Role of oestrogen in the regulation of bone turnover at the menarche

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Abstract
The rise in oestrogen levels at menarche in girls is associated with a large reduction in bone turnover markers. This reduction reflects the closure of the epiphyseal growth plates, the reduction in periosteal apposition and endosteal resorption within cortical bone, and in bone remodelling within cortical and cancellous bone. Oestrogen promotes these changes, in part, by promoting apoptosis of chondrocytes in the growth plate and osteoclasts within cortical and cancellous bone. The period of early puberty is associated with an increased risk of fracture, particularly of the distal forearm, and this may be related to the high rate of bone turnover. A late menarche is a consistent risk factor for fracture and low bone mineral density in the postmenopausal period; models that might explain this association are considered.

Introduction
The decrease in the levels of oestrogen at the time of the menopause results in an increase in the rate of bone remodelling and this plays a major role in the development of postmenopausal osteoporosis. These changes are dwarfed by the large increase in bone turnover that occurs at the menarche (Fig. 1). Indeed, the changes in bone turnover are matched by the changes in bone mineral – up to 25% of total bone mineral accrual occurs over two years at the time of peak height velocity (Bailey et al. 1999). This review article will consider the role of oestrogen in the changes in bone turnover at the menarche, and the consequence of these changes to an individual’s risk of fracture.

The study of bone turnover

Methods
Most of the information about the changes in bone turnover at menarche and menopause come from the measurement of biochemical markers of bone turnover. These markers are measured in serum and urine and thus have the advantages that they can be measured repeatedly in an individual, they are relatively inexpensive, are non-invasive and reflect bone turnover in the whole skeleton. They have drawbacks in that they may not be specific to bone, and their levels may be determined by changes in the rate of clearance from the circulation.

The alternative methods for studying bone turnover include bone histomorphometry, calcium balance with kinetics, and radioisotope tracer methods. Bone histomorphometry is based on a bone biopsy, usually taken from the iliac crest. This method has the advantage that it allows the study of changes to the two major cell types, osteoblasts and osteoclasts, and if tetracycline labels are administered, it allows the rate of bone remodelling to be measured. The drawbacks are that the biopsy is invasive, that the biopsy site may not be representative of the entire skeleton, and that only two biopsies may be taken in an individual’s lifetime. Calcium balance studies involve an in-patient stay of several weeks on a fixed diet with the administration of a radioactive or stable tracer of calcium (or strontium). This method has the advantage that it relates to the whole body and that it gives an accurate estimate of balance, in contrast to the other two methods. However, the long hospital stay means this is a very expensive approach and requires great attention to detail, and there are few places which currently conduct such studies. Also, the mathematical models to estimate calcium kinetics have never been standardised.

Bone turnover markers
The bone turnover markers are products of the osteoblasts or osteoclasts. The osteoblasts synthesise many proteins,
and several of these are unique to the osteoblasts. The immature osteoblasts secrete proteins such as the bone isoform of alkaline phosphatase (bone ALP) which is involved in the mineralisation of bone and the type I collagen propeptides from the C- and N-terminal (PICP, PINP). The latter are cleaved from type I procollagen after its secretion from the osteoblasts. The mature osteoblasts secrete osteocalcin (OC), the marker that is most specific to the osteoblasts.

The bone resorption markers are either degradation products of type I collagen, or the enzyme tartrate resistant acid phosphatase (TRACP). Hydroxyproline makes up a large part of the weight of collagen, but this amino acid is derived from other proteins, is absorbed from the diet, and there is no immunoassay and so its use as a bone resorption marker has fallen out of favour. The pyridinium crosslinks are formed between and within collagen molecules from lysine and hydroxylysine residues once the collagen fibres line up in a quarter stagger array. The crosslinks include pyridinoline (PYD) and deoxypyridinoline (DPD); these may be excreted in the urine in the free form, or attached to the terminal residues of the collagen molecule, the so called C- and N-terminal telopeptides (CTX and NTX).

These bone turnover markers may be measured in serum and urine (Table 1). Their measurement has been validated by the similar pattern with age in children (Fig. 1), the relationship to height velocity (see below), and by comparison with calcium kinetics, and compares well, even in children (Weaver et al. 1997).

Changes in bone turnover across life

There has been a great deal of investigation into the increase of 20 to 100% in bone turnover that occurs at the menopause. However, the levels of bone turnover at the menarche are up to ten times higher than in premenopausal women, and the levels in the neonate are even higher. The reason for the very high levels of bone turnover markers in childhood is that they reflect not only the process of bone remodelling, but also that of growth.

