BONE MECHANOADAPTATION AND THE INFLUENCE OF MUSCULAR ACTION ON BONE ACROSS THE LIFESPAN

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Abstract:

It is unknown how physical activity and exercise affect bone throughout life, and to what extent this is caused by changes in muscle. Therefore bone strength and muscle size were examined in a prospective cohort study of fifty-three young children to assess the influence of early locomotory activities on bone. In addition, upper limb muscle and bone size and strength of fifty adolescent and eighty-eight older adult tennis players were examined in two cross-sectional studies. Seventeen sprinters, fifteen tennis players and nineteen physically inactive controls were also examined in a case-control study to reveal how age and exercise type influence exercise benefits to bone. Finally, a case-control study examined muscle and bone asymmetries in a veteran tennis player with an unconventional bilateral playing style and twelve conventional players to assess relative effects of service and ground strokes on bone. Strong positive effects of early locomotion on tibial bone strength and of regular tennis play on upper and lower limb bone strength were observed. Exercise benefits to bone were greater in younger and male tennis players, and those who had begun to play in childhood. Strong muscle-bone relationships in all cohorts and concurrent loss of muscle and bone suggest an important role of muscular action in mechanoadaptation throughout life. Evidence for torsional strains as a potent osteogenic stimulus was observed in both the upper and lower limbs. In addition, the sizeable humeral hypertrophy observed in tennis players appears to be attributable to the service stroke. In conclusion, whilst exercise benefits to bone appear greatest

in adolescence the body appears to retain a large capacity for bone mechanoadaptation throughout life - driven largely by muscular action.

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‘The natural potential of each arm is just about the same, and the difference between them is our own fault, because we’ve habitually misused them‘

Plato

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Abbreviations/definitions:

BMC - Bone mineral content

BMD - Bone mineral density

CT - Computed tomography

DXA - Dual energy x-ray absorptiometry

I_p - Polar moment of inertia, calculated as cortical bone area multiplied by the square of the distance of each pixel from the centre of bone mass indicating bone stiffness in torsion

MRI – Magnetic resonance imaging

pQCT - Peripheral quantitative CT

R_p - Polar moment of resistance, calculated as I_p divided by periosteal circumference indicating bone strength in torsion – also referred to as section modulus

Strain (ϵ) - Change in object length with respect to original length as percentage. The unit microstrain (με, or 10^{-6}ϵ) is commonly used in bone research

Stress (σ) – Force per unit area, commonly described in Pascals (Pa, or N/m^2).

1. INTRODUCTION

1.1 General Introduction:

Bone fractures are highly prevalent throughout life, with annual incidence as high as 5% dependent on age and gender (Landin, 1983, Donaldson et al., 1990). This presents a large burden to health services; for example, the cost of osteoporotic fractures in females to the NHS has been estimated to be nearly £1 billion annually (Dolan and Torgerson, 1998). Bone strength (as indicated by densitometry) has been shown to be predictive of fracture risk (Clark et al., 2006, Schuit et al., 2004) with a standard deviation decrease in bone strength associated with a 50-90% greater risk. Therefore understanding factors which influence bone strength throughout the lifespan is of key clinical importance.

Bones respond to the level of habitual loading they experience. As muscles work with short lever arms, the internal muscle forces are far greater than external reaction forces during movement (Rittweger, 2007) – therefore muscular action is an important factor in determining habitual bone loading. In disuse cases such as long-term spinal cord injury up to 50% of bone mass can be lost (Eser et al., 2004, Rittweger et al., 2010). Conversely, bone mass in the lower limbs of sprinters or the racquet arm of tennis players can be 25-35% greater than in sedentary controls or the non-racquet arm respectively (Haapasalo et al., 2000, Wilks et al., 2009a). There is consequently great interest in physical activity and exercise as therapeutic

interventions to improve bone strength. However, the effects of prolonged controlled interventions are currently unimpressive – particularly in older adults (Nikander et al., 2010b). Understanding bone mechanoadaptation, the influence of muscular action on bone during exercise and factors (*e.g.* age, gender or starting age) affecting the osteogenic potential of exercise throughout life is important in improving effectiveness of interventions.

1.2 Bone Physiology:

1.2.1 Functions of bone

Bones serve a number of essential functions within the human body. They protect vital organs such as the brain, heart and lungs, and host the primary site for white and red blood cell production. Bones also function as a store of fat and essential minerals – *e.g.* over 99% of the body's calcium (essential for blood clotting, function of the central nervous system, excitation-contraction coupling, *etc.*) is stored in the bones (mainly as a material similar to hydroxylapatite). Finally, bones act as rigid structures, providing levers for muscles to act against, thereby permitting movement such as standing and locomotion.

1.2.2 Types of bone

The number of bones within the human body changes from 350 at birth to ~200 at adulthood as certain bones fuse – the final number varying based on the number of sesamoid bones which can be as many as 42 (Sarin et al., 1999). There are a number of different types of bone – classified broadly by their shape, which is connected to their function. Flat bones (*e.g.* scapula, bones of the skull, sternum, *etc.*) provide either extensive protection to underlying soft tissue, or a broad surface for muscular attachment. Short bones (such as the tarsals and carpals) provide support for movement. Long bones (*e.g.* femur, tibia and humerus) provide

a high lever arm ratio for muscles to act against – allowing rapid limb movements in *e.g.* sprinting. Irregular bones are those with distinct shapes and purpose, and whose functions are multifluous – from the hyoid's role in supporting tongue movement, to the vertebrae providing protection for the otherwise vulnerable spinal cord. Finally, sesamoid bones (such as patella or pisiform) are found at certain locations where a tendon crosses a joint. They act to protect the tendon and to offer a mechanical advantage in movement by increasing the moment arm of the joint.

