

Short-term low-dose heparin plus bedrest impairs bone metabolism in pregnant women

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Abstract

Objective: To assess the osteoporotic risk of short-term low-dose heparin plus bedrest in pregnancy. **Study design:** In a prospective case-control study, 10 pregnant women on bedrest receiving prophylactic unfractionated heparin 10,000 IU per day for 7–46 days pre-study and 28 days per-study were compared with 6 normal pregnant controls of similar maternal and gestational age and 10 nonpregnant women of similar age. Serum ionised calcium, 1,25-dihydroxyvitamin D₃, osteocalcin, and urinary calcium/creatinine ratio were determined three times at 2-week intervals. **Results:** 1,25-Dihydroxyvitamin D₃ was lower in the treated group than in pregnant controls throughout ($P < 0.03$). Osteocalcin was lower at study start than end in both pregnant groups ($P < 0.05$), and lower in the treated group than in either pregnant (n.s.) or nonpregnant controls ($P < 0.005$). Calcium/creatinine ratio differences were non significant (n.s.). **Conclusion:** Short-term low-dose heparin plus bedrest suppresses 1,25-dihydroxyvitamin D₃ and osteocalcin levels in pregnancy.

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Keywords: Pregnancy; Bedrest; Heparin; 1,25-Dihydroxyvitamin D₃; Osteoporosis

1. Introduction

The osteoporotic implications of long-term high-dose heparin in pregnant women are well-documented [1–4], but it is unknown whether short-term low-dose regimens have a similar effect. The mechanism is believed to involve the chelation of calcium ions [5] and may be compounded by the physiological changes in bone metabolism during pregnancy, in particular the increased mobilisation of calcium to form the fetal skeleton. Although the compensatory increase in 1,25-dihydroxyvitamin D₃ during pregnancy [6–8] promotes enteral calcium absorption, thus slightly raising the free calcium level, there is a counterregulatory fall in parathyroid hormone (PTH) levels, with resultant inhibition of tubular calcium reabsorption, i.e. normal pregnancy is associated with hypercalciuria [8]. In addition, immobilisation is in itself a major risk factor for demineralisation [9]. Pregnant women under prophylactic heparin treatment confined to bedrest thus risk osteoporosis from three sources: heparin, pregnancy and immobilisation.

2. Materials and methods

2.1. Design

Pregnant women under prophylactic heparin treatment confined to bedrest (group 1), normal pregnant women (group 2) and nonpregnant women (group 3) were recruited over 1 year on a case-control basis: at least one woman was matched for maternal age (range: 1 January of one year to 31 Dec of following year = 24 months) across all three groups, and for gestational age (range: 30 days) across the two pregnant groups. The aim was to include 10 consecutively numbered women per group, with subjects 11 (group 2) and 21 (group 3) matching patient 1 in group 1, etc.

Following local ethics committee approval of the protocol, informed consent was obtained from each participant after a thorough explanation of the study aim and content. Venous blood and spontaneously voided urine were collected as far as possible at the same time of day (07:30) on three occasions at 2-week intervals (0, 2 and 4 weeks). Sera for ionised calcium determination and urine samples for creatinine and calcium determination were processed and analysed forthwith. Serum aliquots were stored at -70° for 1,25-dihydroxyvitamin D₃ and osteocalcin analysis.

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2.2. Subjects

Pregnant inpatients under prophylactic heparin treatment (group 1, n = 10) confined to three grades of bedrest: absolute (bedpan and blanket bath), strict (allowed up to toilet and wash-basin only), or relaxed (allowed up, but required to spend most of the day in bed). Nine patients received prophylactic unfractionated heparin 7500–15,000 IU subcutaneously (s.c) daily, and one received 5000 IU s.c daily for two of the four study weeks. Group age was 31.4 (22–38) years. Gestational age at study start was 28.7 (17.3–31.7) weeks. The duration of pre-blood sample bedrest was 18.1 (7–46) days. The indications for bedrest were marginal or complete placenta previa ($n = 5$) and preterm labour with cervical changes ($n = 5$). The commonest pre- and per-study comedications, apart from iron, vitamin and magnesium preparations, were β -agonists, corticosteroids, narcotic analgesics and antipyretics, anti-infective agents and in one case supplemental calcium.