Changes in bone turnover at the menarche

Description

Bone turnover markers are high during childhood (relative to adulthood) and there is a further increase during puberty. With the onset of menarche, the markers all decline despite the high levels of insulin-like growth factor-I (IGF-I). Many studies have investigated markers of bone formation and resorption during childhood (Table 1). The changes in bone turnover with age are similar to those reported by bone histomorphometry (Parfitt et al. 2000). These studies consistently show that bone metabolism rates are greater in children compared with adults, with markers of bone formation and resorption being several times higher compared with adults. The magnitude of this increase can vary greatly between markers (Blumsohn et al. 1994). In the study by Blumsohn et al. (1994) bone ALP increased tenfold but PICP only increased threefold in pubertal girls compared with the adult reference range (Fig. 2).

Thus, the bone resorption marker, urinary NTX, is 330 nmol bone collagen equivalents (BCE)/mmol creatinine (Cr) in mid-puberty (Tanner (T) stages II and III), 80 at the end of puberty (stage V), and 30 in premenopausal women (Fig. 1).

A significant positive correlation between biochemical markers of bone turnover and height velocity has been described in several studies (Schiel et al. 1983, Kanzaki et al. 1992, Hertel et al. 1993, Bollen & Eyre 1994, Kubo et al. 1995, Marowska et al. 1996, Rauch et al. 1996,
Table 1 List of the bone turnover markers and the supporting evidence for changes during childhood and puberty

<table>
<thead>
<tr>
<th>Bone turnover marker</th>
<th>Evidence for increased levels of bone turnover markers during childhood (relative to adulthood) and a further increase during puberty</th>
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<td>Bone formation</td>
<td>(Blumsohn et al. 1994), (Eastell et al. 1992), (Cadogan et al. 1998), (Mora et al. 1999), (Magmusson et al. 1995), (Sorva et al. 1997), (Schiele et al. 1983), (van Hoof et al. 1990), (Trivedi et al. 1991), (Hertel et al. 1993), (Kanzaki et al. 1992), (Tobiume et al. 1997), (Kubo et al. 1995), (Libanati et al. 1999), (Slemenda et al. 1997), (Crofton et al. 1997), (Cole et al. 1985), (Tommasi et al. 1996), (Kikuchi et al. 1998), (Riis et al. 1985), (Johansen et al. 1988), (Gundberg et al. 1983), (Sabatier et al. 1996), (Bass et al. 1999), (Sen et al. 2000)</td>
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Bone resorption

Hydroxyproline (Hyp)
Galactosyl hydroxlysien (Gal-Hyl)
Pyridinoline (PYD)
Deoxypyridinoline (DPD)
N- and C-telopeptides of type I collagen (NTX-I, CTX-I and CTX-MMP (ICTP))
Tartrate-resistant acid phosphatase (TRACP)

(Blumsohn et al. 1994), (Eastell et al. 1992), (Cadogan et al. 1998), (Beardsworth et al. 1990), (Ohishi et al. 1993), (Rauch et al. 1995, 1996), (Fujimoto et al. 1995), (Libanati et al. 1999), (Slemenda et al. 1997), (Crofton et al. 1997), (Shaw & Bishop 1995), (Tommasi et al. 1996), (Kikuchi et al. 1998), (Conti et al. 1998), (Bollen & Eyre 1994), (Bass et al. 1999), (Marowska et al. 1996)

Figure 2 Biochemical markers of bone formation (shaded boxes) and resorption (open boxes) in mid-puberty (Tanner stages II and III) in girls relative to the mean level in adults. ALP, alkaline phosphatase; i, immunoreactive; w, wheat germ lectin; ICTP, type I collagen C-telopeptide; uGal-Hyl/Cr, urinary galactosyl hydroxlysine to creatine ratio. Data from Blumsohn et al. (1994).
Sorva et al. 1997, Cadogan et al. 1998, Kikuchi et al. 1998, Bollen 2000). Longitudinal studies indicate that bone turnover markers are maximal during early puberty (TI to TIII) but the greatest mineral accrual occurs during mid to late puberty when markers are declining (Riis et al. 1985, Theintz et al. 1992, Del et al. 1994, Slemenda et al. 1994,}

Figure 3 (A) and (B).
Cadogan et al. (1998, Magarey et al. 1999). Cadogan et al. (1998) reported that bone turnover markers were correlated to height velocity and not with bone gain (Fig. 3). Therefore Szulc et al. (2000) suggest that bone turnover markers are likely to reflect statural growth rather than bone mineral accrual.

