

Acute Effect of 1,25-Dihydroxyvitamin D₃, Prednisone, and 1,25-Dihydroxyvitamin D₃ Plus Prednisone on Serum Osteocalcin in Normal Individuals

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ABSTRACT

Suppression of osteoblastic function plays an important pathogenic role for the development of glucocorticoid-induced osteoporosis. Serum osteocalcin (OC) is a sensitive marker of bone formation. The diurnal rhythm in serum OC can be changed by administration of single doses of either 1,25-(OH)₂D₃ or prednisone. However, the two steroids have opposing effects: 1,25-(OH)₂D₃ increases and prednisone decreases serum OC. The aim of the present study was to examine whether 1,25-(OH)₂D₃ can oppose the acute suppressive effect of prednisone on serum OC in normal subjects. We compared the effect of a combined dose of 2 μg 1,25-(OH)₂D₃ and 10 mg prednisone on the diurnal rhythm of serum OC with the effect of 2 μg 1,25-(OH)₂D₃ + placebo in a crossover study. Seven normal subjects aged 23–36 years were investigated twice at an interval of 1 week. Blood samples were collected every 60 minutes from 1900 until 1100 h the following day. Study drugs were given at 2000 h. The data from the present investigation were compared with data obtained from a similar study with placebo and prednisone in the same subjects. After administration of 1,25-(OH)₂D₃ serum OC followed the placebo curve during the first 8 h, but in contrast to the placebo curve it then continued to increase and remained elevated throughout the observation period ($p < 0.05$). Prednisone inhibited and reversed the nocturnal rise in serum OC levels ($p < 0.01$). The course of serum OC after administration of 1,25-(OH)₂D₃ + prednisone almost paralleled the course after placebo. We conclude that 1,25-(OH)₂D₃ and prednisone have opposing effects on serum OC.

INTRODUCTION

OSTEOCALCIN (OC), or bone γ -carboxyglutamic acid-containing protein (bone gla protein, BGP), is the most abundant noncollagenous bone matrix protein.^(1,2) It is localized to bone and dentine⁽³⁾ and is believed to be exclusively synthesized and secreted by bone cells of osteoblastic phenotype. OC also circulates in the blood,⁽⁴⁾ and serum OC has been validated in numerous clinical studies as a valuable biochemical marker of osteoblastic activity in both normal subjects and patients with various metabolic bone diseases.^(5–14) The metabolic clearance rate is mainly dependent on renal function,⁽¹⁵⁾ and the serum half-life of

OC is approximately 20 minutes in humans.⁽¹⁶⁾ These qualities make serum OC a specific, sensitive, and rapidly responding marker of osteoblastic activity, as long as the metabolic clearance of OC remains unaltered. Several investigators have reported a characteristic circadian rhythm for serum OC with maximum levels during the early night and minimum levels around noon.^(17–19) This diurnal variation seems relatively unaffected by age, gender, smoking, and season,⁽²⁰⁾ but it can be significantly changed by exogenous administration of single doses of 1,25-dihydroxyvitamin D₃ [1,25-(OH)₂D₃]⁽¹⁸⁾ or glucocorticoids (GC).⁽¹⁹⁾ GC suppress serum OC,⁽¹⁹⁾ but 1,25-(OH)₂D₃ exerts a stimulatory effect.⁽¹⁸⁾ This is in accordance with studies

showing profound effects of GC and 1,25-(OH)₂D₃ on serum OC after several days of treatment.⁽²¹⁻²³⁾

Suppression of osteoblastic function plays an important pathogenic role for the development of GC-induced osteoporosis⁽²⁴⁾ along with decreased intestinal absorption of calcium⁽²⁵⁾ and enhanced parathyroid hormone (PTH) secretion.⁽²⁴⁾ The decreased intestinal absorption can be corrected by administration of 1,25-(OH)₂D₃.⁽²⁶⁾ Moreover, a recent *in vitro* experiment demonstrated that 1,25-(OH)₂D₃ is capable of antagonizing the GC-induced enhancement of PTH secretion from cultured parathyroid cells.⁽²⁷⁾ Finally, 1,25-(OH)₂D₃ may have a beneficial effect on GC-induced osteopenia in humans,⁽²⁸⁾ but it is not known whether 1,25-(OH)₂D₃ ameliorates the GC-induced depression of osteoblastic activity. Administration of 1,25-(OH)₂D₃ to patients during chronic GC treatment has been reported to stimulate⁽²⁹⁾ or to have no effect on serum OC.⁽³⁰⁾ In rats, however, 1,25-(OH)₂D₃ opposes the deleterious effect of GC on bone⁽³¹⁾ when initiated simultaneously with GC treatment.