1.2.3 Bone – organ, tissue and material

Bone is an ambiguous term, describing the organ as a whole, the tissue which comprises it, and the material from which that tissue is constructed. At the tissue level, bone consists of both an organic collagen component, and an inorganic mineral component. The collagen has high tensile strength, due to its triple-helix aminic bond structure (Rittweger, 2011), and is flexible. Bone mineral is predominately made up of calcium phosphate crystals, although either or both of these principal components are replaced by other elements in a small percentage of crystals. Whilst bone mineral plays an important role in mineral homeostasis within the body, its main function mechanically is in affording stiffness and compressive strength to bone. Human bone is ~60% mineral by mass, and is a composite material offering a balance between stiffness and toughness provided by the two components. However, there are some differences in bone composition

where function demands it – *e.g.* the high (~90%) mineral content of the auditory ossicles conveys the stiffness necessary for the propagation of vibrations required for sound transmission. Bone mineral also considerably attenuates X-rays and X-ray based scanning is hence a very widely used method of assessing bone strength.

In addition to the material component, bone as a tissue contains bone cells (described later within the bone metabolism section) and blood vessels. Bone tissue can be either cortical or trabecular. Human cortical (or compact) bone has ~90% bone volume fraction (*i.e.* conversely it has ~10% porosity) whereas trabecular (or cancellous) bone has around 20% bone fraction volume (Jee, 1983). Both cortical and trabecular bone are composed of stacked layers of bone called lamellae. In lamellar compact bone, lamellae are stacked in a staggered parallel fashion, similar to the staggering of vertical joints in layers of brickwork. However, in woven bone (a response to trauma or severe overloading of bone) the organization of these layers is lost – thereby compromising bone strength. On a larger scale, lamellae make up Haversian bone – a series of concentric cylindrical lamellae around a central Haversian canal that contains nerve and blood supply for the bone (Figure 1). Transverse Volkmann's canals connect these Haversian systems (or osteons) via the Haversian canal.

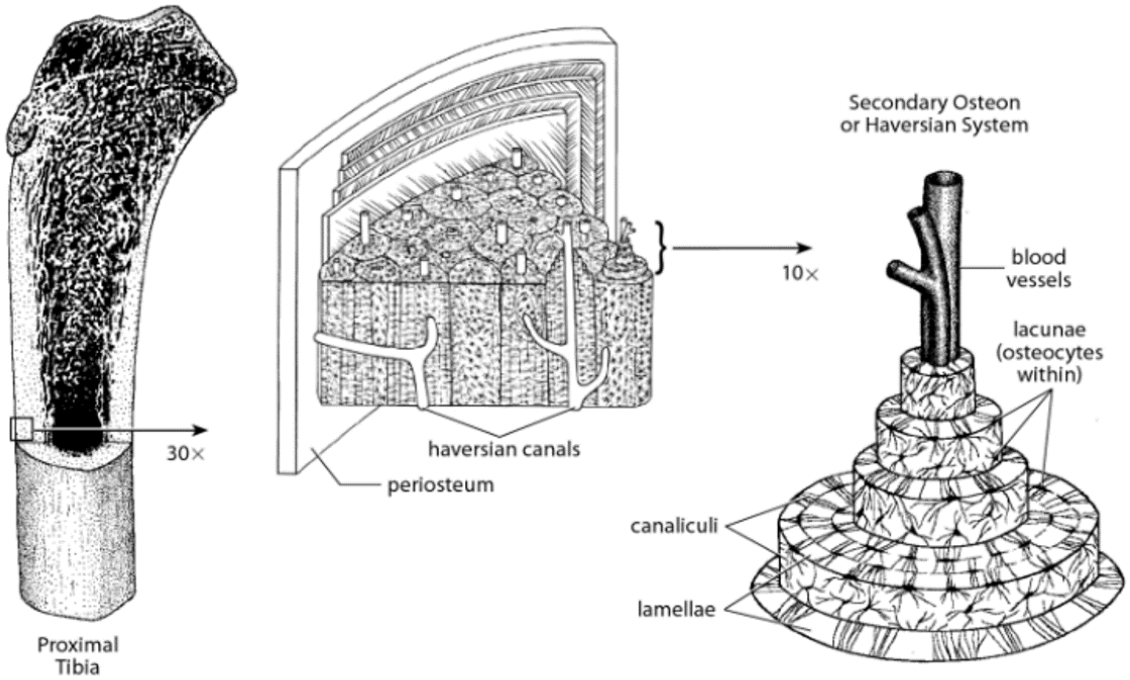


Figure 1. Gross and microscopic bone structures (White et al., 2011). Reproduced with permission.

Bones are covered with a membranous sheath on both the inner and outer cortical borders. Both sheathes contain osteoblasts and osteoclasts (responsible for bone modeling and remodeling – discussed later), and blood vessels supplying the bone. There is an additional layer on the outer cortical surface called the periosteum, which also contains connective tissue, blood vessels and nerve fibres including nociceptors. Long bones are made up of three sections – firstly, a long, hollow cylindrical diaphysis composed mostly of a cortical bone shell with bone marrow contained within the inner cavity. Second, the metaphysis – a region towards the ends of the bone containing both cortical and trabecular bone where

– during childhood – longitudinal growth of the bone occurs. Finally, the wide, mainly trabecular epiphysis – its broad surface area minimises compressive stress applied to the hyaline cartilage which covers the bone ends and is the interface between articulating bones.

