Regular review

Bone disease in preterm infants

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New work in bone physiology and cell biology during the last decade has made it possible to construct a model for the bone disease of preterm infants variously labelled 'rickets', 'osteopenia', and 'metabolic bone disease of prematurity'. The model proposed here explains its pathogenesis and its outcomes, and suggests a sequence of appropriate investigations as well as a scheme of management. The figure illustrates the basic processes of bone mineral metabolism in the perinatal period, and provides a framework against which the derangement in mineral homeostasis leading to bone disease can be more clearly visualised.

Fetal bone mineral homeostasis

Mineral accretion rates for both calcium and phosphorus increase throughout pregnancy, reaching a maximum during the third trimester of 3.0–3.7 mmol/kg/day for calcium and 2.4–2.7 mmol/kg/day for phosphorus.¹

Fetal plasma calcitonin concentrations are also raised in utero²; though this peptide hormone is known principally for its hypocalcaemic action, there is substantial evidence that it has appreciable anabolic, mineral accreting effects on bone.

In contrast, the principal hormones mediating bone resorption in later life, parathormone, and 1, 25 dihydroxycholecalciferol, are found in low concentrations in the fetus.^{2,3} Interestingly, however, prolonged low dose administration of these hormones in animals results in increased bone mass and thus both may be actively concerned in the accretion rather than resorption of mineral in utero.⁴

The first 48 hours

After ligation of the cord, the supply of calcium, phosphorus, and all other nutrients ceases abruptly. The continuing demand by bone for calcium entrains a rapid fall in blood calcium concentrations; the nadir for ionised calcium is usually at about 18 hours of age, slightly before that for total calcium.⁵

In well preterm infants, hypocalcaemia will usually begin to improve by 24–30 hours of age, with values in the adult normal range attained by 48–60 hours. Factors unrelated to bone metabolism, particularly tissue hypoxia with subsequent calcium influx, may contribute to the low calcium concentrations seen in the sickest infants, in whom hypocalcaemia is more likely to persist and be more pronounced.

Previous workers have suggested, however, that the plasma parathormone concentration does not rise after birth, and that parathormone 'resistance' is likely to occur in preterm infants. Much of the early work on parathormone in the perinatal period was carried out using radioimmunoassays for either the carbon or the nitrogen terminal moiety of the molecule. Parathormone is an 84 amino acid peptide that is rapidly and continuously synthesised and almost immediately subjected to intracellular degradation.⁶ Inactive fragments and intact molecules are stored together and then released, principally in response to hypocalcaemia. An increase in the amount of active hormone secreted could remain undetected, as the total terminal specific assay activity might not change appreciably. More recent studies, however, carried out with intact-molecule and active fragment (residues 1–34) assays, have shown a two fold to five fold increase in active parathormone secretion during the first 48 hours after birth.⁵ The principal target organs for the parathormone molecules thus released are bone and kidney. In response to parathormone the kidney reabsorbs calcium and actively excretes phosphorus. A reasonable indication of the response to parathormone would therefore be to monitor urinary output of phosphorus over the first days after birth.

The longitudinal changes in whole blood ionised calcium up to the age of 72 hours, and in urinary phosphorus excretion up to the age of 5 days were studied in 18 preterm infants. High urinary concentrations of phosphorus on days 2 and 3 after birth were observed; subsequently, the phosphorus loss diminished rapidly and by day 5 phosphorus excretion had ceased in most of the infants studied

Figure Outline of processes of bone mineral metabolism in the perinatal period.

(unpublished observations). These observations parallel results from animal studies of the effects of exogenously administered parathormone on phosphate metabolism in states of phosphorus repletion and depletion.⁴

Another important consequence of the action of parathormone on the kidney is the enhancement of 1,25 dihydroxycholecalciferol synthesis. 1,25 dihydroxycholecalciferol is the most active metabolite of vitamin D₃ affecting not only the gastrointestinal

absorption of calcium and phosphorus, but also the mobilisation of calcium from bone. 1,25 dihydroxycholecalciferol has a central role in the maintenance of calcium homeostasis, which is discussed in detail below.

The release of parathormone is probably potentiated by the apparently paradoxical release of calcitonin almost immediately after birth.⁷ In post-natal life calcitonin is secreted postprandially in response to gastrin production, and the initial surge

of gastrin after the first feed may be responsible for the increase in plasma calcitonin concentrations seen at this time.⁸ Calcitonin inhibits the resorptive response of osteoclasts and so delays the supply of calcium from bone to the circulation. The postnatal regulation of calcium homeostasis is principally achieved by the interlocking actions of parathormone and 1,25 dihydroxycholecalciferol on bone, and by their separate effects on absorption and retention of mineral substrate.