The aim of the present study was to examine whether 1,25-(OH)₂D₃ is capable of opposing the acute suppressive effect of prednisone on serum OC in normal subjects. For this purpose the diurnal rhythm in serum OC was studied in seven normal subjects after administration of 1,25-(OH)₂D₃ and 1,25-(OH)₂D₃ + prednisone in a crossover design. The subjects earlier participated in a study on the effects of a single dose of prednisone or placebo,⁽¹⁹⁾ and the results from the present study were compared with the previously obtained data.

SUBJECTS AND METHODS

Subjects

A group of four women and three men aged 23 to 36 years (mean 28) volunteered to the study. The subjects were selected from a group of subjects who 12 months earlier participated in a similar study examining the effect of small oral doses of prednisone (2.5 and 10 mg) on the circadian variation in serum OC.⁽¹⁹⁾ They were again carefully screened by physical examination and routine biochemistry. None had any history of intercurrent endocrine, renal, or metabolic disease or was taking any medications at the time of the study. The protocol was reviewed by the local ethical committee and the National Board of Health. Each subject gave informed consent before the study.

Design

In the present study all subjects were studied on 2 days in March at an interval of 1 week. Subjects received 2 µg 1,25-(OH)₂D₃ on both study days. In addition they received 10 mg prednisone or placebo in a double-blind, crossover design. In the previous study conducted 12 months earlier, the same subjects were examined after prednisone only or placebo only in a double-blind, crossover design. Five subjects received 10 mg prednisone and two subjects received 2.5 mg prednisone. In both studies each subject was given a single dose of each drug and was not pretreated.

Except for the medication, the conditions on the 4 study days during the two studies were kept as constant as possible. Volunteers were admitted to the hospital at 1600 h. Blood samples were collected every 60 minutes through an indwelling venous catheter until 1100 h the following day. Drugs were given orally at 2000 h. Meals were offered at 1800 and 0830 h. Normal indoor activities were allowed, and lights were turned off at midnight. The subjects slept during blood collection from midnight until 0730 h.

Biochemistry

Serum OC was analyzed by a radioimmunoassay (RIA) modified from that described by Price and Nishimoto.⁽⁴⁾ Purified bovine OC, generously supplied by Dr. J. Poser (Procter and Gamble Co., Cincinnati, OH), was used for standard and tracer. To each assay tube was added 100 µl rabbit antiovine OC serum (final dilution, 1:5000). After 1 h 100 µl ¹²⁵I-OC (10,000 cpm in assay buffer) was added. After an additional 48 h incubation at 4°C the assay was terminated by precipitation of ¹²⁵I-OC bound to antibody by adding 500 µl 25% (wt/vol) polyethylene glycol to each tube. The tubes were centrifuged for 10 minutes at 2000 × g, followed by a second precipitation with 500 µl 12.5% (wt/vol) polyethylene glycol. The supernatants were decanted, and the tubes were counted in a LKB rack minigamma counter. The sensitivity was 1.4 ng/ml. The intraassay and interassay variations were 5 and 10%, respectively. In both studies all samples from an individual subject were analyzed in duplicate in the same run.

