A longitudinal study of the effect of heparin thromboprophylaxis during pregnancy on maternal bone metabolism

O Ogueh MBBS MRCOG*, M R Johnson PhD MRCOG[†] and A Benjamin MD*

*Department of Obstetrics and Gynecology, McGill University, Royal Victoria Hospital, 687 Pine Avenue West, Montreal, Canada H3A 1A1;

[†]Academic Obstetrics and Gynaecology, Imperial College School of Medicine, Chelsea and Westminster Hospital, 369 Fulham Road, London SW10 9NH, UK

Summary: The aim of this study was to determine the effect of heparin thromboprophylaxis during pregnancy on maternal biochemical markers of bone metabolism. This was a prospective longitudinal study of carboxy terminal pro-peptide of type I collagen (PICP) and cross-linked carboxyterminal telopeptide of type I collagen (ICTP) levels in 15 women, who had heparin thromboprophylaxis during pregnancy compared with those of 18 normal pregnant controls. During pregnancy, the rate of change of PICP and ICTP in women who had heparin thromboprophylaxis was similar to those of women who did not ($P = 0.184$ for PICP, and $P = 0.129$ for ICTP), and PICP and ICTP levels at individual time points were similar in both groups. Therefore, heparin thromboprophylaxis during pregnancy does not affect maternal biochemical markers of bone metabolism.

Keywords: pregnancy, heparin, PICP, ICTP

INTRODUCTION

Heparin thromboprophylaxis is increasingly being used in pregnancy because it has been shown to improve pregnancy outcome and prevent thromboembolism in women with antiphospholipid syndrome, hereditary thrombophilia or with a previous history of recurrent thromboembolism.^{1–4} In non-pregnant rats heparin treatment for 32 days has been shown to cause osteoporosis by decreasing the rate of bone formation and increasing the rate of bone absorption.⁵ Heparin therapy in pregnancy has been found to be associated with a decrease in postpartum lumbar spine bone density.⁶ However, there is also a decrease in lumbar spine bone density in normal pregnancies where heparin was not used.⁷

It is now possible to directly study bone metabolism by assaying specific biochemical markers of bone turnover. The circulating levels of the carboxy terminal pro-peptide of type I collagen (PICP) directly correlates with the rate of bone formation while the levels of the cross-linked carboxy-terminal telopeptide of type I collagen (ICTP) reflects bone resorption.^{8–10} Monitoring these bone markers may reveal sub-clinical changes in bone metabolism that might indicate a future risk of osteoporosis. Therefore, the aim of this study was to monitor the effect of heparin thromboprophylaxis during pregnancy on the circulating levels of PICP and ICTP.

METHODS

This was a prospective longitudinal study. We studied 15 women who were treated with unfractionated heparin 5000 units twice daily from the date of positive pregnancy test (performed from day 25 of a 28-day menstrual cycle) till six weeks postpartum between January 2000 and August 2001. The control group consisted of 18 untreated women with uncomplicated pregnancies. We did not perform sample size calculation, but aimed for, and achieved, numbers similar to those used in previous studies on maternal bone metabolism.^{11–13} The indications for heparin therapy included antiphospholipid syndrome ($n = 5$), previous thromboembolism ($n = 4$, one with factor V Leiden mutation and one with low antithrombin III), and recurrent miscarriage ($n = 4$) and previous intrauterine fetal death ($n = 2$) without antiphospholipid antibodies or thrombophilic defects. All the women conceived spontaneously, and had no history of endocrine, metabolic or renal disease that might have affected their bone turnover. They all gave informed consent to the study, and the study was approved by the hospital's ethics committee.

Seven treated and 12 untreated women were recruited before pregnancy, and blood was obtained pre-pregnancy and at 6–8, 10–12, 16–18, 26–28 and 36–38 weeks gestation and six weeks postdelivery. The blood samples were collected into bottles with lithium heparin, centrifuged immediately and the serum stored at -20°C until analysed in one batch. The serum was analysed for PICP and ICTP by an equilibrium radioimmunoassay (RIA) (DiaSorin, Stillwater, Minnesota, USA). Cross-reaction with the carboxyterminal peptides of other procollagens by the assay kits was minimized by careful purification of the antigen and selection of antiserum.

