Quantitative Ultrasound Assessment of Bone Health in the Neonate

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Key Words
Metabolic bone disease · Osteopaenia · Speed of sound · Prematurity

Abstract
For a number of reasons there is a need to explore reliable non-invasive methods for assessing bone health in neonates and young infants. Epidemiological studies suggest that early events in life may predispose the adult to degenerative diseases such as osteoporosis. Preterm infants have an increased risk of low bone mass because of limited bone mass accretion in utero and a greater need for bone nutrients. Despite improvements in neonatal care fractures still occur. The diagnosis of osteopaenia of prematurity remains difficult as there is no screening test which is both sensitive and specific. Biochemical indices are non-diagnostic, and plain X-rays in the absence of fractures are poor at diagnosing bone disease. Although dual energy X-ray absorptiometry is increasingly used to assess bone mineral status in newborn infants, the size and immobility of the scanner, the length of time to perform the scan and use of ionising radiation make it unsuitable for routine use in the setting of the fragile very low birth weight infant. Quantitative ultrasound (QUS) was first developed in 1984, as a non-ionising, portable and low cost method of assessing bone health. The measurements obtained from QUS are thought to be related not only to the mineral density of the bone but also to reflect parameters of bone quality and strength. Preliminary studies suggest that this technique may be a useful method of assessing changes in bone health in preterm infants, but the data need to be interpreted carefully. This review will concentrate on the methodology of QUS and the studies that have already been performed in neonates.

Introduction
Premature restriction of the in utero process of bone mass accretion and a greater ex utero need for bone nutrients predisposes the preterm infant to adverse bone health. Current methods of assessing bone health in the neonate have a low specificity and with an increasing survival rate of very low birthweight (VLBW) preterm infants [1] there is a need to explore novel, reliable, non-invasive methods for assessing bone health in this group of patients. Quantitative ultrasound (QUS) was developed in the early 1980’s, as a non-ionising, portable and low cost method of assessing bone health. Preliminary studies suggest that this technique may have a role in assessing changes in bone health in preterm infants and this review will concentrate on the methodology of QUS and the studies that have already been performed in neonates. However, a thorough understanding of these issues requires some knowledge of bone development, especially in the context of the preterm neonate.
Bone Biology

The embryonic primordiae of the appendicular skeleton are the limb buds, which are mesodermal structures covered by ectoderm. The first visible outline of the embryonic limb follows a condensation of mesenchymal cells which subsequently differentiate into cartilage cells, the chondrocytes. These cells secrete a matrix and thus produce cartilaginous models of the future bones. Surrounding this cartilage is the perichondrium, the outer layer of which becomes a connective tissue sheath while the inner cells remain pluripotential (fig. 1). This cartilage rudiment grows by interstitial and appositional growth, and a vascular system develops to invade the perichondrium. A collar of bone is then laid down around the mid-shaft of the bone. This ossification is a result of the inner perichondrial cells differentiating into bone forming cells, the osteoblasts and by this time the three regions of the developing long bone, diaphysis, epiphysis and metaphysis are evident. At the same time, increasing vascular development at the site where the cartilage cells and matrix have begun to disintegrate allows osteoblasts to invade the centre of the shaft to form a primary ossification centre. Trabecular bone is then deposited on cartilaginous remnants. In humans, primary ossification centres in the femur and humerus are visible by week 6 of gestation [2].

The epiphysis contains the growth plates and is responsible for the transverse and spherical growth of the ends of the bone, the shaping of the articular surfaces, and the longitudinal growth of the metaphysis and the diaphysis. The cells within the growth plate (chondrocytes) go through a sequential process of cell proliferation, synthesis of extracellular matrix, cellular hypertrophy, mineralisation of the matrix, localised vascular invasion, and apoptosis [3]. The growth plate replenishes itself through the germinal zone and is continually replaced with bone at the physeal–metaphyseal junction. Not only is the hypertrophic chondrocyte crucial for longitudinal growth, it also prepares the matrix for calcification and vascularisation and directs the mineralisation of the adjacent matrix and attracts vessels by producing vascular growth factors.