1.2.4 Bone metabolism

The process of bone metabolism is accomplished via the opposing actions of osteoblasts and osteoclasts. Osteoblasts are directly responsible for osteogenesis, laying down a collagen matrix called osteoid which is then mineralized to form new bone. Conversely, osteoclasts break down and resorb bone. Osteocytes are osteoblasts which have become trapped within the osteoid they have produced. They are thought to play an important role in regulation of osteoblast and osteoclast activity. During growth, modeling is the predominate mode of bone regulation and is characterized by drifts – whereby bone surfaces undergoing formation oppose those where bone is being resorbed. This is displayed well in the shape of long bone cross-sections during growth prior to physeal closure – whereby both periosteal and endocortical borders increase in diameter. This allows increases in bone mass and size, whilst allowing the bone to retain a cross-section shape optimized for the forces which habitually act upon it. Longitudinal growth of bone is accomplished by endochondral ossification (formation of bone within cartilage), whereas increasing bone width results from intramembranous ossification (formation of bone within the organic matrix

membrane) and appositional ossification (formation of bone on the outer surface of existing bone) (Buckwalter et al., 1996). Whilst it is thought that joint size is largely fixed at the end of puberty or epiphyseal closure (Frost, 2004c, Rittweger, 2008a) a modest amount of periosteal and endocortical expansion appears to continue throughout life (Riggs et al., 2004).

The other method of bone turnover is remodeling, or the replacement of old bone tissue with new bone. Taking 90-120 days, this process is again completed by the opposing actions of first osteoclast and then osteoblasts. Bone remodeling follows a sequence of quiescence, activation, resorption, reversal and formation, and occurs on periosteal and endocortical surfaces, as well as the surface of trabeculae and Haversian canals (Parfitt, 1984). Targeted remodeling involves the replacement of damaged bone tissue, in an effort to minimize the accumulation of microdamage. Stochastic modeling also occurs, and is thought to be related to regulation of calcium. Over 99% of the body's 1-2kg of calcium is contained within bone – total blood calcium outside the range 300-750mg (in a typical 70kg human) is usually fatal. Therefore modelling to incorporate in or release calcium from the bone plays an important role in the regulation of circulating calcium levels within this narrow range. By the same token it follows from these numbers that any bone adaptive process will take months and years. Bone turnover – through either modeling or remodeling - occurs continuously throughout life, the two processes often occurring simultaneously, being responsible for bone repair following disease or injury (Buckwalter et al., 1996). Bone turnover can be measured via markers

of osteoblast and osteoclast activity, and markers of hormones and molecules related to mineral homeostasis such as vitamin D. It is usually taken from blood or urine, hence no anatomical information is contained within it, which would allow site-specific response of bone to be assessed.

1.2.5 Bone mechanics

Bones' roles as levers for muscular actuators to work against necessitates a marriage of great strength (due to the short levers muscles work against (Özkaya and Nordin, 1998)) and lightness, as they must also support their own weight. The forces acting upon a material, and their effect upon the bonds within the material are described by stress and strain. Stress (σ) is the result of the strain within a material when force is applied – commonly described in Pascals (Pa, or N/m^2). Stress can be either compressive, tensile or shear. Compressive stress acts to push atom bonds together, and tension acts to pull them further apart. In shear, opposing forces act perpendicular to the inter-atomic bond. The type of stress experienced may well vary within the same bone – *e.g.* as the tibia is bent anteriorly during foot contact in sprinting, the anterior aspect of the tibia will primarily experience tension, and the posterior aspect compression.

The result of an applied force is a change in the length of the body, due to lengthening or shortening of its inter-atomic bonds, known as strain (ε). Strain – being an intensive property - is given as a relative measure of this change with

respect to the object's original length (*i.e.* a percentage). Typically in bone research, the term microstrain (με) is used – referring to a 0.0001% change in bone length – *e.g.* a stress of 1-2MPa results in 50-100 με in healthy mammalian bone (Frost, 2004b). The strain caused by a given stress indicates a material's intrinsic stiffness (Young's modulus). The Young's modulus of cortical bone is ~20GPa, which is similar to concrete - making it much stiffer than rubber (<0.1GPa) but much less stiff than steel (~200GPa). The relationship between stress and strain for a material remains linear until the yield point, and upon removal of the stressing force the material will return to its original shape. After the yield point, permanent (plastic) deformation of the material occurs – depending on the extent of the deformation damage, cracks or fracture may occur (Figure 2). External loading of the bone can be assessed via force plates (in the case of ground or other reaction forces) and tendon strain gauges (for muscular forces) (Fukashiro et al., 1995, Komi et al., 1992). However, stress will vary throughout the bone due to material and geometrical anisotropy. Strain within bone has traditionally been examined through use of strain gauges, although they have several limitations such as the inability to measure whole bone strain in multiple directions (Yang et al., 2011). Recently, an optical tracking method has been developed (Ganse et al., 2014, Yang et al., 2014a, Yang et al., 2012) which may give greater insight into bone strains *in vivo* (Yang et al., 2015, Yang et al., 2014b).