What processes do the markers reflect?

Bone remodelling is about three times higher in children as compared with adults (Parfitt et al. 2000). Thus, the increase in markers must be reflecting other processes. These include linear growth, which occurs at the epiphyseal growth plate, and modelling which includes changes in shape and axis. The growth plate results in the production of new trabecular bone, and modelling results in the production of new cortical bone by periosteal or endosteal apposition. The processes are directed by the daily stress stimulus; bone growth during puberty is a mechanically driven process, modulated by hormonal and dietary factors (Beaupre et al. 1990, van der Meulen et al. 2001). These mechanical forces ensure that the section modulus of bone.

Figure 3 The increase in serum oestradiol levels precede menarche and are associated with a slowing of growth velocity and a decrease in bone turnover markers. Note how the IGF-I levels continue to increase, despite the deceleration in height velocity. The maximum gain in bone mineral content occurs at menarche and subsequently serum PTH levels decline. (A) Change (Δ) in height (a), total body bone mineral content (TBBMC) (b) and bone mineral density (TBBMD) (c). (B) Levels of and change in serum bone ALP (immunoreactive (ib)AP) (a and b) and urinary free DPD (ifDPd) (c and d). (C) Levels of and change in serum parathyroid hormone (PTH) (a and b), oestradiol (E2) (c and d), and insulin-like growth factor I (IGF-I) (e and f) in healthy girls entering the study at ages 11 to 12 years, with 18 months of follow-up. Adapted from Cadogan et al. (1998) with permission from the American Society for Bone and Mineral Research.
closely follows body weight (and thus, muscle mass). The exquisite sensitivity of bone growth to the daily stress stimulus may explain why the benefits of physical activity before menarche are so much greater than after menarche (Kannus et al. 1995).

Linear growth continues until age 16 in girls and 18 in boys (Tanner 1975). Boys enter puberty two years later than girls and the accelerated growth phase lasts one year longer and hence boys are 10% taller than girls. Presumably, the greater increase in body weight and muscle mass in boys is providing a greater mechanical stimulus to bone and thus the total body bone mineral is 25% higher in girls than in boys at the end of puberty (Riggs et al. 2002). Some growth plates remain open until the age of 25, e.g. those in the posterior spinous processes. Modelling is maximal during puberty, continues until the third decade, and probably continues to a smaller extent throughout life. The continued opening of some growth plates and the continued increase in size of bones by modelling, such as the vertebrae (Henry et al. 2004), accounts for the higher levels of bone turnover markers in women in the third decade as compared with the fourth and fifth decades (Khosla et al. 1998).

**Why do the changes differ between markers?**

Not all markers behave the same. The increase in urinary free crosslinks (e.g. immunoreactive (i)FDPD) increase less than telopeptides (conjugated crosslinks e.g. NTX). This may relate to renal handling of free and conjugated crosslinks. It appears that up to half of free crosslinks are generated within the renal tubule from conjugated crosslinks and that this process is rate limiting (Colwell & Eastell 1996). Thus the changes in free DPD tend to be damped. PICP increases less than PINP. This may relate to the induction of the mannose receptor by IGF-I; PINP is cleared by a different pathway, the scavenger receptor (Smedsrod et al. 1990). Finally, the markers may differ in their distribution — OC is rich in cortical bone (Ninomiya et al. 1990, Magnusson et al. 1999) while bone ALP is present in the growth plate (Tuckermann et al. 2000).

**What is the role of oestrogen?**

**Growth, modelling and remodelling**

Oestrogen has a biphasic effect on growth. By stimulating growth hormone (GH) production, it results in an increase in growth during puberty. This was well shown by Ross et al. (1983) who treated girls with Turner’s syndrome with ethinyl oestradiol. They found that low doses accelerated growth, whereas high doses slowed growth. Thus, at low doses, growth is accelerated but at high doses there is inhibition of growth and stimulation of fusion of the epiphyses (Parfitt 2002). The importance of oestrogen for the arrest of growth and closure of the growth plate is now recognised by the report of cases of delayed closure in men with oestrogen receptor deficiency (Smith et al. 1994) or aromatase deficiency (Morishima et al. 1995, Carani et al. 1997).

The effects of oestrogen on modelling are to inhibit periostal apposition and stimulate endosteal apposition. It is thus a key mediator of the sexual dimorphism of bone and is the reason (along with the differences in linear growth, see above) why women have smaller bones relative to their size than men. The main effect of oestrogen on bone remodelling is to decrease it (see below).