The foetus has a high rate of mineral accretion, with high serum phosphate, calcium, calcitonin and a low PTH and low circulating levels of the active vitamin D
metabolites. Approximately 99% of body calcium and 80% of phosphorus is in the skeleton at birth, at least 80% of this mineral deposition occurs between 25 weeks gestation and term [4].

At the same time as longitudinal growth, there is radial growth of the diaphysis and the metaphysis caused by direct apposition of cortical bone by periosseum-derived osteoblasts. This is coordinated closely with resorption of bone by osteoclasts on the inner cortical endosteal (medullary) surfaces and lateral metaphyseal surfaces to maintain the relative proportions of the marrow cavity to the cortices and the overall shape of the bone as it grows. The metaphyseal cortical bone is formed by the coalescence of peripheral endochondral trabecular bone from the physis with intramembranous bone from the inner osteogenic layer of the periosteum. This dynamic process is under the influence of a number of intrinsic and extrinsic factors that include, growth factors, sex steroids and external biomechanical stressors.

Detailed studies of foetal long bones from 21 to 41 weeks gestation show that the bone geometry changes over the gestational period such that the medullary diameter increases at a greater rate than the outer cortical diameter growth rate thus resulting in a wider diaphyseal width with a greater medullary cavity and a relatively narrower cortex [5]. Although, the velocity of medullary diameter growth was similar (0.05 mm/week) in three long bones (tibia, femur, humerus) studied, the velocity of outer cortical diameter growth was greater in the tibia (0.16 mm/week) than in the femur (0.14 mm/week) or humerus (0.13 mm/week). The diaphyseal growth rate decreased in the second half of the period studied in all three bones. This process of endocortical resorption and expansion of the marrow cavity, which in densitometric terms corresponds to a decrease in volumetric bone mineral density becomes even more pronounced after birth and, in densitometric terms, results in an effective reduction in bone density [6]. Some factors such as oestrogen promote endocortical bone formation and it is possible that premature birth may hasten the process of endocortical resorption. The change in geometry of the neonatal bone may be regarded as an adaptation process and defects in this process may predispose the infant to adverse bone health.

Overall, skeletal development does not depend solely on bone mineral accretion but also bone mass accrual which may be influenced by an imbalance between bone formation and resorption [7], as well as mechanical stimulation which is higher in utero with the muscular resistance of the uterine wall, compared to movements against little resistance after birth, putting smaller loads on the skeleton. Furthermore, the hormonal situation is also different postnatally, with the placental supply of oestrogen and other hormones cut off.

Osteopaenia of Prematurity

At birth, preterm infants have low skeletal mineral stores as a consequence of delivery prior to completion of the third trimester when mineral accretion would be exponentially increasing. These infants are then often dependent on total parenteral nutrition which has a low mineral content. The supply of both calcium and phosphate is low, but a critical factor leading to osteopaenia of prematurity (OP) is lack of phosphate [8]. In addition, preterm infants are frequently ill and immobile, and often require drugs, such as steroids and loop diuretics that may alter bone mineralisation [9, 10]. Disturbed mineral metabolism is followed by reduced bone mineralisation leading to abnormal bone remodelling and reduced linear growth. Lack of mechanical stimulation may lead to less new bone formation and reduced osteoid. Therefore, it is a combination of reduced osteoid and reduced mineral, and the relative defect of these two factors that may vary from one case to another. Studies in the 1980’s found the incidence of radiological rickets and osteopaenia in VLBW and extremely low birthweight infants to be 32% [11] and 54% [12], respectively. Despite improved nutrition and the regular use of oral phosphate supplements, fractures still occur with one recent study reporting that 10% of VLBW infants may still be at risk of fractures [13].