Correspondence to: Dr Onome Ogueh, Department of Obstetrics and Gynaecology, Brighton and Sussex University Hospitals NHS Trust, The Princess Royal Hospital, Lewes Road, Haywards Heath, West Sussex RH16 4EX, UK
Email: Onome.ogueh@bsuh.nhs.uk

Samples were incubated with the ^{125}I PICP/ICTP tracers and PICP/ICTP primary antibodies respectively for two hours at 37°C . After separation of bound from free tracer, the assay was centrifuged and decanted. The bound tracer in the pellet was counted with a gamma counter. Counts are inversely proportional to the amount of PICP or ICTP present in each sample. The reference interval for adults aged 20–49 years for PICP is 108–117 $\mu\text{g/L}$ and for ICTP is 1.6–5.0 $\mu\text{g/L}$.

Statistical analysis was performed using the Statistics Package for Social Sciences (SPSS). To compare the rate of change during pregnancy between the two groups, analysis of summary measures was made. Linear regression line was fitted for each subject's data to determine the slope that represents the rate of change with gestation (summary measure), and the summary measures were then analysed as though they were raw data.¹⁴ This method is statistically valid and more relevant for medical research data collected serially including variables that start from a baseline (sometimes zero), rise to a peak and then return to baseline, and variables that steadily increase or decrease with time and do not start to return to baseline over the period of the study.¹⁴ The difference in the rate of change PICP and ICTP during pregnancy and circulating levels of PICP and ICTP at the seven individual time points between the women who had heparin thromboprophylaxis and those who did not were assessed using independent-samples *t*-test.

RESULTS

The age, weight, height, body mass index and parity of the women who had heparin thromboprophylaxis during pregnancy were similar to those of women who did not have heparin thromboprophylaxis (Table 1). In the untreated group, the PICP level during pregnancy initially fell and was significantly lower than the prepregnancy level at 10 and 16 weeks ($P = 0.038$ at 10 weeks and $P = 0.034$ at 16 weeks gestation). The PICP level subsequently rose and became significantly higher than the prepregnancy level at six weeks postdelivery ($P = 0.044$) (Figure 1). There was no difference in the PICP levels between the two groups at the individual time points, and the rate of change of PICP in the two groups was similar ($P = 0.184$).

In the untreated group, the ICTP levels during pregnancy was higher than the prepregnancy levels at all time points, and this rise was significant at 10 and 36 weeks gestation ($P = 0.023$ at 10 weeks and $P = 0.004$ at 36 weeks). At six weeks postpartum, the ICTP level was also significantly higher than the prepregnancy levels ($P < 0.001$) (Figure 2). There was no difference in the ICTP levels between the two groups at the individual time points (Figure 2), and the rate

Figure 1 The circulating levels of PICP pre-pregnancy, during pregnancy and post-pregnancy in women who had heparin thromboprophylaxis during pregnancy and those who did not

of change of ICTP levels in the two groups during pregnancy was similar ($P = 0.129$).

DISCUSSION

We have shown that during pregnancy PICP levels initially fall by the second trimester and then rise in the third trimester, rising further in the postpartum period. In contrast, ICTP levels were higher than prepregnancy levels throughout pregnancy and the postpartum period. This is similar to the findings of Ulrich *et al.*¹¹ who studied 15 women before, during and after pregnancy and found that the markers of bone formation showed a biphasic pattern with a decrease from prepregnancy to the first (total and bone specific alkaline phosphatase) or second (osteocalcin) trimester, while markers of bone resorption increased with peak levels in the third trimester (N-telopeptides) or postpartum (C-telopeptides). Similarly, Hellmeyer *et al.*¹⁵ found that the biochemical markers of bone

Figure 2 The circulating levels of ICTP pre-pregnancy, during pregnancy and post-pregnancy in women who had heparin thromboprophylaxis during pregnancy and those who did not

