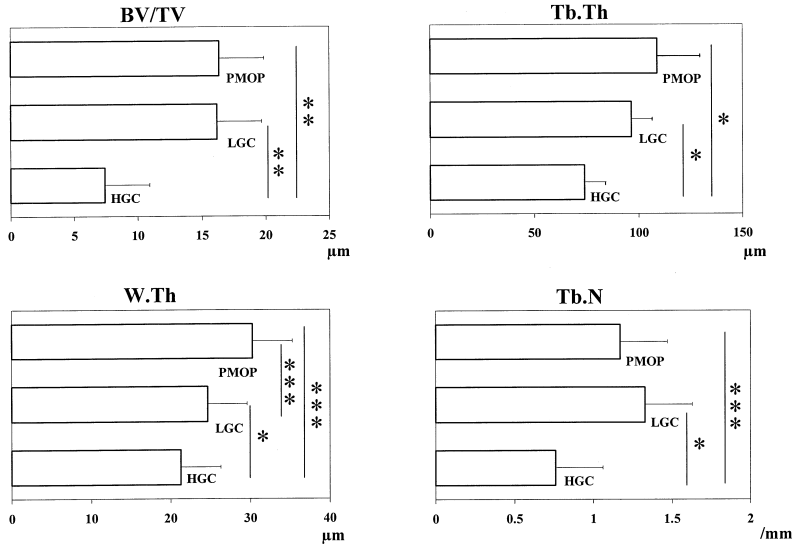


FIG. 1. Bone microarchitecture in PMOP and GIOP caused by LGC and HGC (mean ± SD; *$p < 0.05$; **$p < 0.001$;***$p < 0.0005$).

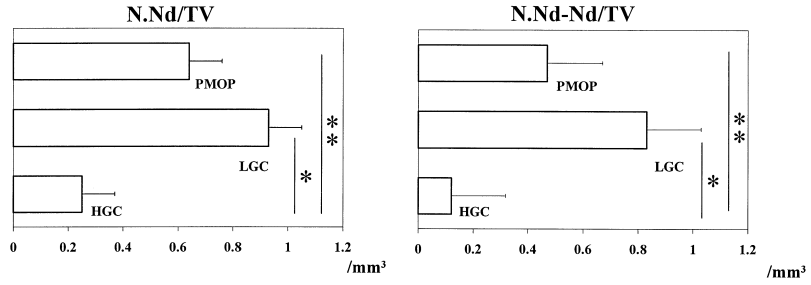
and Nd/Tm and higher TBPf, E/TV, ICI, and MaSV (Figs. 1–3). Remodeling parameters were similar in HGC and LGC groups, except for FP(a+), which was significantly lower in HGC than in LGC (36 ± 10 days vs. 53 ± 14 days, respectively; $p < 0.05$).

LGC, when compared with PMOP, only showed significant lower W.Th (24.6 ± 4.5 μm vs. 30.3 ± 2.5 μm, respectively; $p < 0.0005$), Aj.AR/BS (0.22 ± 0.17 μm/day vs. 0.83 ± 0.73 μm /day, respectively; $p < 0.05$), and BFR/BS (0.016 ± 0.022 $\mu m^3/\mu m^2$ per day vs. 0.037 ± 0.016 $\mu m^3/\mu m^2$ per day, respectively; $p < 0.05$), with increased N.Oc/BS (0.13 ± 0.12 mm^{-1} vs. 0.06 ± 0.10 mm^{-1}, respectively; $p < 0.05$). In addition, the HGC group compared with PMOP showed decreased Tb.N and FP(a+) (36 ± 10 days vs. 53 ± 11 days; $p < 0.05$) and increased MaSV. After adjustment for BV/TV, ICI was the only parameter significantly higher in HGC, among those reflecting microarchitecture, with respect to the two other groups ($p < 0.0005$).