Normal pregnant women (group 2, n = 6). Group age was 32.3 (28–37) years; the age difference versus group 1 was 9.8 (2–19) months. Gestational age at study start was 30.4 (26.3–32.9) weeks; the mean difference versus group 1 was 9.8 (2–26) days. No control subject received heparin or was confined to bedrest; all were healthy and taking regular iron and vitamins, and in two cases supplemental calcium. Blood samples were taken as part of their routine antenatal care at times between 07:30 and 15:00. The difficulty of finding women matching a group 1 patient in maternal and gestational age prevented the recruitment of more than six normal pregnant controls during the study period.

Nonpregnant women (group 3, n = 10). Group age was 31.4 (21–39) years; the age difference versus the group 1 case-controls was 7.2 (2–13) months. None was pregnant or had pre-existing disease; none received heparin or was confined to bedrest; none took regular medication, except hormonal contraception ($n = 2$).

2.3. Analytical procedure

Ionised calcium (serum) samples were centrifuged within 20 min and analysed using ion-selective electrode potentiometry [10]. The results were corrected for sample pH, which has a strong effect on albumin ionisation [11].

1,25-Dihydroxyvitamin D₃ (serum) samples from each subject were processed in the same run of an ¹²⁵I radioimmunoassay (Nichols Institute Diagnostics, San Juan Capistrano, California 92675¹) [12]. The intra-assay coefficient of variation was 5.4–10.6% and the inter-assay coefficient of variation 9.3–15.3%.

Osteocalcin (serum) samples from each subject were processed in the same run of an ¹²⁵I immunoradiometric assay

(Nichols Institute Diagnostics) which uses two different polyclonal antibodies to achieve higher sensitivity than with monoclonal antibodies [13]. The intra-assay coefficient of variation was 3.6–5.3% and the inter-assay coefficient of variation 4.4–5.7%.

Calcium/creatinine ratios (urine) show a highly significant correlation with total calcium excretion [14] and served to avoid 24 h urine collection. Before processing, samples were acidified with HCl to prevent calcium salt precipitation, in particular calcium oxalate. Calcium and creatinine were determined using a Boehringer Mannheim/Hitachi 747 analyser (Roche Diagnostics, Rotkreuz, 6343 Switzerland) [15].

2.4. Statistical analysis

Data entry and statistical analysis were performed in Stat-View 4.51 (Macintosh). Descriptive data (maternal and gestational age, bedrest duration) were expressed as means and standard deviations. Hypothesis-related data were expressed as medians and 25th and 75th percentiles. Intergroup differences were tested using the Kruskal–Wallis or Mann–Whitney test and intra-group changes using Wilcoxon's signed rank test ($P < 0.05$ in all cases).

3. Results

All samples were collected at the scheduled time points except one set of blood samples from one group 1 patient (in the afternoon) and all three blood samples in one group 3 subject (also in the afternoon).

Ionised calcium differences between and within groups were non-significant over the 4 weeks. All group 1 and 3 values were within the assay's normal range (1.10–1.30 mmol/l). Values were above normal in four group 2 samples (including two from the same patient), and median group 2 values were higher than those of the other groups at each data point (Fig. 1).

1,25-Dihydroxyvitamin D₃ levels underwent no significant within-group changes. At weeks 0, 2 and 4, they were lower in group 1 than in group 2 ($P = 0.0227$, 0.0197 and 0.0147, respectively) and higher than in group 3 ($P = 0.0015$, 0.0003, 0.0025, respectively). Groups 2 and 3 also differed from each other on all three occasions ($P = 0.0011$, 0.0011, 0.0011, respectively; Mann–Whitney test; Fig. 2).

Osteocalcin levels increased from week 0 to week 4 in group 1 ($P = 0.0051$; Fig. 3) and from week 0 to weeks 2 and 4 in group 2 ($P = 0.0464$, 0.0277, respectively; Wilcoxon's signed rank test). Levels were higher in group 3 than in group 1 at weeks 0 and 2 ($P = 0.0041$, 0.0126, respectively; Mann–Whitney test) (Fig. 4). Only one sample from one group 1 patient was below the reference range (2.4–10.0 $\mu\text{g/l}$). All levels were below normal in one group 2 subject taking supplemental calcium.