Table 1 Subject characteristics

	Heparin	Control	<i>P</i> value
Age (years)	32.87 (4.64)	33.72 (4.85)	0.611
Weight (kg)	66.92 (11.01)	62.67 (7.41)	0.223
Height (m)	1.67 (0.05)	1.66 (0.06)	0.806
Body mass index (kg/m ²)	23.16 (3.32)	22.94 (2.97)	0.859
Parity*	0 (0–3)	0 (0–3)	0.602

Mean (standard deviation), except parity* that is median (range)
Comparison was by independent-samples *t*-test

formation, N-terminal propeptide of type 1 collagen (PINP) showed a significant decrease from the first to the second trimester followed by a significant increase from the second to the third trimester, but another biochemical marker of bone formation, bone alkaline phosphatase and biochemical markers of bone resorption, type 1 collagen C-telopeptide (CTX) and ICTP showed an overall increase from the first to the third trimester. Also, Kaur *et al.*¹⁶ observed an initial decrease in PINP and CTX and a subsequent rise during pregnancy. Yamaga *et al.*¹² measured biochemical markers of bone turnover in 18 healthy women throughout pregnancy and observed that both markers of bone formation (intact-osteocalcin and alkaline phosphatase) and bone resorption (tartrate-resistant acid phosphatase, urinary pyridinoline, deoxypyridinoline and hydroxyproline) increased with gestation. Anim-Nyame *et al.*¹³ found that PICP and ICTP levels increased progressively in 17 normal pregnant women, but their study started at 16 weeks gestation when the initial fall in PICP levels may already have occurred. Physiological haemodilution and weight gain of pregnancy may be responsible for the initial decrease in the levels of biochemical markers of bone metabolism demonstrated by some, rather than a true change in bone turnover.¹⁶ But the overall acceleration of bone metabolism demonstrated by this and other studies may reflect the fetal calcium demand that peaks in the third trimester, and maternal calcium decreases as maternal bone resorption peaks in late pregnancy.^{11,17}

Short-term high-dose heparin has a temporary effect on bone metabolism characterized by inhibition of osteoblast function as suggested by reduction of serum osteocalcin levels that revert to basal levels four months after completion of anticoagulant therapy.¹⁸ Short-term low-dose heparin has also been associated with a small significant suppressing effect on serum alkaline phosphatase levels (marker of bone formation), but it has no effect on osteocalcin (another marker of bone formation) or the urinary excretion of hydroxyproline and serum ICTP levels (markers of bone resorption).¹⁹ However, in pregnant women confined to bedrest, even short-term unfractionated heparin of 10,000 IU/day induces significant changes in the counter regulatory mechanisms of bone metabolism, particularly a decrease in osteocalcin levels.²⁰

A potential risk of long-term heparin therapy is osteoporosis, but osteoporosis has not been described when dosages of less than 10,000 IU/day are given or when the treatment period is less than 10 weeks.¹⁹ Heparin therapy in pregnancy has been found to be associated with a decrease in postpartum bone mineral density (BMD).^{2,6,21} However, Shefras and Farquharson⁷ reported a decrease in lumbar spine BMD of 5% during pregnancy in 17 women given low molecular weight heparin (LMWH), but this was not significantly greater than the 3% decrease in a control group of eight untreated pregnant women. Also, Pettila *et al.*²² found that the postpartum BMD of the lumbar spine of women who had LMWH (dalteparin) from before 20 weeks gestation till six weeks after delivery was not different from that of healthy postpartum women who did not receive heparin treatment. Therefore, the bone loss associated with the use of long-term LMWH during pregnancy was not significantly different from physiological bone loss that may occur during pregnancy.²³