In the short-term, OP might impair respiratory status [14] and has been postulated to be a factor in the development of myopia of prematurity related to altered bone growth of the skull [15]. Neonatal osteopathy might also adversely affect linear growth. Studies have shown that former preterm infants tend to be shorter and lighter than their term counterparts [16, 17] and high neonatal alkaline phosphatase (ALP) has been significantly associated with short stature at 18 months and 10 years of age [17,18]. Total body BMC and cortical area of the distal tibia have been reported to be lower in ex-preterm preschool children, even after adjusting for current body weight [19]. Lower bone mineral content and density has also been found in school aged children born preterm [20, 21]. Ex-preterm children have been shown to have increased urinary calcium excretion at age 8 years associated with a reduction in height and hip bone mineral density [22].
Current Assessment of Bone Health

The diagnosis of OP remains difficult. Although the combination of low-serum phosphate and high-ALP markedly improves sensitivity, these biochemical indices as well as serum calcium and urinary phosphate excretion correlate poorly with bone mineralisation [23–26]. Plain X-rays in the absence of fractures are poor at diagnosing bone disease due to the subjectiveness of the interpretation and the fact that changes are not seen until there is a significant reduction in mineralisation [27]. Although dual energy X-ray absorptiometry (DXA) is increasingly used to assess bone mineral status in newborn infants, the size and immobility of the scanner, the length of time to perform the scan and use of ionising radiation make it unsuitable for routine use in the setting of the fragile VLBW infant, in addition the data obtained from DXA can be difficult to interpret in newborns due to both movement artefact [28] and variations in technique [29]. Quantitative computed tomography (QCT) has the advantage of measuring true volumetric bone density (so not size dependent), however both axial QCT and peripheral QCT have similar limitations to DXA when considering their use on a neonatal unit.

Quantitative Ultrasound

QUS was first developed in 1984, as a non-ionising, portable and low-cost method of assessing bone health. The measurements obtained from QUS are based upon the attenuation of the ultrasound beam as it passes through the specified region of interest, most commonly the broadband ultrasound attenuation (dB/MHz) or the speed of sound (SOS/VOS, m/s) are measured, some devices also measure bone transmission time (BTT). These measurements are thought to be related not only to the mineral density of the bone but also to reflect parameters of bone quality and strength [30, 31].

From a technical point of view QUS has many advantages, it does not involve ionising radiation and it is easily accessible and transportable. There are now new devices with a dedicated hardware design and a well-defined QA/QC procedure, the measurements are relatively fast, and the overall cost is low.

QUS may only be applied to the peripheral skeleton and sites for measurement are the calcaneus, phalanges, patella and tibia. The most commonly measured site is the calcaneus; it is rich in metabolically active trabecular bone, weight bearing and has little surrounding soft tissue, making it ideal for ultrasound measurements. The application of calcaneal ultrasound in children is difficult due to lack of a dedicated paediatric transducer.

QUS is reported to measure both qualitative bone properties, such as bone mineralisation, and quantitative properties such as cortical thickness, elasticity and microarchitecture [32–35]. In adults, QUS does not diagnose osteoporosis, but does (using calcaneal transverse transmission) predict clinical fractures independent of BMD [36, 37]. A recent study in adult women found tibial SOS correlated with site matched QCT and DXA [38]. Similarly, in children tibial ultrasonography has been found to correlate with bone mineral assessment by DXA [39]. The impact of skeletal properties on QUS variables assessed by measuring the proximal phalanges using QUS (transverse transmission), QCT and MRI, found SOS was affected most by cortical area, cortical bone density and cortical porosity [40]. In vitro studies of both newborns (gestational age 20–41 weeks) [41] and adults have shown forearm QUS correlates significantly with bone strength [42].

Devices Currently in Use

**DBM Sonic BP (IGEA, Italy)**

The DBM Sonic BP (IGEA, Italy), originally designed to measure adult phalanges, can be used to measure the phalanges or humerus of children and infants. It uses two probes, mounted coaxial on two separate branches of a calliper. The device measures the distance between the probes and the time elapsing between emission and reception to calculate the SOS (fig. 2). As well as SOS, this device also measures BTT. The speed of ultrasound in...
soft tissue is taken as 1,570 m/s, and the signal travelling faster than 1,570 m/s is thus the SOS through bone. The BTT attempts to minimise the confounding effect of soft tissue as it represents the time interval between the instant that the fast wave is detected and that at which the signal travelling at 1,570 m/s received [43].