Bone resorption

The isolation and culture of pure cell lines of osteoblasts and osteoclasts, have enabled rapid advances in our understanding of the underlying processes in bone.⁹⁻¹¹ It is now clear that osteoblasts and osteoclasts act together to undertake bone resorption, and that osteoclasts, having no parathormone receptors, exert resorptive activity in response to signals from osteoblasts. The specific effects of parathormone on bone are⁴: increased osteoblast permeability to calcium; release of collagenase from osteoblasts; and release of osteoclast activating factor(s) from osteoblasts, as a result of which osteoclasts increase in number and activity.

In addition, 1,25 dihydroxycholecalciferol, produced in response to the increased concentrations of parathormone, exerts synergistic effects on bone.⁹⁻¹¹ These are: activation of an osteoblastic calcium pump; increased activation and fusion of the monocyte/macrophage precursors of osteoclasts; production by osteoblasts of osteocalcin (Gla protein), which is chemotactic for osteoclasts; effects on immune cell function, particularly lymphocytes, with reduced interleukin 2, and increased interleukin 1 production, which enhances osteoclast formation and activity; and possibly a reduced response of osteoblasts to parathormone (reduced cyclicadenosine monophosphate response).

Thus parathormone and 1,25 dihydroxycholecalciferol have complementary actions; the increase in osteoblast permeability to calcium flux with the activation of a specific calcium pump provides an acute response to falling ionised calcium concentrations. The recruitment, activation, and fusion of osteoclast precursors, and their subsequent activity in response to osteoblast derived humoral factors, provides a longer term source of calcium.

New bone formation

The result of the resorptive process is to produce calcium, phosphorus, and breakdown products of bone matrix. These breakdown products are thought to act locally to promote new bone forma-

tion by osteoblasts. The increase in resorptive activity initiated by the surge of parathormone after birth is therefore matched by a concurrent increase in new bone formation. Though matrix volume is not reduced during this period of intense activity, net loss of bone mineral will occur if the exogenous supply of mineral substrate is inadequate.

In addition to the supply of adequate mineral substrate to normally functioning osteoblasts, a favourable local environment for bone mineralisation is also crucial to the remodelling and growth of bone; many factors have been identified in laboratory studies as having a role. In particular, there is a growing body of evidence to support the part played by matrix vesicles in the initiation and propagation of crystallisation.¹²

Matrix vesicles are discrete sacs that are derived from the osteoblast cell membrane. They are composed of a phospholipid bilayer rich in phosphatase enzymes including alkaline phosphatase, and they accumulate at the growing front of bone. At the pH of the mineralisation front, alkaline phosphatase functions principally as a phosphotransferase, transporting phosphate residues that have been cleaved by other phosphatase enzymes into the vesicle's sap.

Calcium enters the vesicle by diffusion, and is trapped by phosphatidyl serine. The additional accumulation of phosphate raises the saturation of the vesicle sap to the point where the calcium/phosphate solubility product is exceeded and crystallisation begins. Electron microscopic pictures have shown the growth of crystals on the inner leaflet of the vesicle that leads to its subsequent disruption as the ends of the crystal pierce the bilayered membrane.

These crystals then seed into the fluid at the mineralisation front and, given adequate mineral substrate there, act as foci for further crystallisation. The rate of turnover of matrix vesicles with the release of their membrane constituents, therefore, reflects the rate of initiation of crystallisation. Laboratory studies have shown that there are greatly increased numbers of matrix vesicles in rachitic growth plates¹³; this lends support to the concept that increased alkaline phosphatase activity in plasma may represent increased vesicle turnover in substrate or vitamin D deficient states.

During the early neonatal period the main determinants of bone remodelling, mineralisation, and growth are those that have been discussed in detail above. There are, however, many other factors affecting the fine control of bone homeostasis,⁹⁻¹¹ of which two are of particular relevance to the preterm infant.

Aluminium is a potent inhibitor of bone minerali-

sation, and is present as a contaminant in parenteral nutrition solutions.¹³ Up to 80% of the intravenously administered load may be retained, and significant deposition was found in bone after three weeks of parenteral feeding. It is possible that aluminium may exacerbate bone disease in preterm infants fed intravenously.