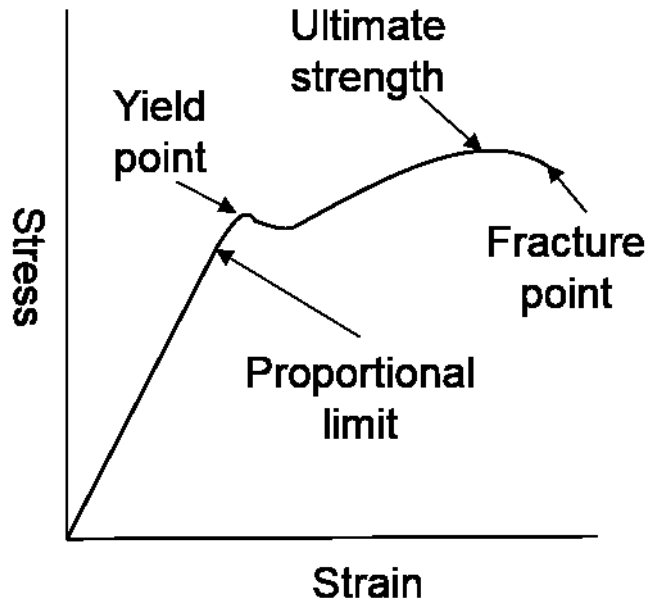
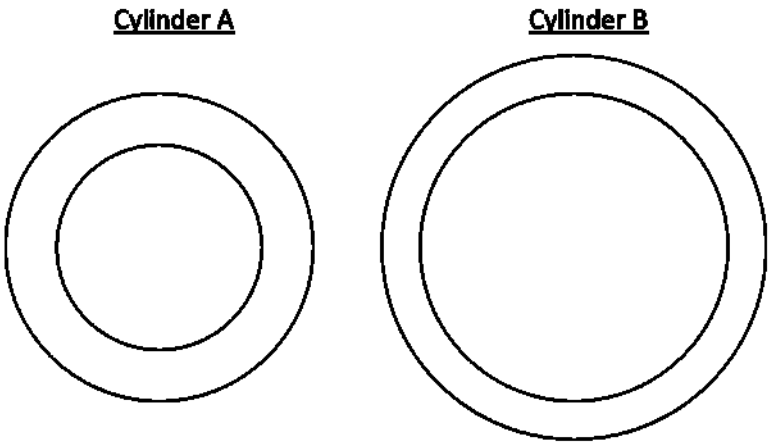


Figure 2. Typical stress-strain curve

The ultimate strength of a material is the stress (and hence strain) at which fracture occurs, whilst the area under a stress/strain curve represents the energy required to cause material failure, known as material toughness. Strength, stiffness and toughness are material-level properties and hence not related to the size of the object the material forms (Turner, 2006). It is important to realize that material stiffness is probably the most important property in relation to bone mechanoadaptation, whereas material strength is the property that is most relevant to fracture.

Within the context of bone, strength and stiffness are often used to describe bone health. However, in the case of trauma energy is transferred into the bone – if the bone is not capable of absorbing this energy then fracture will occur. This ability of a material to absorb energy and deform without fracturing is known as toughness. Therefore a bone with lower stiffness and strength (but greater toughness) may not fracture in circumstances where a stiffer, stronger bone would.

Stiffness, strength and toughness of bone are dictated by a number of factors. In cortical bone the density and size of mineral crystals incorporated into bone affect tissue properties – *e.g.* in ageing, crystals increase in size which is associated with increased brittleness (Boskey, 2003). The porosity of bone also differs between individuals, with ageing and exercise/disuse – pore size and number explains the majority of variation in bone yield stress and stiffness (Dong and Guo, 2004). Microcracks accumulate as a result of prolonged or heavy loading – whilst they can be repaired, their prevalence is inversely related to bone stiffness and ultimate strength (Zioupos, 2001). As an organ, strength of bones is dependent on a number of factors aside from material properties: orientation of trabeculae and lamellae, bone mass and geometry. Bone is anisotropic both structurally and at the material level, and the orientation of trabeculae can alter in response to changes in the direction of joint loading (Barak et al., 2011). Similarly, osteons in the cortical area of long bones are predominately orientated longitudinally and accordingly cortical bone is stronger along this axis.



Variable	Cylinder A	Cylinder B	Difference A-B (%)
Cross-sectional area (cm^2)	20.0	20.0	0%
Inner Circumference (cm)	4.0	6.0	50%
Outer Circumference (cm)	6.0	7.483	24.7%
Polar moment of resistance (R_p, cm^3)	86.7	122.9	41.8%
Polar moment of inertia (I_p, cm^4)	520	920	76.9%

Figure 3. Effects of geometry on torsional strength and stiffness of cylinders of equal cross-sectional area.

Bone mass orthogonal to the direction of applied force affects whole bone strength, as (assuming the same applied force) a lower stress – and hence strain – will be produced in a bone of greater mass. Finally, the shape of a bone of given mass greatly affects its stiffness and strength in bending and torsion. Whilst resistance to compressive or tensile stresses is proportional to the mass of the cross-section, resistance to bending and torsional stress increases the further mass is distributed

away from the central axis (Figure 3). However, as material is distributed further away from the central axis, the wall thickness decreases. The ratio of bone radius to cortical thickness is known as the buckling ratio, and has a strong positive relationship with fracture risk (Melton et al., 2005).

1.2.6 Bone assessment

In comparison to testing methods available to those researching virtually any other organ, the battery of tests available for bone is limited. Direct measurement of bone strength can only occur through mechanical testing. These tests can be completed in cadaveric or animal samples, but within clinical practice and research assessment of living humans a less invasive approach is required. Bone mineral attenuates X-rays strongly, and hence X-ray based methods are a common method of assessment of bone strength.

Single and dual photon absorptiometry (used to assess bone mineral density) were replaced with the quicker single and dual energy X-ray absorptiometry (SXA/DXA) machines in the 1990's, which also permitted whole body scanning and measurement of bone area/mineral content as well as lean and fat mass. However, DXA measurements are based on assessment of bone mineral content (BMC) and projected area, from which areal bone mineral density is derived. Therefore given the relationship between BMC and area, bias in measured BMD can be introduced particularly when comparing individuals of different size. The

hip structural analysis (HSA) algorithm gives better estimates of bone geometry from DXA but does not allow for a true 3D representation of bone.