Statistical analysis

The mean values of serum OC (raw data) for each study day were plotted against time (Fig. 1A or 2A). The effect of time on the variations in serum OC, the effect of treatment on the time course, and the effect of treatment on the overall serum OC levels were analyzed with repeated-measures analysis of variance (ANOVA). The effect of placebo, 1,25-(OH)₂D₃, and 1,25-(OH)₂D₃ + prednisone was tested using data from all seven subjects. The effect of placebo, 1,25-(OH)₂D₃, prednisone, and 1,25-(OH)₂D₃ + prednisone was tested using data from the five subjects who received 10 mg prednisone in the previous study. Furthermore, individual raw data from each study day were transformed by subtracting the 2000 h value (pretreatment value) from all other time points of that day. This procedure reduces the interindividual variation and assay-related variations in the level of serum OC. The transformed data indicate changes in serum OC from pretreatment values (Δ serum OC). The effect of time and treatment on the variations of Δ serum OC were also analyzed with repeated-measures analysis of variance.

To make the differences in the time course patterns more illustrative the Δ curves of each individual were then smoothed using a moving average technique⁽¹⁸⁾ and the mean values plotted against time (Figs. 1B and 2B).

Finally, areas under the curves (AUC) were calculated for the intervals 2000-0400 h and 0400-1100 h by trapezoidal integration and transformed to integrated serum OC concentrations [S-OC(I)] by division with the appropriate

time intervals. Statistical analysis was performed using the SPSS (Statistical Package for Social Sciences). *P* values less than 0.05 were considered significant.

RESULTS

Figure 1A shows mean serum OC plotted against time after a single dose of 1,25-(OH)₂D₃, 1,25-(OH)₂D₃ + prednisone, and placebo. On all three occasions serum OC varied significantly with time (*p* < 0.01). Following placebo, serum OC gradually increased around bedtime (2330 h) to a maximal plateau phase around 0200 h. It then decreased toward minimum at 1100 h. After administration of 1,25-(OH)₂D₃, serum OC followed the placebo curve during the first 6–8 h, but in contrast to the placebo curve it continued to increase for the following 3 h and this increase was sustained for the rest of the observation period. The course of serum OC after 1,25-(OH)₂D₃ + prednisone almost paralleled the placebo curve. The time courses during the three treatments were not significantly different. Also, the differences in the overall level of serum OC during the interval 2000–0400 h was statistically insignificant. However, during the time from 0400 to 1100 h, the overall level of serum OC varied significantly between the three treatments (*p* < 0.05). The same results was found when Δ values was used in ANOVA. The smoothed curves are shown in Fig. 1B. Table 1 depicts individual values of S-OC(I) during the interval 0400–1100 h in percentage of S-OC(I) during the initial 8 h (2000–0400 h) for each treatment. After placebo only one subject had a higher S-OC(I) during the last time interval, whereas six subjects had higher levels of S-OC(I) after 1,25-(OH)₂D₃ (*p* < 0.01). The changes in S-OC(I) after 1,25-(OH)₂D₃ + prednisone were not statistically significant from the changes after placebo (*p* = 0.56).

Figure 2 shows the mean curves obtained from the placebo day, the prednisone day, the 1,25-(OH)₂D₃ day, and the 1,25-(OH)₂D₃ + prednisone day in the five subjects who in the previously reported study received 10 mg prednisone. On the prednisone day no increase in serum OC after bedtime was seen. Instead serum OC declined steadily toward minimum levels in the early morning and remained low during the rest of the study period. The variation in serum OC during the last 7 h differed significantly between treatments (*p* < 0.05). S-OC(I) was lower during the last 7 h in all five individuals (Table 1).

DISCUSSION

The pattern of the diurnal rhythm in serum OC is remarkably constant over years⁽¹⁸⁾ and seasons.⁽²⁰⁾ The data obtained in the present study could therefore be analyzed and compared with data after placebo only and prednisone only obtained in an identically designed study, although the previous study was conducted 12 months earlier. We deliberately chose to evaluate the effect of the drugs on the nocturnal increase in serum OC during a period when endogenous levels of cortisol is low to enhance the sensitivity of the test system. Our results demonstrate that 1,25-