Sunlight Omnisense 7000P (Sunlight Medical Ltd., Israel)
The Sunlight Omnisense 2000P (Sunlight Medical Ltd., Israel) is based upon just one probe being used, the ultrasonic wave travelling along the cortical bone and the reflected wave being measured, this technique is called ultrasound critical angle reflectometry (fig. 2). The propagation of an ultrasound wave through a medium, its speed, and its attenuation (change in signal amplitude) are influenced by the physical properties of that medium. In the frequency range used in QUS devices, the ultrasound signal travels faster through bone than through soft tissue and, therefore, the hardware can detect the SOS through bone, and this should not be affected by the amount of soft tissue. This device measures SOS at the radius and tibia and is suitable for measurement in paediatrics having been specifically designed for children and neonates with age and gestational specific reference data.

QUS Studies in Neonates

Twelve published studies were reviewed. Table 1 shows the characteristics of these studies.

Feasibility
Both QUS scanners appeared easy to use, with a short scan time, usually less than 5 min per site. One study reported a single 24 week gestation infant in whom no measurement was possible [44]. Therefore a total of 1,009 infants were successfully scanned amongst the reviewed studies.

Reproducibility
Precision results for tibial SOS measured by the Sunlight Omnisense 7000P ranged from a coefficient of variation (CV) of 0.32% [45] (2 measurements of 35 infants) to 1.2% [46] (3 measurements of 15 infants.) Instrumental accuracy for this machine is stated by the manufacturers as 0.25–0.5%. The technique precision error [47] as determined by the root mean square average CV for tibial SOS measured by the Sunlight Omnisense 7000P was 1.4% [46], and this was similar to that for humeral SOS and BTT, 1.76 and 1.99%, respectively, as measured by the DBM Sonic [43]. The modified DBM Sonic had a CV of 1.0% for SOS, 7.0% for BTT and 7.5% for humeral BTT [48] (7 infants had 6 measurements over 3 consecutive days.) Interobserver error (2 operators, 6 infants) and intersite variation (simultaneous measurements at both radii and tibia in 20 infants) was also evaluated in one study [46]. CV was 1.2% for interobserver error, 2.4% for measurement at all 4 sites, 2.1% for left and right radius, 2.3% for right radius and tibia, 1.8% for left radius and tibia and 1.7% for left and right tibia. No significant site related differences were found when comparing left and right humeral measurements [43]. Making measurements at multiple anatomical sites has obvious potential advantages and it is therefore important to note the absence of any large differential measurement errors between sites.

Influence of Temperature and Humidity
QUS calcaneous measurements in adults have been shown to be influenced by temperature [49]. As neonatal incubator care is routine the potential effects of temperature and humidity are important. Our group has studied the effect on radial SOS in adults by placing the forearm in an incubator. Radial SOS in 15 adults was measured at ambient temperature, 35°C, and 35°C with 95% humidity. The SOS did not change significantly with increasing temperature and humidity. Mean CV (1SD) for all measurements was 2.0% (1.1%), measurements at room temperature and 35°C was 1.7% (1.1%), room temperature and 35°C with 95% humidity was 2.1% (1.8%), and 35°C and 35°C + 95% humidity was 1.7% (1.7%) [50].

The 'Normal Range'
The four studies of tibial SOS using Omnisense 7000P values for term infants [44–46, 51] and the three studies of humeral SOS [43, 48, 52] have reported comparable data. All term infants were measured within the first 7 days of birth (see table 1). The term infants studied were mainly appropriate for gestational age (AGA.) One study included 15 infants who were small for gestational age (SGA) and 12 who were large for gestational age (LGA) [46]. In the preterm population there is also an overlap in the measured range between study groups (see table 1). All studies found that QUS parameters were significantly lower in the preterm infants compared to term infants and a significant correlation was found with gestation, r = 0.4–0.84, p < 0.05.

Four studies included preterm infants with measurements made in the first week of life [44, 46, 52, 53], whilst
four studies performed measurements at a later stage ranging between 1 and 19 weeks postnatal age (corrected gestational age = 31–49 weeks) [43, 45, 51, 53]. One study measured tibial SOS at 1, 4 and 8 weeks and found a significant decrease over this period [53]. This is in agreement with two other longitudinal studies, one with results only in abstract form so far. Both found a significant decrease in tibial SOS in preterm infants from birth to term corrected age, with a fall in median SOS by 121 m/s ($p < 0.05$) in one study group from birth to end of the study period [54] and a within subject correlation $r = -0.28$, $p < 0.001$ in the other [55]. In addition, Ritschl et al. performed QUS in term infants over the first 18 months of life, and in preterm infants from birth to 14 months