DISCUSSION

At the histological level, previous studies have found that PMOP patients with respect to normal women showed a significant decrease of about 40% in cancellous bone volume, Tb.Th, Tb.N, W.Th, and in the FP(a+). In addition, there were no significant differences in bone formation at the tissue and BMU levels. Moreover, a significant increase in Tb.Sp and osteoclast number was observed.[21,22] The same results were observed in GIOP patients compared with normal age-matched controls, even if, in that study, the lower values of Tb.Th and W.Th were attributed principally to depressed formation at the cell level because of decreased lifespan of the cells.[19,20]

All the GC-treated women in our study were menopausal. Thus, an interesting aim of the study was to evaluate possible specific effects of GC on bone loss in addition to the menopause-related ones.[21,32] As for the remodeling, we found increased bone resorption expressed as ES/BS and



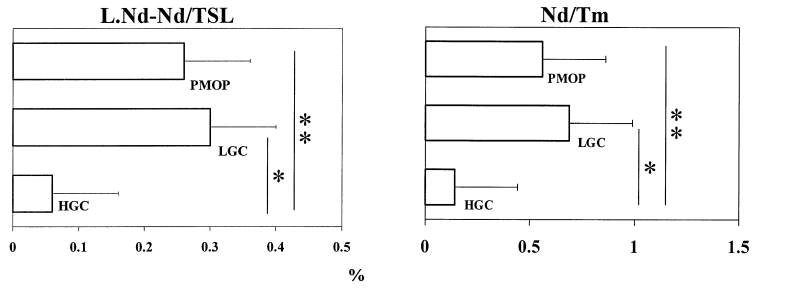
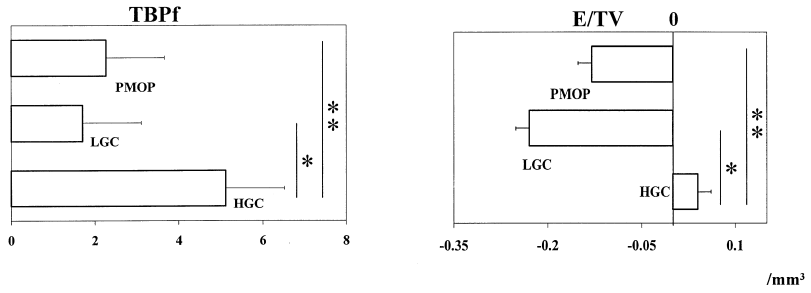


FIG. 2. Node parameters of strut analysis in PMOP and GIOP caused by LGC and HGC (mean ± SD; $*p < 0.05$; $**p < 0.01$).



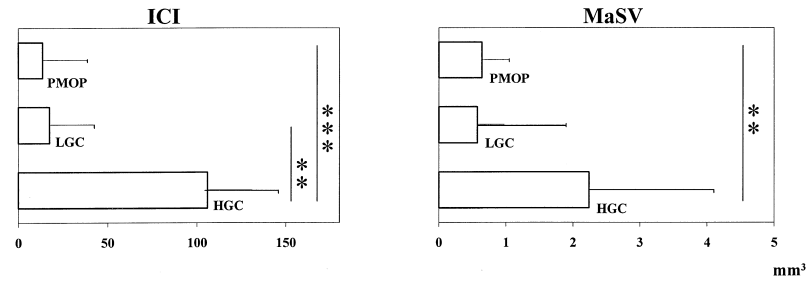


FIG. 3. Indirect parameters of bone microarchitecture in PMOP and GIOP caused by LGC and HGC (mean ± SD; $*p < 0.05$; $**p < 0.005$; $***p < 0.0005$).

N.Oc/BS as in PMOP, but at a significantly higher degree. This could be related to the effect of high doses of GCs in further lowering estrogen levels, which could increase remodeling space.[33]

On the contrary, the bone formation was decreased in GIOP with respect to PMOP at all three levels, cell, BMU, and tissue. These changes were associated with lower W.Th, noted even with the low cumulative dose. This finding was likely to be caused by a lower osteoblastic apposition rate, which resulted from both a reduced O.Th and a trend for a shorter FP(a+), associated with similar MAR. Decreased W.Th has previously been described in GIOP,[18–22] but in those studies neither dose nor time influence were taken into account. In our study, the importance of the cumulative dose was emphasized, 10 g corresponding to a high risk of osteoporosis, as previously shown by Joseph.[34] Similar results in bone remodeling parameters were observed when we compared low and high cumulative dose groups. In addition, the FP(a+) was significantly reduced in the HGC group, suggesting a dose-related toxic effect of GC treatment on the osteoblasts. In fact, these findings suggested that while decreased osteoblastic activity was already associated with low cumulative doses of GC, only prolonged exposure to this treatment could reduce the period of time in which these cells work actively to synthesize bone matrix.