For the past 15 years, peripheral computed tomography (pQCT) has been used in assessing upper and lower limb bone strength. Both DEXA and pQCT involve patient exposure to ionizing radiation and so use must be carefully monitored. However, the dose for each scan is ~1-2μSieverts, equivalent to less than twenty-four hours of background radiation or a short flight. As pQCT scanners reconstruct a three-dimensional volume, the volumetric BMD obtained is a true representation of the mineral density of the bone under examination and is affected little by size. CT scanning can be subject to beam hardening errors, whereby lower-energy X-rays are attenuated more readily than higher-energy X-rays resulting in a change in beam profile which will result in inaccurate assessment of material density. However, pQCT scanners employ corrective algorithms which minimize the influence of beam hardening on measured density values regardless of object size (Augat et al., 1998). pQCT allows the separation and analysis of trabecular and cortical bone compartments, which have been shown to respond differently in ageing, exercise and disuse. In addition, they permit examination of the geometry of bones, which similarly differs both within and between bones and within their response to the influence of age and exercise/disuse.

A methodological concern with pQCT is the partial volume effect – whereby attenuation of the X-ray beam is measured from a series of discrete voxels. At

bone boundaries, bone may only cover part of each voxel and hence the measured attenuation and computed density will be affected. This error will be around 1-2%, which is considerable when considering *e.g.* effects of short-term exercise or comparing similar groups. In addition the effect is more pronounced as the circumference to area ratio increases – hence in children and the elderly, comparisons of different bones or regions within a bone bias may be introduced. The bias is minimized when the threshold used for identification of the bone contours is set midway between the density of bone and the surrounding tissue (*i.e.* muscle in the case of *in-vivo* analysis of human bone). In adult cortical bone this value is around 1300mg.cm^{-2} but can be greatly reduced in young infants (*unpublished observations*). A corrective algorithm has also been developed (Rittweger et al., 2004) – using knowledge of bone circumference and voxel size to correct the density values obtained. In the last decade, high-resolution pQCT has been developed and used to assess distal limb bone. High-resolution pQCT allows a larger volume of bone to be scanned, and permits greater analysis of trabecular structure – values such as trabeculae number and the ratio of bone/total volume (indicating trabecular porosity) can be assessed. However, the radiation dose for these scans is higher than pQCT and – at present – proximal limb segments cannot be scanned. Although less commonly used, ultrasound can also be employed to assess bone – it is a low-cost method without ionizing radiation, although what the obtained attenuation values then represent is unclear. Ultrasound transmission velocity is affected by bone strength parameters such as bone size and density, and ultrasound measures have correlated with fracture risk.

However, the values obtained do not provide the detail obtained with DXA or pQCT scanning.

Whilst pQCT measurements are only representative of the degree of mineralization of the bone, pQCT-derived bone strength parameters have been shown to correlate strongly with bone ultimate strength as measured mechanically (Kontulainen et al., 2008, Ebbesen et al., 1997). This is also true of both geometric variables (Myers et al., 1993) and density measurements (Moro et al., 1995) calculated from DXA scans, although associations with fracture load in the latter are less strong. Densitometric bone strength indicators are predictive of fracture risk, with a standard deviation decrease in bone mineral density associated with a 50% greater risk of all non-vertebral fractures and greater than two-fold risk of hip fracture (Schuit et al., 2004). Similar results are also observed in children (Clark et al., 2006). Increases in bone mineral density following treatment with antiresorptive agents (Cummings et al., 2002) are associated with sizeable reductions in fracture incidence. In the public consciousness, bone mineral density (BMD) is well known and the impression is that high BMD is beneficial. However, in osteogenesis imperfecta, it is now thought that the problems are caused by an increased BMD at the material level (Boyde et al., 1999). Similarly, it has been suggested that high BMD may be deleterious for joint health (Turner, 1998a) – recent evidence has shown increased risk of knee and hip osteoarthritis in patients with extremely high bone mass (Hardcastle et al., 2015, Hardcastle et al., 2014).

1.2.7 Regulation of bone strength

The control mechanisms by which bone modeling and remodeling are regulated are a long-established research topic. Galileo speculated that mechanical stimulation contributed to bone strength regulation around 400 years ago, and in the mid 1800's Culmann and Von Meyer observed that trabeculae were orientated along principal stress lines. Julius Wolff then proposed that the form of bone follows its function, and so bone adapts to its mechanical environment (Wolff's Law) – however the mechanisms by which this occurs were not stated (Frost, 2004b). It was not until Glucksman constrained the growing limbs of chick embryos to create bending loads (Glucksmann, 1942) that experimental evidence for these theories was produced. That ossifying tissues were found to align along principal lines of tensile stress, and that these stresses resulted in increased ossification of fibrous tissue were supportive of Wolff's proposal. Harold Frost's work then resulted in the development of his Mechanostat theory (Frost, 1987), whereby it is proposed that the aim of bone adaptation is to keep habitual strain within the bone within defined thresholds. Further to this, that there is a window of strain rates where no net bone strength change occurs – sustained strain above this level lead of increased bone strength, whereas strains below result in net bone strength loss.

Whereas a remodeling threshold of 1000με has been proposed (Schiessl et al., 1998), this appears site-specific according to the typical loading environment of a bone (Hillam et al., 2015). Whilst magnitude of strain appears to be a key factor

in bone mechanoadaptation, a number of other loading variables are important. Strain rate is positively associated with bone adaptation to the same peak strain (O'Connor et al., 1982). Bone's capacity for further adaptation diminishes with increasing cycle number (Turner, 1998b), and the number of loading cycles required to instigate modelling is inversely related to peak strain (Cullen et al., 2001). Recovery periods both within and between bouts of exercise restore bone's mechanical sensitivity (Robling et al., 2001) – therefore short, frequent exercise sessions appear to be maximally beneficial for improving bone mass (Robling et al., 2002). Stresses applied to a bone may vary in type (*e.g.* compressive, tensile, shear) and magnitude within a cross-section, or along a bone's length. Consequently, bones are not homogenous in shape, size and structure, with inter and even intra-bone differences evident. Within tibial cross-sections, significant anterior-posterior differences in BMD and cortical thickness have been found (Lai et al., 2005).