Immobility also causes loss of bone mass. Stress generated electrical potentials have been implicated in the osteogenic process, and prolonged periods of sedation or paralysis during mechanical ventilation increase the possibility of the loss of bone mass.

Mineral substrate insufficiency

Given an adequate nutrient supply, remodelling, mineralisation, and growth of bone should proceed normally in most infants. For bone disease to develop, depletion of mineral substrate must occur. Phosphorus depletion is likely to develop more rapidly as it may initially be lost in the urine, and protoplasmic metabolic requirements for phosphorus are greater than for calcium (extrapolating from data on fetal accretion rates and body composition studies, 0.6–0.7 mmol/kg/day compared with 0.2–0.3 mmol/kg/day).

Inadequate dietary provision of phosphorus—for instance, the exclusive use of unsupplemented human milk—compounded by the initial urinary phosphorus losses will result in low tissue phosphorus stores, and low circulating concentrations of phosphorus.

The reduced delivery of phosphorus to the kidney prevents further appreciable urinary losses and enhances renal production of 1,25 dihydroxycholecalciferol. The increase in circulating 1,25 dihydroxycholecalciferol in turn increases gastrointestinal absorption of both calcium and phosphorus. In addition, the release of parathormone is inhibited, further reducing the risk of phosphorus loss in the urine. As a corollary, however, renal reabsorption of calcium is reduced, with consequent hypercalcuria. The inhibition of parathormone release may also slow the process of bone reabsorption; nevertheless, the potent bone resorbing activity of 1,25 dihydroxycholecalciferol will continue to remove some calcium and phosphorus from bone.

In addition to the phosphorus absorbing and retaining processes detailed above, it is possible that hypophosphataemia is a key factor in accelerating directly or indirectly the turnover of matrix vesicles and hence increasing plasma alkaline phosphatase activity.

If mineral substrate provision continues to be inadequate, further substrate will be lost from bone

in order to supply the needs of other tissues. The biochemical outcomes of these processes are inter-linked; reduced concentrations of phosphate in urine and plasma precede the increasing urinary loss of calcium, and in extreme cases, hypercalcaemia. Raised plasma alkaline phosphatase activity is seen principally after 6 weeks of age.

Radiological and anthropometric changes occur slowly, being seldom evident before 6 weeks of age.^{14 15} In the long term the principle outcomes for bone are linear growth, mineral content, and structural integrity. In a large study of preterm infants receiving different diets during the neonatal period, we found a significant association between the increase in plasma alkaline phosphatase activity and a reduction in height achieved at both 9 and 18 months implying that bone disease, reflected by increased remodelling activity during the first weeks of life, had a lasting effect on the infants' growth potential up to the age of 18 months.¹⁶

If these differences persist, then it is likely that the nutritional deprivation sustained by bone during this apparently critical phase of development has 'programmed' the bone to grow less slowly, as catch up growth would otherwise be observed when dietary sufficiency was achieved.¹⁶

The regulatory mechanisms for this adaptive change remain to be elucidated, but could involve changes in cell number, type, or function, either locally or systemically.

Investigation of early bone disease

Plasma phosphate concentrations fall gradually from 2 mmol/l to 1.0–1.5 mmol/l over the first week, and often reach a nadir during the second week after birth (unpublished observations). In infants depleted of phosphorus as a result of urinary losses and poor intake a further reduction to <1 mmol/l may occur, and this has been reported to be associated with the later development of biochemical and radiological evidence of bone disease. Urinary phosphate excretion initially may be increased but by day 5 is usually negligible. By contrast, urinary calcium losses increase and persist during the period that tissue phosphorus stores remain depleted. A prolonged absence of phosphate from the urine with persisting calciuria would imply continued tissue phosphorus depletion, and might be a useful marker to follow sequentially in an individual infant.

The natural history of plasma alkaline phosphatase activity is to rise over the first 3 weeks and plateau until the age of 5–6 weeks. Rises that occur after this are seen principally in infants with persistently low plasma phosphorus concentrations

receiving low phosphate diets—for instance, un-supplemented human milk. Increased plasma alkaline phosphatase activity is widely quoted as being indicative of bone disease; difficulties arise in the interpretation of results and comparison with other centres because of the use of different assay systems with widely varying ranges and different units of measurement. Peak concentrations of greater than 7.5 times the maximum adult normal value for that particular alkaline phosphate assay have been associated with reduced linear growth velocity in the short term.¹⁴ In the work previously referred to we found an area of demarcation at five times the maximum normal adult value for plasma alkaline phosphatase activity, with appreciable reductions in growth potential for infants with peak concentrations exceeding this limit.¹⁶

Radiological changes are usually not seen until the age of 6 weeks; reduced bone density, and abnormal bone remodelling in the form of cupping, splaying, and fraying of epiphyses may occur,¹⁵ and—in extreme cases—there may be fractures of both ribs and long bones. The interpretation of radiographs is, however, subjective and the use of scoring systems has not improved their predictive value for minor to moderate degrees of demineralisation.