For many years, microarchitecture evaluation has been considered as an interesting approach to histological evaluation of bone. Particularly in the last decade, many parameters have been suggested and evaluated to describe directly or indirectly the trabecular network. However, all these microarchitecture parameters have been evaluated in 2D sections even if the connectivity is a 3D property of bone

structure. Thus, using 2D images, there are some limitations and information about connectivity may only be inferred. Therefore, the strut analysis in our study only focused on the nodes, but we have also evaluated other parameters, some of which were previously found to be correlated to 3D measures.[25] Nevertheless, changes in microarchitecture also have been proposed to explain the disproportional decrease in fracture incidence with respect to relative gain in bone mass with new antiresorptive agents. Recently, it has been found that trabecular microarchitecture was a major and independent determinant of vertebral fractures in middle-aged men with severe osteopenia.[35] From another point of view, it also has been suggested that even if the deterioration of trabecular architecture is a contributory effect to bone fragility, it is not necessary to repair the architectural defects to prevent fractures.[36] Hence, further studies are needed to define the effective relationship between trabecular architecture, bone loss, and fracture risk.

In our evaluation, no microarchitectural differences were observed between GIOP and PMOP. On the contrary, different results were found when the GIOP group was divided according to cumulative doses of GC. In fact, HGCs were associated with important alterations of the trabecular network with respect to LGC and PMOP groups. These microarchitectural changes were characterized by major bone loss with trabecular thinning, which also were less numerous and highly disconnected. The high value of TBPf, MaSV, ICI, node-related parameters, and a positive value of E/TV proved these findings. Indeed, the different parameters that evaluate microarchitecture appeared consistent, according to previous published studies,[24–27] and could well discriminate between different degrees of connectivity of the trabecular network. This is an important finding that confirms microarchitecture as a relevant approach in the assessment of connectivity because of the consistency of different evaluations, which are certainly not casual. These results were similar to those reported in osteoporotic men compared with controls.[20] Even if no significant difference was noted in any parameter of bone microarchitecture between LGC and PMOP groups, there was a trend to a highest number of nodes (higher N.Nd/TV and N.Nd-Nd/TV) and a better connectivity (lower E/TV) in LGC versus PMOP. These findings, according to previous studies,[22] suggested a different pathway of GIOP with respect to PMOP, characterized by progressive thinning of the trabeculae, without early plate perforations, at least after low exposure to GC. This could be caused by transiently lower or depressed osteoclastic activity induced by GCs, despite an increased number of osteoclasts and a length of the resorption cavities (related to secondary hyperparathyroidism). Even if in this context BV/TV was certainly a major determinant to explain the different pattern of the trabecular network among different doses of GC, nevertheless, microarchitecture evaluation provided additional and original information about the shape and organization of trabeculae, even in 2D sections.

From another point of view, the results of this study also can give an interesting suggestion about the possible reversibility of GC effects on bone. Previous studies have shown a densitometric[37,38] and histological[17] recovery after withdrawal of exogenous treatment or successful surgery (in Cushing's syndrome); our results suggested that over a total intake of 10 g, which could be considered as "the point of no return" in this context, the bone recovery could not be possible, because of substantial and nonreversible disruption of the trabecular network. This was suggested by lower values of structure and microarchitecture parameters and also by higher values of indirect microarchitectural parameters in the HGC group, with evidence of a bone characterized by a low number of trabeculae, not connected and separated by a large marrow space (ICI increased).

In summary, GC treatment with respect to PMOP is characterized by a greater reduction in osteoblastic activity at all three levels, cell, BMU, and tissue, which leads to reduced bone formation. This condition was associated with higher resorption parameters.