Mechanotransduction is the conversion of mechanical stimuli into biological information within the body. In the initial mechanocoupling phase, stimuli are sensed and transduced into a mechanical signal, followed by biomechanical coupling whereby the signal is further transduced into a biochemical signal (Turner and Pavalko, 1998). This signal is then transmitted to an effector cell which responds appropriately according to the signal received. When forces are applied to bone, a number of mechanical changes occur which could act as the mechanical signal for bone adaptation. A fluid shear stress upon the osteocytes is created – in

addition, bone is inherently piezoelectric and so electrical fields called streaming potentials are generated (Turner and Pavalko, 1998). Although osteoclasts, osteoblasts, bone lining cells and osteocytes can all sense mechanical stress osteocytes are thought to be the likeliest candidates for the role of sensing cell for a number of reasons including their location within the bone (Cowin, 2002) and that their shape amplifies the mechanical signal (Han et al., 2004). In addition, osteocytes align according to local mechanical loading (Vatsa et al., 2008). Extracellular fluid flow across the osteocytes (caused by the pressure gradients created within bone when strain occurs) is sensed by the osteocytes and is currently thought to be the main signal for adaptation.

1.2.8 Strain modality

Whilst a number of studies have highlighted the importance of strain magnitude (Rubin and Lanyon, 1985), rate (Turner et al., 1995) and duration (dynamic *vs*. static) (Lanyon and Rubin, 1984) for bone mechanoadaptation, the type of deformation *e.g.* compressive, bending or torsional has largely been ignored. However, there is increasing evidence that torsional loading may be highly important for bone development and adaptation. Whilst it was assumed that locomotion was associated with primarily compressive and bending stresses, *in vivo* studies have shown large torsional strains during walking and jogging (Yang et al., 2014b). Recent mathematical modelling work has shown that axial torsion is particularly effective for generation of tube structures like long bones, and that

these strains can remedy misalignment after fracture (neutralization of flexure) (Mittag et al., 2015). In disuse studies, torsional loading is more effective at preventing atrophy of bone than axial loading (Rubin et al., 1996). The most dramatic exercise-based adaptations in bone are observed in the humerii of tennis (Jones et al., 1977) and baseball (Warden et al., 2009) players where large shoulder internal rotation torques (likely to apply large torsional stresses to the humerus) occur (Bahamonde and Knudson, 2003, Fleisig et al., 1995, Elliott et al., 2003, Fleisig et al., 2003). Material eccentricity analysis was devised to assess how bone adaptation to bending stresses was associated with the length of the moment arm (bone strength) for such stresses (Rittweger et al., 2000). Such an index could be adapted to examine bone adaptation to torsion by assessment of torsional stiffness and moment arms. In the lower limbs, turning movements produce far greater knee and ankle torques (likely to result in torsional tibia stress) than straight locomotion (Orendurff et al., 2006, Taylor et al., 2005). Accordingly, bone strength in mice encouraged to perform turning movements was greater than that of those persuaded towards linear locomotion (Wallace et al., 2013). Tennis play involves hundreds of quick turns (Robinson and O'Donoghue, 2008), likely to produce large torsional tibia strains in the absence of large ground reaction forces (Stiles and Dixon, 2007). Therefore study of tennis players could help reveal the effectiveness of turning movements and thereby torsion in improving bone in humans.

	Standing	Hopping
Body Mass [kg]	70	70
Ground reaction force [kN]	0.7	2.5
Torque [Nm]	84	300
Acceleration [g]	1	3
$Force_{AchillesT}$ [kN]	2.1	7.5
$Force_{Tibia}$ [kN]	2.8	10

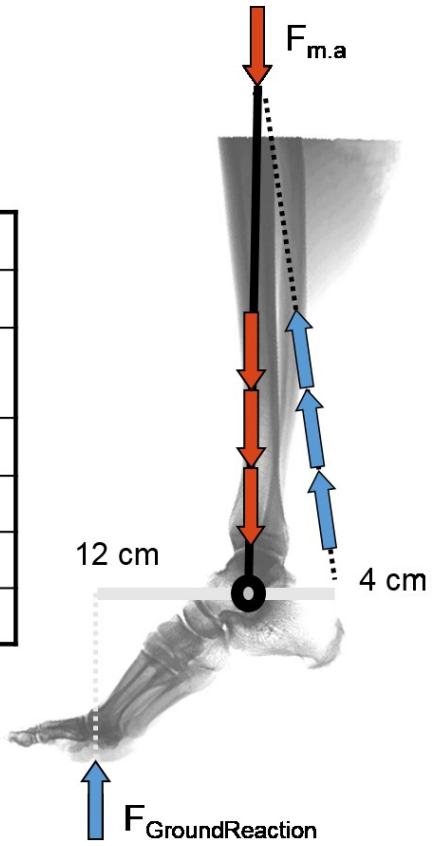


Figure 4. Reaction and tendon forces during standing and hopping (Rittweger, 2007). Reproduced with permission. Torque is that caused by application of ground reaction force (GRF), calculated as GRF multiplied by moment arm (12cm, or 0.12m). Achilles tendon force ($Force_{AchillesT}$) is that required to balance torque created by GRF, calculated as Torque divided by Achilles tendon moment arm (4cm, or 0.04m). Force applied to the tibia ($Force_{Tibia}$) is the sum of GRF and $Force_{AchillesT}$.