¹ Revisers' note: We have supplied the US address as no Swiss distributor is listed on the Nichols website.

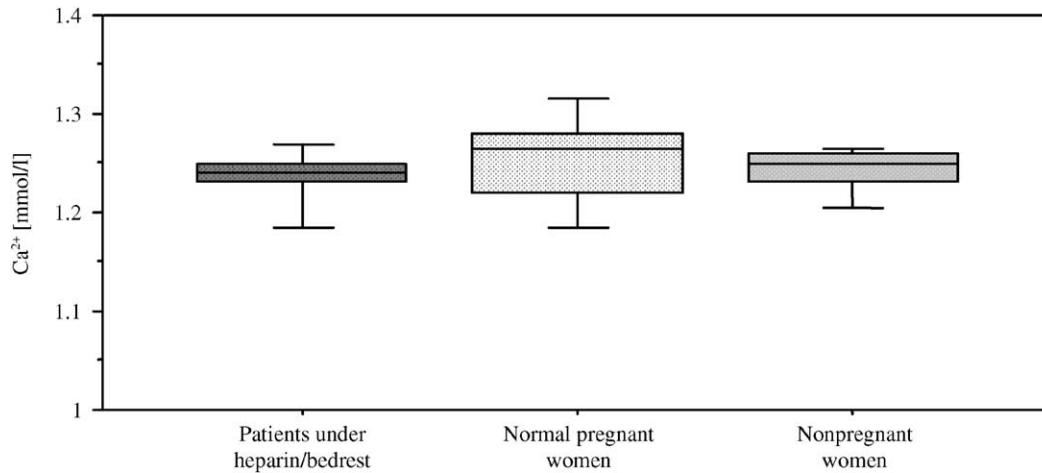


Fig. 1. Serum ionised calcium (week 2).

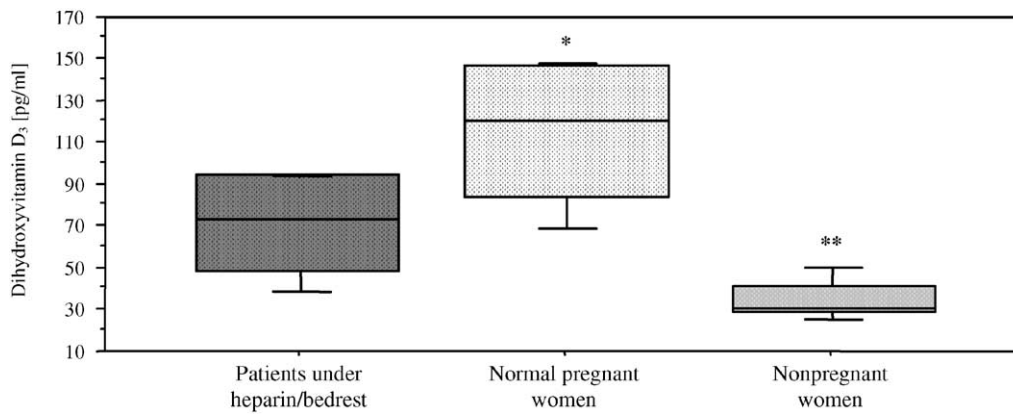


Fig. 2. Serum 1,25-dihydroxyvitamin D₃ (week 0). * $P = 0.0227$ vs. pregnant patients under heparin/bedrest, ** $P = 0.0015$ vs. pregnant patients under heparin/bedrest, $P = 0.0011$ vs. normal pregnant women.

Calcium/creatinine ratios showed no significant within-group changes (Wilcoxon’s signed rank test). They were lower in group 3 at weeks 0, 2 and 4 than in either group 1 ($P = 0.0004$, 0.0052 , 0.0004 , respectively) or group 2

($P = 0.0092$, 0.0048 , 0.0034 , respectively; Mann-Whitney test; Fig. 5).