Table 1. Summary of QUS studies performed in neonates and infants

<table>
<thead>
<tr>
<th>References</th>
<th>Year</th>
<th>QUS device</th>
<th>Site/parameter</th>
<th>Term/preterm</th>
<th>Design</th>
<th>N</th>
<th>SOS</th>
<th>Age at scan, days</th>
<th>Preterm values</th>
<th>Age at scan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ritschl et al.</td>
<td>2005</td>
<td>DBM sonic</td>
<td>metacarp/SOS, BTT</td>
<td>yes/yes</td>
<td>cross-sectional/longitudinal</td>
<td>338</td>
<td>1,683 (27)</td>
<td>&lt;1</td>
<td>1,605 (6)</td>
<td>term CGA</td>
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<td>(GA 23–25 weeks)</td>
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<td>1,614 (12)</td>
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<td>(GA 26–28 weeks)</td>
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<td>1,613 (15)</td>
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<td>(GA 29–31 weeks)</td>
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<td>1,634 (15)</td>
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<td>(GA 32–34 weeks)</td>
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<td>1,643 (14)</td>
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<td></td>
<td>(GA 35–37 weeks)</td>
<td></td>
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<tr>
<td>Ritschl et al.</td>
<td>2005</td>
<td>DBM sonic</td>
<td>hum/SOS, BTT</td>
<td>yes/no</td>
<td>cross-sectional</td>
<td>94</td>
<td>1,734 (28)</td>
<td>≤7</td>
<td>1,664 (42)</td>
<td>1–19 weeks</td>
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<td></td>
<td>PCA</td>
<td></td>
</tr>
<tr>
<td>Gonelli et al.</td>
<td>2004</td>
<td>DBM sonic</td>
<td>hum/SOS, BTT</td>
<td>yes/yes</td>
<td>cross-sectional</td>
<td>140</td>
<td>1,727 (25.7)</td>
<td>≤3</td>
<td>2,630–3,300</td>
<td>within 4 days</td>
</tr>
<tr>
<td>McDevitt et al.</td>
<td>2005</td>
<td>omnisense</td>
<td>radius, tibia/SOS</td>
<td>yes/yes</td>
<td>cross-sectional</td>
<td>110</td>
<td>3,079b</td>
<td>≤7</td>
<td>2,630–3,300</td>
<td>within 4 days</td>
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<td>2004</td>
<td>omnisense</td>
<td>tibia/SOS</td>
<td>yes/yes</td>
<td>cross-sectional</td>
<td>103</td>
<td>3,100 (2,870–3,381)b</td>
<td>≤2</td>
<td>2,630–3,300</td>
<td>within 4 days</td>
</tr>
<tr>
<td>Littner et al.</td>
<td>2003</td>
<td>omnisense</td>
<td>tibia/SOS</td>
<td>yes/yes</td>
<td>cross-sectional</td>
<td>73</td>
<td>2,850–3,300</td>
<td>≤4</td>
<td>2,630–3,300</td>
<td>within 4 days</td>
</tr>
<tr>
<td>Nemet et al.</td>
<td>2001</td>
<td>omnisense</td>
<td>tibia/SOS</td>
<td>yes/yes</td>
<td>cross-sectional</td>
<td>44</td>
<td>3,101 (2,899, 3,314)b</td>
<td>≤2</td>
<td>2,630–3,300</td>
<td>within 4 days</td>
</tr>
<tr>
<td>Lit Manovitz et al.</td>
<td>2004</td>
<td>omnisense</td>
<td>tibia/SOS</td>
<td>no/yes</td>
<td>longitudinal</td>
<td>12</td>
<td>2,886 (29)</td>
<td>birth</td>
<td>2,630–3,300</td>
<td>within 4 days</td>
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<tr>
<td>Tomlinson et al.</td>
<td>2003</td>
<td>omnisense</td>
<td>tibia/SOS</td>
<td>no/yes</td>
<td>longitudinal</td>
<td>18</td>
<td>2,923 (2,672–3,107)b</td>
<td>≤7</td>
<td>2,630–3,300</td>
<td>within 4 days</td>
</tr>
<tr>
<td>Eliakim et al.</td>
<td>2003</td>
<td>omnisense</td>
<td>tibia/SOS</td>
<td>no/yes</td>
<td>intervention</td>
<td>24</td>
<td>2,892 (29.5)</td>
<td>(control group)</td>
<td>2,630–3,300</td>
<td>within 4 days</td>
</tr>
<tr>
<td>May et al.</td>
<td>2002</td>
<td>omnisense</td>
<td>tibia/SOS</td>
<td>no/yes</td>
<td>case study</td>
<td>3</td>
<td>2,892 (29.5)</td>
<td>birth</td>
<td>2,630–3,300</td>
<td>within 4 days</td>
</tr>
</tbody>
</table>