We used unfractionated heparin as it was the prevalent thromboprophylactic agent at the time of this study. While some studies show lower mean postpartum BMD in women who had unfractionated heparin during pregnancy, compared

with those who had LMWH, other studies show similar effect, and overall postpartum BMD remained normal.^{22,24,25} The studies that showed decreased BMD used higher dosage of unfractionated heparin^{21,22} than that used in this study. Casele *et al.*²⁵ found no difference in the change in BMD at the femoral neck or total proximal femur in pregnant women who received a mean dose of 17,380 units unfractionated heparin for a mean of 26.3 weeks compared with those who received a mean of 68.4 mg enoxaparin for a mean of 27.7 weeks. Therefore, the effect of LMWH on PICP and ICTP is likely to be similar to that of unfractionated heparin. LMWH is now commonly used for thromboprophylaxis during pregnancy. LMWH has several well-established advantages over unfractionated heparin including increased bioavailability, more predictable dose response, less intensive coagulation monitoring and a lower probability of causing immune-mediated thrombocytopenia.²⁶

One possible explanation for the failure to demonstrate any difference in either the absolute levels or the rate of change of PICP and ICTP may be the sample size. The study groups were small because of the difficulty in recruiting women to participate in a longitudinal study from preconception to postpartum period.¹¹ In view of the small numbers, it was not possible to correct for seasonal variation, ethnicity, smoking and breast feeding and this may have influenced the results. Future studies addressing these shortcomings are necessary to confirm our findings.

CONCLUSION

This study is the first to attempt to determine the effect of long-term heparin thromboprophylaxis during pregnancy on these maternal biochemical markers of bone metabolism. We have demonstrated that heparin thromboprophylaxis does not appear to affect the levels of biochemical markers of bone metabolism (PICP and ICTP) during pregnancy, and this supports previous clinical data showing no increased risk of osteoporosis.

This study has provided important longitudinal baseline data for bone biomarkers in pregnancy. Future studies will ideally be large, prospective studies assessing bone biomarkers and parameters such as calcium, parathormone and vitamin D during pregnancy, and bone densitometry before and after pregnancy in women treated with LMWH during pregnancy.

Disclosure of interests

None.

Contribution to authorship

All authors met the criteria for authorship including substantial contribution to concept and design, interpretation of data, drafting and revision of manuscript, and approval of the final version of the manuscript.

Details of ethics approval

The study was approved by the Royal Victoria Hospital, Montreal ethics committee.

Funding

We are also grateful to the *Lupus Erythematosus of Saskatchewan, Canada* for their financial support.

ACKNOWLEDGEMENT

Our gratitude goes to Professor SL Tan and the Department of Obstetrics and Gynecology, McGill University, Montreal Canada for making the study possible.