At the BV/TV constant, even LGC induced a reduction in osteoblastic activity associated with trabecular thinning and increased resorption. With HCG, these changes were associated with a more dramatic bone loss caused by a major loss of trabecular connectivity. Nevertheless, the number of subjects in this study is small and there is large variability in the measurements. Thus, larger studies should be done to confirm these results.

REFERENCES

1. Villareal DT, Civitelli R, Gennari C, Avioli LV 1991 Is there an effective treatment for glucocorticoid-induced osteoporosis? Calcif Tissue Int **49:**141–142.
2. Cooper C, Coupland C, Mitchell M 1995 Rheumatoid arthritis, corticoid therapy and hip fracture. Ann Rheum Dis **54:**49–52.
3. Reid IR 1997 Steroid-induced osteoporosis. Osteoporos Int **7:**S213–S216.
4. Adinoff AD, Hollister JR 1983 Steroid-induced fractures and bone loss in patients with asthma. N Engl J Med **309:**265–268.
5. Ross EJ, Linch DC 1982 Cushing's syndrome-killing disease: Discriminatory value of signs and symptoms aiding early diagnosis. Lancet **2:**646–649.
6. Reid IR 1997 Glucocorticoid osteoporosis—mechanisms and management. Eur J Endocrinol **137:**209–217.
7. Epstein S, Shane E, Bilezikian JP 1995 Organ transplantation and osteoporosis. Curr Opin Rheumatol **7:**255–261.
8. Rodino MA, Shane E 1998 Osteoporosis after organ transplantation. Am J Med **104:**459–469.
9. Brandli DW, Golde G, Greenwald M, Silverman SL 1991 Glucocorticoid-induced osteoporosis: A cross-sectional study. Steroids **56:**518–23.
10. Laan RF, van Riel PL, van de Putte LB, van Erning LJ, van't Hof MA, Lemmens JA 1993 Low-dose prednisone induce rapid reversible axial bone loss in patients with rheumatoid arthritis. Ann Intern Med **119:**963–968.
11. Lukert BP, Raisz LG 1990 Glucocorticoid-induced osteoporosis: Pathogenesis and management. Ann Intern Med **112:**352–364.
12. Chavassieux P, Pastoureau P, Chapuy MC, Delmas PD, Meunier PJ 1993 Glucocorticoid-induced inhibition of osteoblastic bone formation in ewes: Biochemical and histomorphometric study. Osteoporos Int **3:**97–102.
13. Mitchell DR, Lyles KW 1990 Glucocorticoid-induced osteoporosis: Mechanism for bone loss; evaluation of strategies for prevention. J Gerontol **45:**M153–M158.
14. Klein RG, Arnaud SB, Gallagher JC, Deluca HF, Riggs BL 1977 Intestinal calcium absorption in exogenous hypercorti-