1.2.9 Muscle and Bone

External reaction forces are certainly a stressor of bones and can vary greatly dependent on the type of physical activity undertaken. Walking results in ground reaction forces roughly equal to body mass (Keller et al., 1996), whereas peak

ground reaction forces of up to 15 times body mass have been recorded during the step phase of triple jump (Perttunen et al., 2000). However, given the short levers that muscles work against internal muscular forces (often produced in reaction to an applied external load) are far greater than the external load applied. For example, Achilles tendon force during standing and hopping is approximately three times greater than the ground reaction force (Figure 4) – this disparity can be even more pronounced in other limb segments dependent upon the relative length of the lever and moment arm. Due to several factors such as tendon compliance, pre-activation of the muscle and rate of force development the time course of bone loading by muscle (via the tendon) and by external forces may differ. For instance, during walking two peaks in ground reaction force are normally seen, where Achilles force only sees a single peak (Komi et al., 1992). Inter-joint differences in muscular loading in reaction to applied external forces may also differ temporally; loading patterns in Achilles tendon and patellar tendon differ during squat and counter-movement jumping (Finni et al., 2000). Given the importance of muscular force to bone loading, it is of little surprise that strong associations of muscle mass (as a surrogate for maximal strength) and bone size, mass or strength have been found in children, adolescents (Schoenau et al., 2002), adults and older individuals (Ferretti et al., 1998). In addition, close relationships between maximal muscular strength and power and bone strength have been found (Ashe et al., 2008).

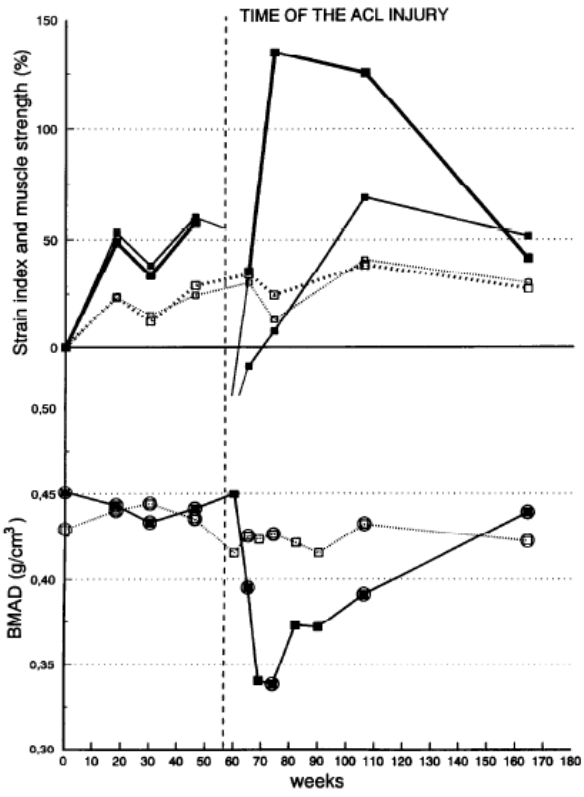


Figure 5. Time course of changes in strain index, muscle strength and patellar bone mineral density following anterior cruciate ligament (ACL) injury and subsequent rehabilitation (Sievänen et al., 1996). Upper panel: Wide solid line indicates changes in patella strain index in the injury limb, and narrow solid line indicates isometric knee extension strength. Changes in uninjured limb strain index and strength are illustrated by wide and narrow dotted lines respectively. Lower panel: Changes in the bone mineral apparent density (BMAD) of the injured (thick line) and uninjured (thin line) patella are shown. Reproduced with permission.

Whilst these relationships may – at least in part – be due to allometric associations, the temporal nature of the relationship between muscle and bone (*i.e.* that changes in muscle should precede those in bone) has been well demonstrated in several studies. For example, in the case of a woman suffering cruciate ligament injury,

the instantaneous loss of muscle force upon ligament rupture preceded a delayed reduction in bone strength (Sievänen et al., 1996). In addition, during rehabilitation the increase in muscle strength then again preceded the return of BMD to pre-injury values (Figure 5). Also, as observed during the pubertal growth spurt, peak rate of lean body mass accrual occurs before peak bone mass velocity (Rauch et al., 2004). These associations of muscle and bone remain even in the extreme case of spinal cord injury patients (Bass et al., 2005).

1.3 Factors Influencing bone strength

1.3.1 Growth and ageing

Bone strength increases greatly with maturational growth – adult bone mass being 4-5 times greater than that of young children (van der Sluis et al., 2002). Whilst there is a strong association of bone mass and height (and hence long bone length) the size and mass of bone cross-sections also greatly increases - young adult distal tibial bone mass being around 10 times greater than bone mass at the same site at birth (Capozza et al., 2010, Viljakainen et al., 2011). Puberty is associated with pronounced bone accrual - bone mass more than doubles in females and nearly triples in males between the ages of 8 and 18 (Maynard et al., 1998), with peak bone accrual rate occurring at ~12 years of age in females and ~14 years in males (Rauch et al., 2004). Changes in bone length and mass do not occur synchronously during puberty, with peak height velocity preceding peak bone mass accrual by around 9 months (and peak lean mass accrual rate by around six months, with these peak differences more pronounced in females (Rauch et al., 2004). Longitudinal bone growth occurs at the metaphyses, and during rapid growth (such as during puberty) a less pronounced age gradient in metaphyseal bone occurs (Figure 6). This results in much younger, weaker bone at these sites (Rauch, 2012), and may well be a contributing factor (together with asynchronous muscle:bone development) to the increased incidence of long bone fracture during early puberty (Landin, 1983).