Photonabsorptiometry is a quick and accurate method of assessing sequentially the changes in bone mineral content at a specific site, usually the distal radius.¹⁷ Photonabsorptiometry has shown that in infants receiving diets containing little mineral substrate, bone mineral content may remain unchanged, or even decrease initially, and then increase at a rate much less than that attained in the uterus. By contrast, infants supplied with mineral in amounts approaching the intrauterine rate can maintain the fetal rate of mineral accretion.¹⁸ The use of photonabsorptiometry is restricted to a few selected centres, however, and its principle use at present is for research rather than as an aid to diagnosis.

Radiographic densitometry is a low dose whole body technique that provides accurate information about the overall mineral state of the skeleton. As yet it has not been applied to preterm infants, but it could provide valuable data for body composition and mineral metabolism studies.

It has been often noted that peak alkaline phosphatase activity rarely occurs at the same time as radiological evidence of abnormal bone remodelling, or the degree of bone demineralisation as measured by photonabsorptiometry. This is essentially a reflection of the intrinsic properties of each investigation—plasma alkaline phosphatase activity is a measure of bone activity, possibly of the

rate of mineral crystallisation; photonabsorptiometry gives an estimation of the amount of mineral actually in bone; and radiographs best show the abnormal remodelling resulting from an inadequate provision of mineral substrate for bones that are continuing to increase their matrix volume.

Short term anthropometry is useful as a non-specific adjunct to the radiological and biochemical investigations in that a reduced linear growth velocity at the age of 6 weeks would provide further evidence to support the diagnosis of bone disease.¹⁶

For practical purposes, sequential analysis of urinary calcium and phosphorus losses is likely to provide the earliest evidence of incipient metabolic bone disease. If by the age of 3 weeks calcium excretion is continuing, with no phosphorus appearing in the urine despite adopting the prophylactic measures outlined below, further mineral supplementation should be instituted.

Management

The management of this condition should essentially follow the dictum 'prevention is better than cure'. The degree and duration of mineral, and in particular phosphorus, depletion that will result in bone disease and the amount of supplementation that will prevent it are unknown. It is nevertheless possible to look at the provision of substrate by current feeding practices, formulate estimates of comparative bone mineral accretion rates, and so assess the minimum 'preventative' amounts of substrate intake required.

Unsupplemented human milk contains 0.5 mmol/100 ml of phosphorus. For infants receiving 200 ml/kg/day, and assuming 90–95% retention, 0.9–0.95 mmol/kg/day of phosphorus will be delivered. After allowing for basal protoplasmic requirements, approximately 0.3 mmol of phosphorus will be available for deposition in bone mineral. Calcium and phosphorus accrete at a ratio of 5:3 in bone; up to 0.5 mmol/kg of calcium might therefore be deposited—about 15% of the intrauterine accretion rate.

By contrast, a preterm formula containing 1 mmol/100 ml of phosphorus supplied at 180 ml/kg/day should result in a phosphorus retention of 1.6–1.7 mmol/kg/day. After allowing for protoplasmic requirements, about 1 mmol/kg/day of phosphorus is available for bone mineralisation, complexing with 1.6 mmol/kg/day of calcium—approximately 50% of the intrauterine accretion rate. Formulas with higher calcium and phosphorus contents are available in some countries, and have been used widely without adverse effects. Reported mineral accretion rates for infants fed these milks approach those achieved in the uterus,¹⁸ but the

reports are usually of well infants, fed fully by the enteral route by the age of 1 week. In addition, concern has been expressed generally that not all of the calcium and phosphate in these milks is available for absorption, possibly as a result of precipitation before feeding.

Given that protoplasmic requirements may be increased because of pre-existing tissue phosphorus depletion, particularly in infants who have previously been intravenously fed, the provision of 1 mmol/100 ml of phosphorus in milk given to preterm infants should be regarded as an absolute minimum.

The addition of phosphorus to expressed human milk is already common. Buffered neutral phosphate can safely be admixed with human milk over a 24 hour period to raise the phosphorus content from 0.5 mmol/100 ml to 1.0 mmol/100 ml.