Parameter interrelationships. The relationship between 1,25-dihydroxyvitamin D₃ and ionised calcium levels was

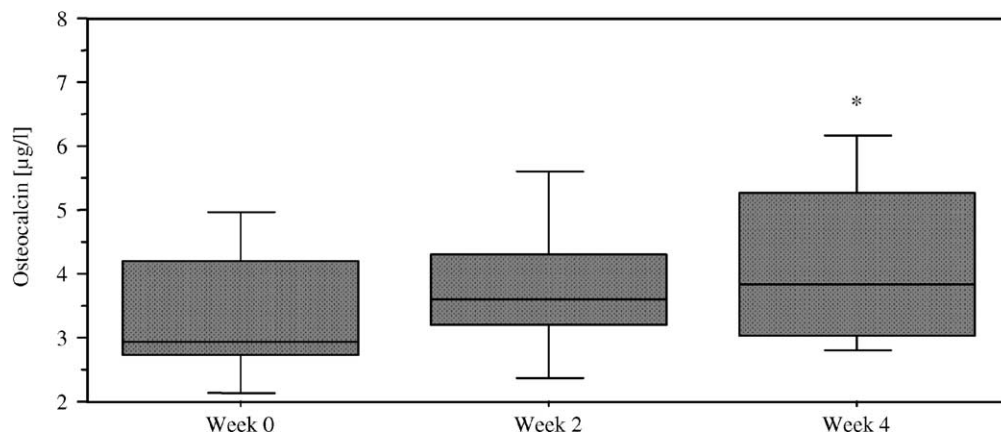


Fig. 3. Serum osteocalcin in pregnant patients under heparin/bedrest. * $P = 0.0051$ vs. week 0.

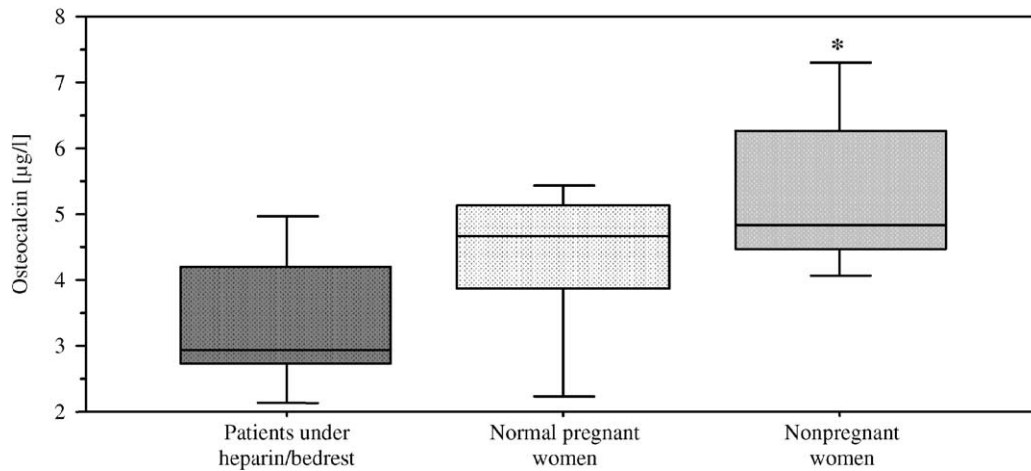


Fig. 4. Serum osteocalcin (week 0). * $P = 0.0041$ vs. pregnant patients under heparin/bedrest.