Some studies report a strong negative correlation between oestradiol and bone turnover markers in girls during puberty (Blumsohn et al. 1994, Cadogan et al. 1998) although the finding is not universal (Rotteveel et al. 1997). This observation would be consistent with the known effects of oestradiol on the growth plate, the periosteum and remodelling.

**How does oestrogen work?**

The effect of oestrogen on bone remodelling is to decrease activation frequency and subsequent decrease in the numbers of osteoclasts and osteoblasts. The effects of oestrogen on the osteoclast are probably mainly indirect and mediated by products secreted by the osteoblast. These products include RANK-L (the ligand of the receptor activator of nuclear factor kappa B) and colony stimulating factor 1 (CSF-1). They regulate the differentiation of osteoclast precursors to osteoclasts, and then modulate the activity of the mature osteoclast and regulate its rate of apoptosis. Oestrogen binds to receptors on the osteoblasts and increases directly the production of osteoprotegerin (OPG) and decreases the production of CSF-1. Oestrogen decreases the secretion of the pro-inflammatory cytokines interleukin-1 and tumour necrosis factor-alpha by marrow monocytes and this results in decreased production of OPG and RANK-L by the osteoblasts, thereby decreasing the rate of production of osteoclasts, their activity and their survival (Riggs et al. 2002). It is possible that oestrogens also exert an anabolic effect on osteoblasts. The evidence for this action comes from histological and biochemical studies in oestrogen-deficient mice and humans (Tobias & Compton 1999). The effects on the growth plate have been studied in ovariectomised rabbits. Here oestrogen induces apoptosis of chondrocytes, resulting in arrest of linear growth (Weise et al. 2001). Oestrogen receptors (ER) (both α and β) have been identified on chondrocytes in the human growth plate cartilage and on osteoblasts on trabecular bone surfaces, which implies a direct action of oestrogen on longitudinal bone growth and bone formation (Kusec et al. 1998, Nilsson et al. 1999).

The effect of oestrogen on bone cells is mediated by both ERα and ERβ. Using ER knockout mouse experiments it is believed that the remodelling effects are...
mainly mediated by ERα, as are the growth plate effects, but the effects on the periosteum may be mediated by the ERβ (Riggs et al. 2002). Interestingly, the effects of biomechanical forces appear to be mediated by the oestrogen receptor α (Lanyon et al. 2004).

How does oestrogen interact with GH and IGF-I?
There are a number of ways in which oestrogen and GH interact. (1) Oestrogen increases GH secretion by the pituitary at puberty, and this increased production has direct and indirect effects (by increasing IGF-I) on bone growth, modelling and remodelling. The effect of oestrogen appears to be to augment growth hormone pulse amplitude (Veldhuis et al. 2004). (2) Growth hormone has a major effect on bone, mainly through the local generation of IGF-I. This stimulates the growth plate to increase the rate of growth. There are increases in IGF-I during childhood (Fig. 3), with peak levels during pubertal development in boys and girls (Luna et al. 1983, Silbergeld et al. 1986, Cara et al. 1987, Johansen et al. 1988, Costin et al. 1989, Argente et al. 1993, Blumsohn et al. 1994, Moreira-Andres et al. 1995, Sabatier et al. 1996, Cadogan et al. 1998, Libanati et al. 1999), and correlation with height velocity (Silbergeld et al. 1986, Juul et al. 1994). These studies also report that IGF-I correlates better with Tanner stage than with chronological age. GH and IGF-I increase the rate of bone remodelling. IGF-I stimulates periosteal apposition. (3) Oestrogen antagonises the effects of GH and of IGF-I on the growth plate by stimulating fusion of the epiphysis (Ho et al. 1987, Stanhope et al. 1988, Matkovic 1996, Caufriez 1997). It opposes its effects at the periosteum and on remodelling. (4) IGF-I lowers the levels of sex-hormone binding globulin, thus increasing bio-available oestradiol (Pfeilschifter et al. 1996); the consequence of this is to limit the actions of GH and IGF-I on bone.

What is the relevance to fracture risk?
Fractures at puberty relate to bone mineral density
Fracture rate increases greatly at puberty in boys and girls. It has been proposed that there is an asynchrony between the peak height velocity and peak increase in bone mineral accrual, that latter following the former by about one year (Cadogan et al. 1998). This could be the case, but there is a pitfall here – height is one dimensional, whereas bone mineral content is three-dimensional (it is distributed within the volume of bone).