* Mean (SD); b median (range); c range.
and described a fall in metacarpal SOS from 1 month old, reaching a nadir at 6 months in term infants; in the preterm infants there was also a fall in metacarpal SOS but the nadir was reached earlier and was lower in the most preterm infants [52]. Metacarpal BTT in the same study group did not change significantly, but remained stable in the term infants, however preterm infants had an increasing BTT after birth, only reaching the age matched term BTT values at the age of 4–6 months. This emphasises that preterm infants have a different SOS trajectory from term infants.

**Anthropometry**

Gonelli et al. [48] demonstrated a significant correlation between BTT and birthweight (r = 0.2, p < 0.05), and BTT and head circumference (r = 0.22, p < 0.05), but no significant correlation with length, and no correlation between hSOS with any anthropometric data. One other study considered the relationship between birthweight and SOS in term infants separately from that in preterm infants and found a non-significant correlation, r = 0.25 [46]. Within this same study there was a strong correlation with SOS and birthweight within the preterm group (r = 0.59). However, the correlation of SOS SD score to birthweight was lower (r = 0.4, NS) indicating only a small effect of birthweight independent of gestation [46]. The remaining studies evaluated the effect of birthweight on SOS in the study group as a whole and found a significant correlation (r = 0.5–0.8, p < 0.05). Only one group found no correlation of QUS parameters with birthweight [51]. One of the five studies measuring preterm infants at discharge reported a similar correlation between QUS parameters and weight/length at birth and weight/length at time of scan [43]. One study measured knee-sole length and this correlated significantly with SOS (r = 0.5, p < 0.001) [44].

Most studies so far have excluded infants who were not AGA, however McDevitt et al. also considered the influence of abnormal growth on SOS. Comparison of gestationally matched infants who were AGA/SGA/LGA revealed no significant growth effect except in the infants who were less than 32 weeks gestation in which the two SGA infants had a significantly higher SOS than those who were AGA [46]. This cohort included five sets of twins with discordant growth where one twin was SGA and the other was AGA. Four of the five twin pairs were the same sex. There were no significant differences between the SOS of the twin sibs. Soft tissue thickness is decreased in AGA infants, the similar SOS values in these twin pairs suggests that QUS outcome is not significantly affected by soft tissue thickness. This is important as changes in body mass occur after preterm birth.

**Gender and Ethnicity**

Gender [43, 44, 46, 48, 51] and ethnicity [44, 46, 51] were universally found to have no effect on QUS parameters.

**Perinatal and Maternal History**

Gonelli et al. [48] measured QUS parameters in mothers as well as their offspring. None of the QUS parameters were influenced by maternal smoking, calcium intake or positive family history of osteoporosis, nor was there any significant relationship between QUS results in mothers and their children [48]. However, the rate of maternal smoking in this study was lower than that in studies using Dxa which have demonstrated an effect of maternal smoking on BMD [56].

**Osteopaenia and Bone Markers**

Litmanovitz et al. reported changes in bone specific alkaline phosphatase (BSAP), a marker of bone formation, and carboxy terminal cross-links telopeptide of type-I collagen (ICTP), a marker of bone resorption, and investigated their association to SOS changes. Ultrasound scans and serum samples were obtained at 1, 4 and 8 weeks of age. There was a significant increase in BSAP and a significant decrease in ICTP, but both remained within the normal range. During the 8-week period, SOS fell but there was no statistically significant correlation between levels of either BSAP or ICTP and SOS [53]. This further questions the role of ALP as the sole screening test for OP. It should also be borne in mind that serum ALP is the sum of three isoforms (liver, intestine and bone) and some of the changes may reflect disorder of liver function. However, the bone isoform contributes to the largest proportion of serum ALP in infants and children and changes in that isoform generally mirror those in total serum ALP [57]. BSAP in the neonate has not been found to improve sensitivity for OP [24].