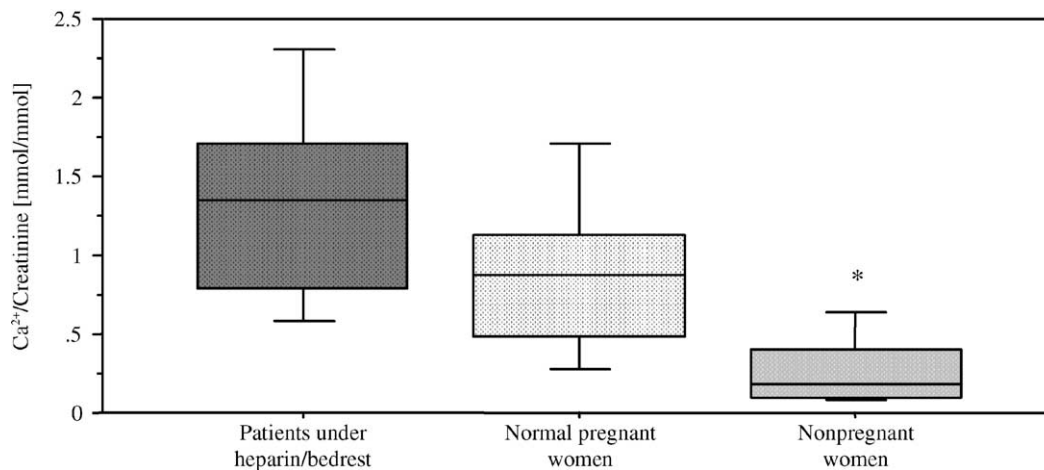


Fig. 5. Urinary calcium/creatinine ratio (week 2). * $P = 0.0004$ vs. pregnant patients under heparin/bedrest, $P = 0.0092$ vs. normal pregnant women.

linear in group 2 ($y = 0.002x - 1.423$, $r = 0.608$, $P = 0.0075$, $n = 10$; Fig. 6a). Osteocalcin and ionised calcium levels were also related in groups 1 and 2 ($y = 0.011x + 1.192$, $r = 0.471$, $P = 0.009$ and $y = 0.023x + 1.135$, $r = 0.558$, $P = 0.016$; Fig. 6b).

4. Comment

These results show that in pregnant women confined to bedrest even short-term treatment with unfractionated heparin 10,000 IU per day induces significant changes in the counterregulatory mechanisms of bone metabolism, in particular a decrease in osteocalcin, possibly due to lower 1,25-dihydroxyvitamin D₃ production than in normal pregnant women. Although our case-control design using two control groups excluded changes purely due to pregnancy, the clinical reality is that the effect of heparin prophylaxis cannot be separated from that of bedrest, i.e. the results reflect the combination of both measures. However, this does not invalidate the practical implications.

Ionised calcium levels were slightly elevated in normal pregnant women, although the range was wider than in the other two groups. This may reflect the smaller size of this group, but it also confirms the contradictory results in the literature [6,8,16]. One explanation for an elevated ionised calcium level is that the increasing calcium requirement in normal pregnancy is offset by augmented 1,25-dihydroxyvitamin D₃ production [7,8]. Thus, we found a correlation between low ionised calcium levels and high 1,25-dihydroxyvitamin D₃ levels in normal pregnant women but not in the pregnant patients under prophylactic heparin treatment confined to bedrest in whom counterregulation was clearly in part abolished. This may be due to a difference in the hypercalciuric effect of PTH [7,8,17]: hypercalciuria, defined as a calcium/creatinine ratio ≥ 0.5 , was more marked in patients under prophylactic heparin treatment confined to bedrest than in the normal pregnant controls.

However, heparin itself inhibits 1,25-dihydroxyvitamin D₃ production. In a case report and a study in 10 pregnant women with heparin 15,000 IU per day, Aarskog et al. [1] found lower 1,25-dihydroxyvitamin D₃ levels than in normal