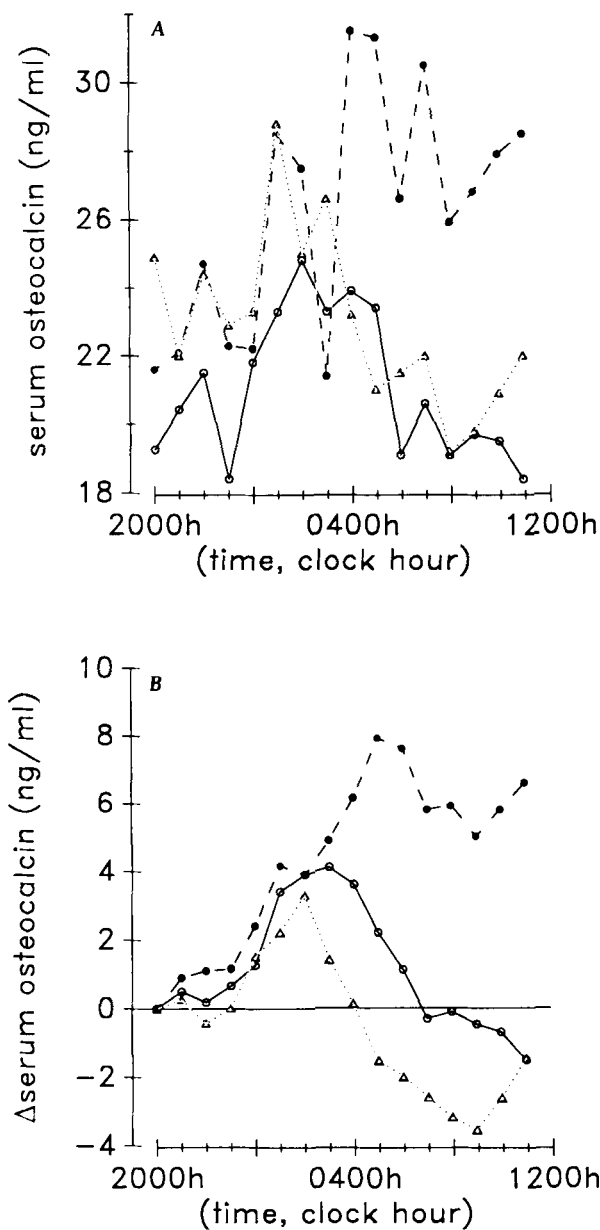


FIG. 1. Mean serum osteocalcin levels against time in seven normal subjects. (A) Mean curves using raw data; (B) smoothed (moving average technique) data transformed by subtracting the 2000 h values of each day from all subsequent time points: (○) placebo; (●) 2 μ g 1,25-(OH)₂D₃ + placebo; (△) 2 μ g 1,25-(OH)₂D₃ + 10 mg prednisone. Drugs were given at 2000 h.

(OH)₂D₃ increased serum OC in comparison to placebo. The exact time before the initiation of the effect is difficult to determine. Inspection of the curves and the statistical analysis gives the impression that stimulation of serum OC becomes apparent 6–8 h after ingestion. This is in agreement with an in vitro experiment using cultured human osteoblasts in which OC appeared in the medium 6 h after the addition of 1,25-(OH)₂D₃.⁽³²⁾ Only one other study has thus far been reported on the effects of single doses of

TABLE 1. INDIVIDUAL INTEGRATED SERUM OC LEVELS DURING THE INTERVAL 0400–1100 H AS A PERCENTAGE OF THAT DURING THE INITIAL 8 H (2000–0400 H) FOR EACH TREATMENT^a

Subject (no.)	Placebo	Treatment		
		1,25-(OH) ₂ D ₃	1,25-(OH) ₂ D ₃ + prednisone	Prednisone
1	98.6	109.0	81.8	85.1
2	97.3	133.7	101.2	72.7
3	83.3	99.1	100.8	80.9
4	76.7	111.9	97.8	55.7
5	101.5	101.8	83.8	64.7
6	96.4	138.4	70.3	—
7	94.8	116.0	82.7	—
Mean	92.7	115.7	88.4	71.8
SEM	3.4	5.7	4.4	5.3

^aIntegrated serum OC [S-OC(I)] levels are calculated from the areas under the curves and divided by the appropriate time intervals.