The fracture rate around puberty (Fig. 4) is the highest during life (Bailey et al. 1989) – about one half of children sustain a fracture, and half of these are forearm fractures, mainly occurring during early puberty (Jones et al. 2002b). Also, these fractures have increased by between 30 and 60% in the past 30 years (Khosla et al. 2003).

What is the mechanism for these fractures? It was proposed that these resulted from high levels of physical activity, but this relationship is not proven (Blimkie et al. 1993). However, the risk of fracture relates to the level of bone mineral density (BMD) (Jones et al. 2002a, Ma & Jones 2003) and to low BMD in mothers. Indeed, the low BMD might be explained by a thin cortical shell due to endosteal resorption (Blimkie et al. 1993, Parfitt 1994, Ma & Jones 2003). The timing of the increase in fractures may relate to this increased cortical porosity in the distal radius, with calcium demand for bone growth being so high. Presumably, the signal for increased cortical remodelling is

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parathyroid hormone (PTH), as the levels of this hormone are higher in early than in late puberty (Cadogan et al. 1998) (Fig. 3). An increase in PTH would indicate that calcium supplementation might be beneficial; there have been several clinical trials of calcium or milk supplementation at this age showing benefits on bone mass (Johnston et al. 1992, Lloyd et al. 1993, Lee et al. 1994, Chan et al. 1995, Bonjour et al. 1997, Cadogan et al. 1997, Dibba et al. 2000, Rozen et al. 2003, Stear et al. 2003). These studies showed consistent results despite the differences in the ages of the subjects, forms of calcium used and in habitual calcium intakes. As yet, there have been no clinical trials with fracture as the endpoint.

Age at menarche is a major determinant of later fracture
A late menarche is a determinant of BMD in the older woman and a strong risk factor for fracture risk. For example, the relative risk of hip fracture in women who had their menarche after the age of 17 was 2·1 (Fujiwara et al. 1997), the relative risk of vertebral fracture in women who had their menarche after the age of 16 was 1·8 (Roy et al. 2003) and the relative risk of distal forearm fracture in women who had their menarche after the age of 15 was 1·5 (Silman 2003).

Late menarche was associated with low BMD at the lumbar spine (Rosenthal et al. 1989, Ito et al. 1995, Varenna et al. 1999), femoral neck (Gerdhem & Obrant 2004) and distal radius (Fox et al. 1993). Some of these effects could be mediated by changes in bone geometry, with a larger bone marrow cavity at the radius (Rauch et al. 1999) but no changes to femoral neck dimensions (Pasco et al. 1999).

There are two hypotheses that might explain this association (Fig. 5). The first of these relates to lifetime exposure to oestrogen. Thus, a woman with an early menarche would have more years of exposure to oestrogen. However, in the above studies age at menarche appears to have a stronger effect on fracture risk than age at menopause (Fujiwara et al. 1997, Roy et al. 2003, Silman 2003) and there is evidence that age at menarche is more strongly related to BMD than age at menopause (Gerdhem & Obrant 2004) although some work does not support this relationship (Ito et al. 1995, Varenna et al. 1999).

The second hypothesis is that the body habitus that is associated with early menarche is associated with low risk of fracture. Thus, girls who enter menarche earlier have higher body mass index (and thus serum leptin (Matkovic et al. 1997)). They soon establish regular menses (Vihko & Apter 1984) (supporting hypothesis one) and they tend to be shorter and heavier (by 4 kg, on average) (Garn et al. 1986). Slender stature is an important risk factor for hip and spine fracture (Meyer et al. 1995, Roy et al. 2003), and tallness is a risk factor for hip fracture (Meyer et al. 1995).
Conclusions

Are measurements of bone turnover markers helpful for a better understanding of the changes in bone during puberty? The case for supporting their use is based on the similarity in the changes across childhood and puberty with the changes assessed by bone histomorphometry (Fig. 1), on the strong relationship between the level of bone turnover markers and height velocity, and on the expected response of bone turnover markers to stimuli such as growth hormone therapy (Kanzaki et al. 1992). The case against supporting their use is that different markers increase to different extents during puberty, the correlation of bone turnover markers with calcium kinetic measurements is not as strong as in adults, and bone turnover markers do not correlate well with changes in bone mineral content. These tools are therefore useful, but their interpretation needs to be treated with caution and the measurement of several different markers in the same study is advised.

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