cism: Role of 25-hydroxyvitamin D and corticosteroid dose. J Clin Invest **60:**253–259.
15. Suzuki Y, Ichikawa Y, Saito E, Homma M 1983 Importance of increased urinary calcium excretion in the development of secondary hyperparathyroidism of patients under glucocorticoid therapy. Metabolism **32:**151–156.
16. Adachi JD 1997 Corticoid-induced osteoporosis. Am J Med Sci **313:**41–49.
17. Manolagas SC, Weinstein RS 1999 New developments in the pathogenesis and treatment of steroid-induced osteoporosis. J Bone Miner Res **14:**1061–1066.
18. Bressot C, Meunier PJ, Chapuy MC, Lejeune E, Edouard C, Darby AJ 1979 Histomorphometric profile, pathophysiology and reversibility of corticosteroid-induced osteoporosis. Metab Dis Rel Res **1:**303–311.
19. Dempster DW, Arlot MA, Meunier PJ 1983 Mean wall thickness and formation period of trabecular bone packets in corticosteroids osteoporosis. Calcif Tissue Int **35:**410–417.
20. Chappard D, Legrand E, Basle MF, Fromont P, Racineux JL, Rebel A, Audran M 1996 Altered trabecular architecture induced by corticosteroids: A bone histomorphometric study. J Bone Miner Res **11:**676–685.
21. Arlot ME, Delmas PD, Chappard D, Meunier PJ 1990 Trabecular and endocortical bone remodeling in postmenopausal osteoporosis: Comparison with normal postmenopausal women. Osteoporos Int **1:**41–49.
22. Aaron JE, Francis RM, Peacock M, Makins NB 1989 Contrasting microanatomy of idiopathic and corticosteroid-induced osteoporosis. Clin Orthop **243:**294–305.
23. Mellish RW, Ferguson-Pell MW, Cochran GV, Lindsay R, Dempster DW 1991 A new manual method for assessing two-dimensional cancellous bone structure: Comparison between iliac crest and lumbar vertebra. J Bone Miner Res **6:**689–696.
24. Vesterby A, Gundersen HJG, Melsen F 1989 Star volume of marrow space and trabeculae of the first lumbar vertebra: Sampling efficiency and biological variation. Bone **10:**7–13.
25. Feldkamp LA, Goldstein SA, Parfitt AM, Jesion G, Kleerekoper M 1989 The direct examination of three-dimensional bone architecture in vitro by computed tomography. J Bone Miner Res **4:**3–11.
26. Hahn M, Vogel M, Pompesius-Kempa M, Delling G 1992 Trabecular bone pattern factor—a new parameter for simple quantification of bone microarchitecture. Bone **13:**327–330.
27. Le HM, Holmes RE, Shors EC, Rosenstein DA 1992 Computerized quantitative analysis of the interconnectivity of porous biomaterials. Acta Stereol **11**(Suppl 1)**:**267–272.
28. Parfitt AM, Drezner MK, Glorieux FH, Kanis JA, Malluche H, Meunier PJ, Ott SM, Recker RR 1987 Bone histomorphometry: Standardization of nomenclature, symbols, and units. J Bone Miner Res **2:**595–610.
29. Parfitt AM, Mathews CHE, Villanueva AR, Kleerekoper M, Frame B 1983 Relationships between surface, volume, and thickness of iliac trabecular bone in aging and in osteoporosis. J Clin Invest **72:**1396–1409.
30. Birkenhäger-Frenkel DH, Nigg AL, Hens CJJ, Birkenhäger JC 1993 Changes of interstitial bone thickness with age in men and women. Bone **14:**211–216.
31. Levitz P, Tchoubar D 1992 Disordered porous solids: From chord distributions to small angle scattering. J Physiol France **2:**771–790.
32. Gallagher JC 1996 Estrogen: Prevention and treatment of osteoporosis. In: Marcus R, Feldman D, Kelsey J (eds.) Osteoporosis. Academic Press, San Diego, CA, USA, pp. 1191–1208.
33. Crilly R, Cawood M, Marshall DH, Nordin BE 1978 Hormonal status in normal, osteoporotic and corticosteroid-treated postmenopausal women. J R Soc Med **71:**733–736.
34. Joseph CJ 1994 Corticosteroid-induced osteoporosis. Am J Hosp Pharm **51:**118–197.
35. Legrand E, Chappard D, Pascaretti C, Duquenne M, Krebs S, Rohmer V, Basle M-F, Audran M 2000 Trabecular bone microarchitecture, bone mineral density, and vertebral fractures in male osteoporosis. J Bone Miner Res **15:**13–19.
36. Dempster DW 2000 The contribution of trabecular architecture to cancellous bone quality. J Bone Miner Res **15:**20–23.
37. Rizzato G, Montemurro L 1993 Reversibility of exogenous corticosteroid-induced bone loss. Eur Respir J **6:**116–119.
38. Manning P, Evans MC, Reid IR 1990 Axial bone density following cure of Cushing's syndrome. Evidence for reversibility of steroid osteoporosis. In: Christiansen C, Overgaard K (eds.) Osteoporosis. vol 3. Handelstrykkeriet Lo Aalburg ApS, Aalborg, Denmark, pp. 1585–1586.

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