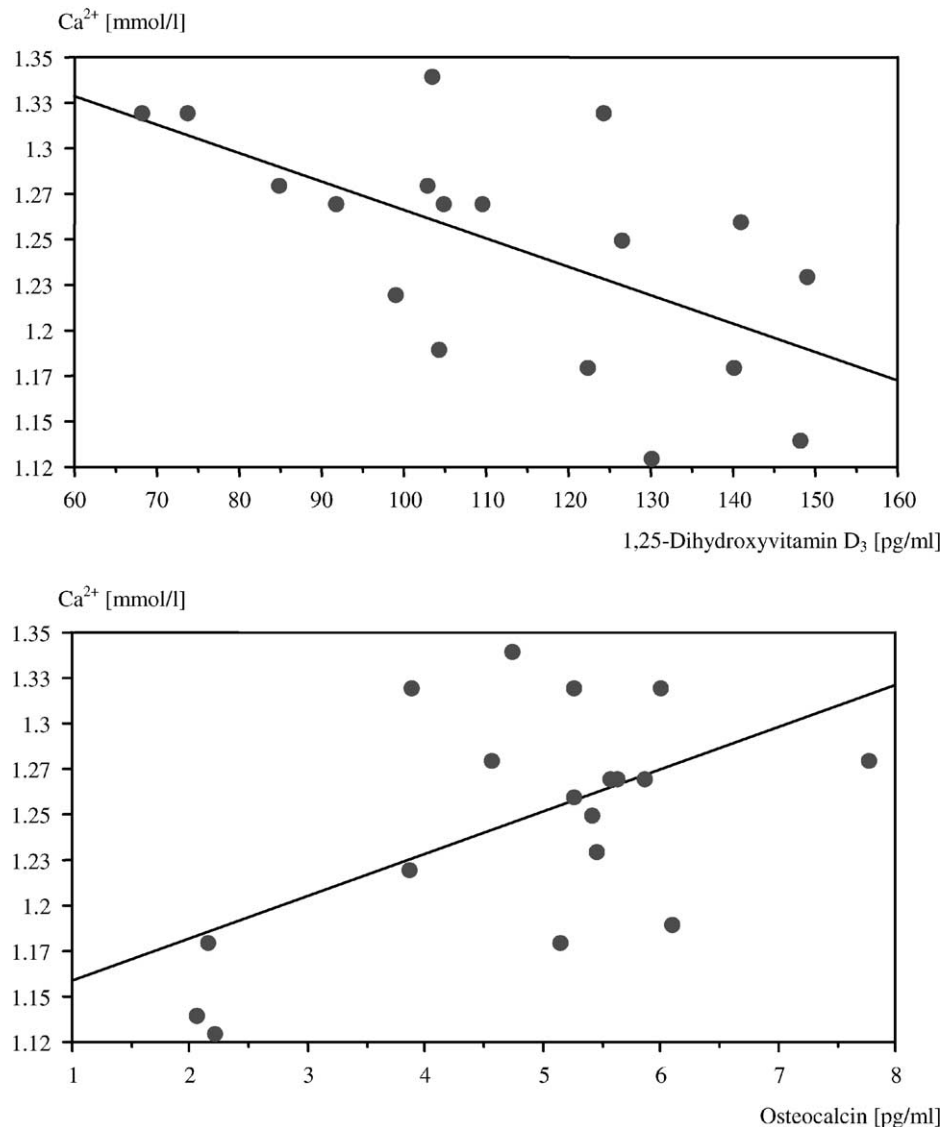


Fig. 6. (a) Correlation between ionised calcium and 1,25-dihydroxyvitamin D₃ in normal pregnant women ($y = 0.002x - 1.423$, $r = 0.608$, $P = 0.0075$); (b) correlation between ionised calcium and osteocalcin in normal pregnant women ($y = 0.023x + 1.135$, $r = 0.556$, $P = 0.016$).

pregnant women; 25-hydroxyvitamin D₃ and 24,25-dihydroxyvitamin D₃ levels were in the normal range, suggesting adequate dietary vitamin D. Haram et al. [3] reported vertebral compression fracture in a pregnant woman treated for thrombosis with heparin 15,000 IU per day from week 11 of gestation: 1,25-dihydroxyvitamin D₃ levels were low from week 15 until heparin was withdrawn 6 weeks post partum.

A surprising feature of our results compared to the literature data is that short-term therapy with a relatively low dose of heparin—9/10 patients received <10,000 IU per day—sufficed to cause significant changes in calcium and bone metabolism. Thus, after only a mean of 18 days preceding the 4-week study, 1,25-dihydroxyvitamin D₃ levels were already significantly lower than in normal pregnant women. Based on their study with Japanese quail

kidney homogenates, van der Vijgh et al. [18] suggested that heparin decreases 1,25-dihydroxyvitamin D₃ production by inhibiting renal 25-hydroxyvitamin D₃ 1 α -hydroxylase. Decreased 1,25-dihydroxyvitamin D₃ production also lowers osteocalcin production by osteoblasts [19,20]. Thus, at every data point, including at week 0 after only 18 days' therapy, we found that osteocalcin levels in pregnant women under prophylactic heparin treatment confined to bedrest were lower than in normal pregnant controls.

We conclude that heparin plus bedrest induces rapid and significant bone loss or decreased bone formation in pregnant women regardless of gestational age. Our results imply that therapeutic stimulation of bone formation is required, in particular in women in whom physiological depression of bone formation continues after delivery, e.g. due to prolonged breast-feeding or early subsequent pregnancy.

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