Nemet et al. [45] found a significant inverse correlation between tibial SOS at birth and serum ALP (r = 0.59, p < 0.005) [45]. Six of the premature infants had a serum ALP of >400 IU/l and the correlation was stronger within this group (r = 0.75, p < 0.05) [45]. In one report, the lowest SOS within the study group [45] was measured in an infant with a serum ALP of 856 IU/l with radiological evidence of osteopaenia. One longitudinal study reported a significant negative correlation of serum ALP and SOS at 35–37 weeks CGA, r = 0.6, p < 0.05, however serum
ALP did not correlate significantly with final SOS SD score in the same patients, $r = 0.3$, $p = 0.23$ [54]. Three infants within the study had a combination of high alkaline phosphatase (859, 1094, 2199 IU/l) and low serum phosphate ($<1.2$ mmol/l) and these infants had the lowest absolute SOS of all study infants [54]. This emphasises the process of OP discussed earlier, mineralisation defects and lack of osteoid contribute to neonatal bone strength. The ‘sick’ environment with inflammation, drugs and immobility and lack of muscle and bone growth all result in a lack of drive to form periosteal bone [58]. Therefore bone strength decreases, and bone SOS decreases in some infants who have normal bone markers as well as those with abnormal bone markers.

**Multiple Pregnancy**

Littner et al. [59] studied 25 pairs of twins between 27 and 40 weeks gestation and found that AGA twins had tibial SOS similar to AGA singletons of the same gestation. This study focused on twins with intrauterine growth equivalent to singletons and suggests that in twins without intrauterine growth restriction bone growth is maintained even if theoretically there is less room for intrauterine movement. There was no significant difference between twin pairs in twins with normal growth [59]. A recent study using DXA suggests that in twin pairs with weight discordances, a difference in body weight is correlated with differences in bone mineral content as well as lean and fat mass [60]. However, as discussed earlier, SOS in five sets of twins with discordant growth was not found to be significantly different between pairs [46].

**Movement and Exercise**

Skeletal growth is driven by functional requirements. Both spontaneous movement and exercise has been related to changes in bone SOS [61, 62]. Three infants with unilaterally reduced spontaneous movements due to central nervous system pathology were shown to have a lower tibial SOS in the non-active limb compared to the normal limb [61]. QUS measurements were taken by a blinded technician. Consistent with these results is the effect of modest daily activity in attenuating the decrease of SOS postnatally in preterm infants described by Litmanovitz et al. [62]. Early exercise intervention of a 5-minute session of extension and flexion range of motion exercises of upper and lower limbs was performed 5 times a week for 4 weeks. The control group’s SOS fell postnatally, whereas the exercise group’s SOS was maintained around the measurement taken shortly after birth. There were no significant differences between the groups in gestation, birthweight, feeding, or oxygen requirement. No harmful effects were reported in the exercise group. There was no effect on early weight gain or bone turnover markers in the exercise group. Exercise therefore does seem to have a role; however, the optimal exercise intervention, i.e. timing of onset, frequency and duration, still needs to be determined.

**Conclusion**

In neonates, QUS can be measured reliably. Whilst the two commonly used ultrasound devices used for this purpose are technically different, the trend in outcome is similar for each device. There is a difference between preterm and term infants at birth, and a fall in SOS when measured longitudinally in preterm infants. These QUS changes may reflect the halt in bone mineral accrual following premature birth, a subsequent increase in cortical porosity and bone loss due to factors such as immobility, inflammation and drugs but this requires further investigation.

QUS remains primarily a research tool. SOS may have a role in non-invasive monitoring of bone health in the preterm infant in combination with other modalities, but currently there are scarce data to indicate that SOS predicts fracture risk in the neonatal population. Its role in long-term follow-up studies of bone health as well as in an epidemiological setting requires further investigation.
References