1,25-(OH)₂D₃ on the circadian serum OC rhythm in normal subjects.⁽¹⁸⁾ In this study a dose of 2 µg 1,25-(OH)₂D₃ administered to four normal subjects at 0800 h prevented the expected morning fall in serum OC within a few hours after ingestion, indicating a biologic response to 1,25-(OH)₂D₃ within 6 h. The apparently slower response to 1,25-(OH)₂D₃ in our study may be explained by differences in the time schedules between the experiments. We gave 1,25-(OH)₂D₃ before the expected nocturnal increase, but Markowitz *et al.*⁽¹⁸⁾ gave 1,25-(OH)₂D₃ before an expected fall in serum OC. In both studies, however, the effect on serum OC was sustained throughout the study period.

The acute suppressive effect of small doses of prednisone on serum OC has been described and discussed earlier.⁽¹⁹⁾ In short, the effect of prednisone in a dose of 10 mg was discernible within 4–5 h after ingestion and serum OC returned to normal levels after approximately 14 h.

In the present study the nocturnal OC profile after simultaneous administration of 2 µg 1,25-(OH)₂D₃ and 10 mg prednisone was statistically indistinguishable from the profile following placebo, but 1,25-(OH)₂D₃ increased and prednisone decreased serum OC. The interaction between the effects of 1,25-(OH)₂D₃ and prednisone has been investigated in several other experiments. In human osteoblast-like cells preexposed to GC for 24 h OC production does not respond to 1,25-(OH)₂D₃ stimulation.⁽³²⁾ In rat osteoblast-like cells preincubation with GC modulates the bioreponse to 1,25-(OH)₂D₃, but in a stimulative direction.⁽³³⁾ Finally, conflicting results were found when 1,25-(OH)₂D₃ was given to patients receiving chronic steroid treatment.^(29,30) One study demonstrated a lack of serum OC response to 5 days of administration of 1,25-(OH)₂D₃ (2 µg/day) in GC-treated patients,⁽²⁹⁾ and another has reported a relative increase in serum OC after 4 days of administration, which was comparable to that of normal sub-

jects.⁽³⁰⁾ The effects of 1,25-(OH)₂D₃, however, may depend on the dose of prednisone. This is indicated in a preliminary report showing that 1,25-(OH)₂D₃ could not stimulate serum OC in normal subjects treated with high-dose prednisone (40 mg/day) for 7 days followed by 7 days of treatment with both prednisone and 2.0 µg 1,25-(OH)₂D₃, whereas a lower dose of prednisone (10 mg/day) did not block the increase in serum OC following 1,25-(OH)₂D₃.⁽³⁴⁾ These studies are not directly comparable with the present, however. Treatment with GC for several days may have altered the osteoblast population by reducing recruitment of osteoblasts from osteoblast progenitors,⁽³⁵⁾ and the present study most likely describes the effects on the present osteoblast population.

Changes in serum OC may be the result of alterations in plasma production rate and/or clearance rate. No data are available from human studies. In sheep 1,25-(OH)₂D₃ increases OC production rate, but it has no effect on the degradation of OC.⁽³⁶⁾ In the same study GC inhibited the OC production rate in a dose-dependent way, and the clearance rate was increased only by large doses of GC.

The 1,25-(OH)₂D₃-induced stimulation of OC production is believed to be a receptor-mediated process involving transcription of the OC gene.⁽³⁷⁾ The mechanism whereby GC exert their effect on OC production is not fully understood. In a recent report it was shown that GC suppress the constitutive level of human OC gene expression. Further, GC are able to inhibit the induction of gene expression by 1,25-(OH)₂D₃ almost completely⁽³⁸⁾ and are capable of regulating the number of 1,25-(OH)₂D₃ receptors, although the direction of this effect appears to be species dependent.⁽³⁹⁾ In humans the serum level of 1,25-(OH)₂D₃ has been shown to be unchanged, increased, or even decreased during treatment with GC.^(21,40–43) Thus the combined action of the two hormones on OC may involve a combination of complex interactions on different levels.