Larger quantities of both calcium and phosphorus can be added to human milk. It is important to add the phosphorus salt (usually disodium phosphate) first so that it can enter the fat micelles. There is an appreciable risk of precipitation if calcium (usually given as calcium gluconate/glibionate) is added before the phosphorus.

As much as 1.6 mmol of phosphorus and 1.35 mmol of calcium can be added in this way.¹⁹ Although some precipitation does occur, balance studies indicate that the absorption and retention of both calcium and phosphorus from such solutions is good.

The multivitamin fortifiers added to breast milk in some countries rely upon 'stabilising agents' to hold large quantities of mineral substrate in solution. Problems with precipitation and reduced absorption and retention have been reported, however, and in one study²⁰ it was found that retention of substrate from a preterm formula exceeded that from a fortified human milk solution. The use of such fortifiers is still the subject of intense research, and they cannot be unreservedly recommended for general use.

The addition of phosphorus to preterm formula is unlikely to be beneficial as the total amount of calcium retained and available for bone mineralisation is already completely utilised. Further addition of phosphate may in fact precipitate calcium from the milk solution, further reducing availability of the substrate.

Vitamin D₃ should be given routinely to all preterm infants.²¹ For infants born in the United Kingdom whose mothers have received vitamin D supplementation during pregnancy the current recommendations are for 400–1000 IU/day, although it should be noted that with adequate dietary provision of calcium and phosphorus as little as 100 IU/day has been given without obvious

adverse outcome for bone. For infants born to mothers not receiving vitamin D supplementation, as much as 1200 IU/day may be required. Increasing the supply of vitamin D₃ further may increase the short term retention of calcium, particularly in those infants in whom the supply of substrate is poor, but may also have longer term consequences for bone resorption and remodelling activities. In addition there may be as yet undefined consequences on cellular differentiation in other tissues; vitamin D₃ is a steroid type molecule, and a potent mitogenic agent.

Most preterm infants will not benefit from being given the active metabolites of vitamin D₃; these should be confined to those patients in whom a vitamin D₃ resistant state, for example, X linked hypophosphataemic rickets, has been confirmed.

Intravenously fed preterm infants are most at risk from poor supplies of mineral substrate. The solubility of calcium and phosphorus in parenteral nutrition solutions depends on a number of factors: the pH and the amino acid composition of the solution, the calcium salt used, and the temperature to which the solution is exposed.

The more acidic the solution, the greater the quantity of mineral substrate it can hold, and for most solutions calcium gluconate is more soluble than calcium chloride. Recently, amino acid solutions 'tailored' to the requirements of infants have been produced, with claims of improved capacity for calcium and phosphate. The testing of these solutions has not, however, taken account of the prolonged exposure to high temperatures (37°C for more than two hours) that may obtain in clinical practice.

The risk of precipitation with line blockage, or even microembolisation of crystalline particles, should not be ignored when using solutions with calcium and phosphate contents near the limit of solubility. The use of microporous filters within the line would reduce these risks appreciably. Using currently available amino acid solutions infused at 150 ml/kg/day, 1.1 mmol/kg/day of phosphorus and 1.5 mmol/kg/day of calcium can be administered at most. Claims of improved solubility and mineral delivery using glucose-1-phosphate have been made, but the use of an intermediary metabolite for this purpose is still under investigation.

In addition to problems with calcium and phosphorus delivery, the presence of high concentrations of aluminium in some solutions has given rise to concern about possible inhibition of osteoblastic function in infants receiving prolonged intravenous feeding.

In clinical practice, the infants likely to require mineral supplementation are those of less than 33

weeks' gestation. A reasonable 'prophylactic' measure would be the addition of 0.5 mmol of neutral phosphate solution to each 100 ml of expressed breast milk, or the use of a preterm formula containing at least 1 mmol/100 ml of phosphorus, and 1.75 mmol/100 ml of calcium. Supplementation should start as soon as enteral feeds are started, and continue until the infant achieves a weight of 2 kg or leaves the nursery. A urinary calcium: phosphorus ratio of >1 at the age of 3 weeks is an indication for further supplementation. For infants receiving preterm formulas, milks with higher mineral densities are available overseas, and may be introduced in the United Kingdom in the near future. Breast milk can be supplemented with both calcium and phosphorus using the previously described method.

It is hoped that early mineral supplementation will result in a reduction in the incidence of metabolic bone disease during the neonatal period, and maximise the potential for subsequent bone growth.

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