REFERENCES

- Branch DW, Silver RM, Blackwell JL, *et al.* Outcome of treated pregnancies in women with antiphospholipid syndrome: an update of the Utah experience. *Obstet Gynecol* 1992;**80**:614–20
- Rai R, Cohen H, Dave M, *et al.* Randomised controlled trial of aspirin and aspirin plus heparin in pregnant women with recurrent miscarriage associated with phospholipid antibodies (or antiphospholipid antibodies). *BMJ* 1997;**314**:253–7
- Macklon NS, Greer IA. Thrombosis prophylaxis in obstetrics and gynaecology. *Vasc Med Rev* 1995;**6**:251–62
- Ogueh O, Chen MF, Spurl G, Benjamin A. Outcome of pregnancy in women with hereditary thrombophilia. *Int J Gynaecol Obstet* 2001;**74**:247–53
- Muir JM, Hirsh J, Weitz JL, *et al.* A histomorphometric comparison of the effects of heparin and low-molecular-weight heparin on cancellous bone in rats. *Blood* 1997;**89**:3236–42
- Douketis JD, Ginsberg JS, Burrows RF, *et al.* The effects of long-term heparin therapy during pregnancy on bone density. A prospective matched cohort study. *Thromb Haemost* 1996;**75**:244–57
- Shefrin J, Farquharson RG. Bone density studies in pregnant women receiving heparin. *Eur J Obstet, Gynecol Reprod Biol* 1996;**65**:171–4
- Melkko J, Niemi S, Risteli J, *et al.* Radioimmunoassay for the carboxy terminal propeptide of human type I Procollagen. *Clin Chem* 1990;**36**:1328–32
- Risteli J, Elomaa I, Neimi S, *et al.* Radioimmunoassay for the pyridoline cross-linked carboxy terminal telopeptide of type I collagen: a new serum marker of bone collagen degradation. *Clin Chem* 1993;**39**:635–40
- Eriksen EF, Charles P, Melsen F, *et al.* Serum markers of type I collagen formation and degradation: correlation to bone histomorphometry. *J Bone Mineral Res* 1993;**8**:127–32
- Ulrich U, Miller PB, Eyre DR, Chesnut CH III, Schlebusch H, Soules MR. Bone remodeling and bone mineral density during pregnancy. *Arch Gynecol Obstet* 2003;**268**:309–16
- Yamaga A, Taga M, Minaguchi H, Sato K. Changes in bone mass as determined by ultrasound and biochemical markers of bone turnover during pregnancy and puerperium: a longitudinal study. *J Clin Endocrinol Metab* 1996;**81**:752–6
- Anim-Nyame N, Sooranna SR, Jones J, Alaghband-Zadeh J, Steer PJ, Johnson MR. A longitudinal study of biochemical markers of bone turnover during normal pregnancy and pregnancies complicated by pre-eclampsia. *BJOG: Int J Obstet Gynaecol* 2002;**109**:708–13
- Matthews JN, Altman DG, Campbell MJ, Royston P. Analysis of serial measurements in medical research. *BMJ* 1990;**300**:230–5
- Hellmeyer L, Ziller V, Anderer G, Ossendorf A, Schmidt S, Hadji P. Biochemical markers of bone turnover during pregnancy: a longitudinal study. *Exp Clin Endocrinol Diabetes* 2006;**114**:506–10
- Kaur M, Godber IM, Lawson N, Baker PN, Pearson D, Hosking DJ. Changes in serum markers of bone turnover during normal pregnancy. *Ann Clin Biochem* 2003;**40**:508–13
- Hosking DJ. Calcium homeostasis in pregnancy. *Clin Endocrinol* 1996;**45**:1–6
- Cantini F, Niccoli L, Bellandi F, Di Munno O. Effects of short-term, high dose, heparin therapy on biochemical markers of bone metabolism. *Clin Rheumatol* 1995;**14**:663–6
- van der Wiel HE, Lips P, Huijgens PC, Netelenbos JC. Effects of short-term low-dose heparin administration on biochemical parameters of bone turnover. *Bone Mineral* 1993;**22**:27–32
- von Mandach U, Aebersold F, Huch R, Huch A. Short-term low-dose heparin plus bedrest impairs bone metabolism in pregnant women. *Eur J Obstet, Gynecol Reprod Biol* 2003;**106**:25–30
- Dahlman TC, Sjöberg HE, Ringertz H. Bone mineral density during long-term prophylaxis with heparin in pregnancy. *Am J Obstet Gynecol* 1994;**170**:1315–20
- Pettit V, Leinonen P, Markkola A, Hiilesmaa V, Kaaja R. Postpartum bone mineral density in women treated for thromboprophylaxis with unfractionated heparin or LMW heparin. *Thromb Haemost* 2002;**87**:182–6
- Carlin AJ, Farquharson RG, Quenby SM, Topping J, Fraser WD. Prospective observational study of bone mineral density during pregnancy: low molecular weight heparin versus control. *Hum Reprod* 2004;**19**:1211–4
- Backos M, Rai R, Thomas E, Murphy M, Dore C, Regan L. Bone density changes in pregnant women treated with heparin: a prospective, longitudinal study. *Hum Reprod* 1999;**14**:2876–80
- Casale H, Haney EL, James A, Rosene-Montella K, Carson M. Bone density changes in women who receive thromboprophylaxis in pregnancy. *Am J Obstet Gynecol* 2006;**195**:1109–13
- Hawkins D, Evans J. Minimising the risk of heparin-induced osteoporosis during pregnancy. *Expert Opin Drug Safety* 2005;**4**:583–90

(Accepted 27 October 2009)