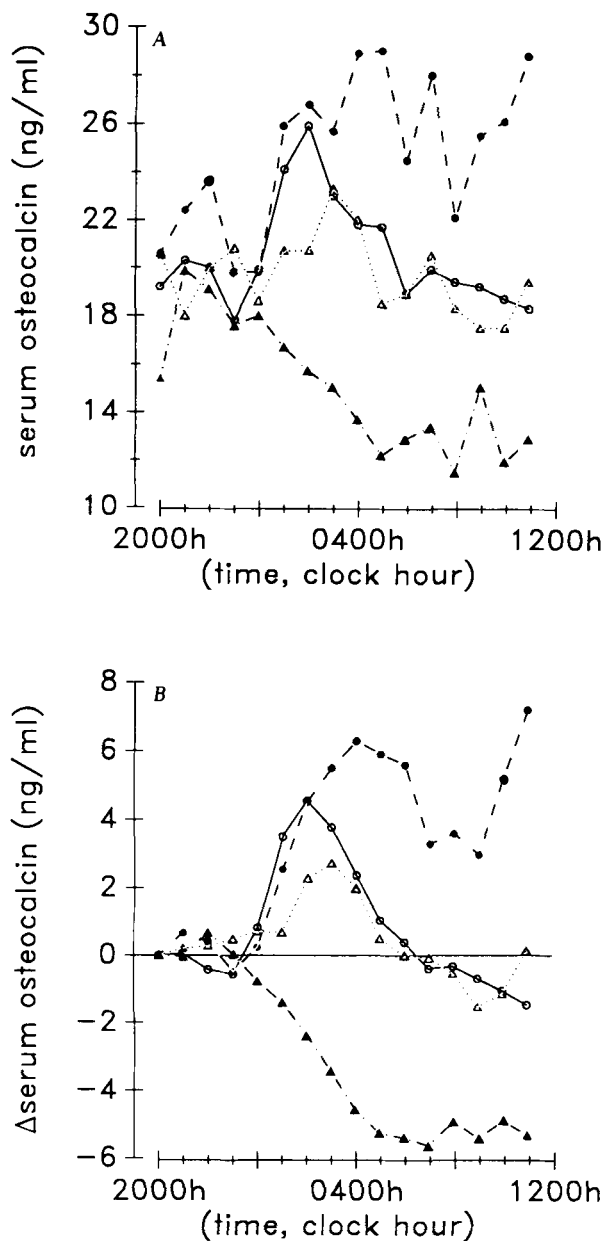


FIG. 2. Mean serum osteocalcin levels against time in five normal subjects. (A) Mean curves using raw data; (B) mean smoothed (moving average technique) data transformed by subtracting the 2000 h values of each day from all subsequent time points: (○) placebo; (●) 2 µg 1,25-(OH)₂D₃ + placebo; (△) 2 µg 1,25-(OH)₂D₃ + 10 mg prednisone; and (▲) 10 mg prednisone. Drugs were given at 2000 h.

Previous studies have described antagonistic effects of GC and 1,25-(OH)₂D₃ on intestinal calcium absorption⁽⁴⁴⁾ and PTH secretion.⁽²⁷⁾ It has been suggested that GC-induced osteopenia might be prevented or treated with the administration of vitamin D metabolites.⁽²⁵⁾ Administration of vitamin D to patients with GC-induced osteopenia has shown a beneficial effect on bone mass in some⁽²⁵⁾ but

not all studies.⁽⁴⁵⁾ Moreover, the risk of hypercalcemia must be carefully considered.⁽⁴⁵⁾ Also, it may be of importance that 1,25-(OH)₂D₃ is given from the start of GC treatment. This is supported by a study in rats in which administration of 1,25-(OH)₂D₃ was able to prevent GC-induced negative effects on bone formation, assessed by bone biopsies and serum OC measurements.⁽³¹⁾ The present study in humans also suggests that GC and 1,25-(OH)₂D₃ have opposing effects on serum OC. However, it is at present unknown whether the increase in serum OC is in fact followed by an improvement in bone formation. Therefore the clinical impact of our results remains to be elucidated.

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