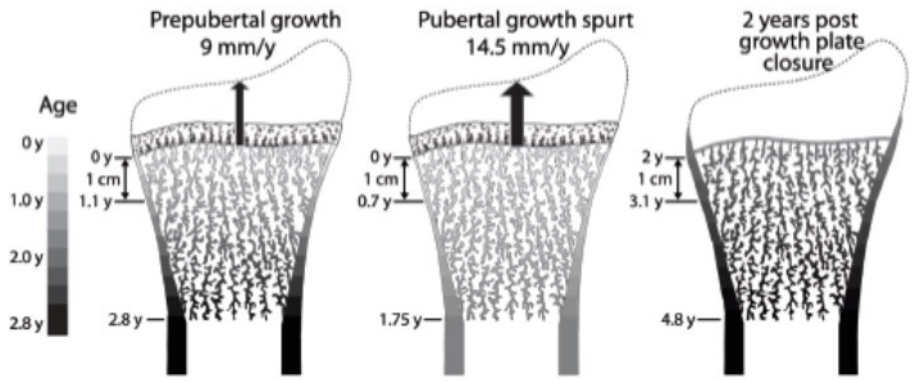


Figure 6. Effect of pubertal growth on age of metaphyseal bone. Age indicates the time since the metaphyseal border with the growth plate has moved from this location. For the sake of simplicity, a constant growth rate was assumed in each case – in the case of the 'post growth' example an abrupt cessation of longitudinal growth was assumed (Rauch, 2012). Figure modified and reproduced with permission.

A peak in bone mass usually occurs in the second or third decade, with age at peak bone mass being site-specific (Matkovic et al., 1994, Lorentzon et al., 2005) – this may be linked to the site-specific age of physeal closure (Cardoso, 2008). Physeal closure is the ossification of the cartilaginous growth plate responsible for increases in bone length, and signals the end of longitudinal bone growth. Thereafter, older age is associated with loss of muscle mass and also strength and power (Runge et al., 2004). Muscle atrophy is a major factor in strength loss, likely primarily resulting from loss of motor units (McNeil et al., 2005). Other neuromuscular factors including changes in specific tension, muscle fibre shortening velocity (Morse et al., 2005), pennation angle (Narici et al., 2003) and tendon stiffness (Reeves, 2006) may also contribute. In addition, there is some evidence that the mechanosensivity and osteogenic response of bone cells to

strain diminishes with age (Kohrt, 2001, Rubin et al., 1992, Klein-Nulend et al., 2002).

Given that the greatest stressor of bone (muscular forces) declines with age and that bone also seems less responsive to mechanical stimuli, it is not surprising that whilst bone size continues to increase slightly with age, more sizeable age-related reductions in BMD suggest that bone strength is reduced in older age (Riggs et al., 2004). Age-related changes in bone strength are driven primarily by increases in osteoclast activity rather than reduction of bone formation by osteoblasts (Kiel et al., 2008). Accordingly, the incidence of osteopenia/osteoporosis (defined by the World Health Organisation as BMD more than one and two-and-a-half standard deviations lower than in 30 year olds) increases with age as does risk of bone fracture (Schuit et al., 2004). However, fracture incidence is strongly related to incidence of falls independent of bone strength (Berry and Miller, 2008) – fall incidence relates also to balance and hence the muscular and tendinous factors mentioned in the previous paragraph as well as *e.g.* age-related decreases in proprioception (Mion et al., 1989).

1.3.2 Sex

Sex also has an effect on bone strength. Whilst sex-dependent differences are not evident at birth (Viljakainen et al., 2010), by 14 months bone mass and size are significantly greater in males (Viljakainen et al., 2011). At adulthood, even

when body size is controlled for males have greater bone strength than females (Riggs et al., 2004). During puberty, extra bone mineral deposition in trabecular bone areas and on the endocortical surface in females (thought to be required to help meet the demands of pregnancy and particularly lactation) results in a higher bone mineral to lean mass ratio than in males (Schiessl et al., 1998). This extra mineral is lost during menopause (Järvinen, 2003), primarily in trabecular bone, leading to high prevalence of early menopausal fractures in these areas. Whilst male bone strength decreases with age, osteoporosis rates are lower than in women (Melton et al., 1998).

1.3.3 Disuse

As habitual strains are a mechanism by which bone strength is regulated, habitual levels of physical activity also affect bone strength. In disuse conditions, bone mass has been shown to decrease markedly. In the extreme example of long-term spinal cord injury patients, lower limb epiphyseal BMD and mass are ~50% lower than that of healthy individuals (Rittweger et al., 2006a, Eser et al., 2004) (Figure 7). In addition, this bone loss is rapid (dependent on the degree of disuse) – and is evident after only 7 days of lower limb suspension (Rittweger et al., 2006b), whereas long-term spaceflight has been shown to result in trabecular BMD loss of >2.5%/month (Lang et al., 2004). These effects are localized to the limb or body segment that is immobilized, with bone in the control leg in unilateral limb suspension studies and upper limbs in spinal cord injury patients remaining

unaffected (Rittweger et al., 2006b, Eser et al., 2004). In addition, bone in the upper limbs seems less responsive to disuse – negative effects on bone mass and muscle size (0.4% and 6.4% respectively) following bed rest being far less pronounced than in the lower limbs (6.0% and 25.6) (Rittweger et al., 2005). Following long-term spaceflight, bone density losses in the lower limbs were 30 times greater than those in the upper limbs, with no significant loss of upper limb lean mass (LeBlanc et al., 2000) - similarly, no bone loss was found in the upper limbs after cast immobilisation (MacIntyre et al., 2001) (although a delayed 1% loss of bone mass was later found). However, in all of these studies upper limb movement was not restricted (unlike that in the lower limbs during bed rest) and so upper limb loading was likely not to differ greatly from habitual levels – hence bone and muscle loss may well have been attenuated.

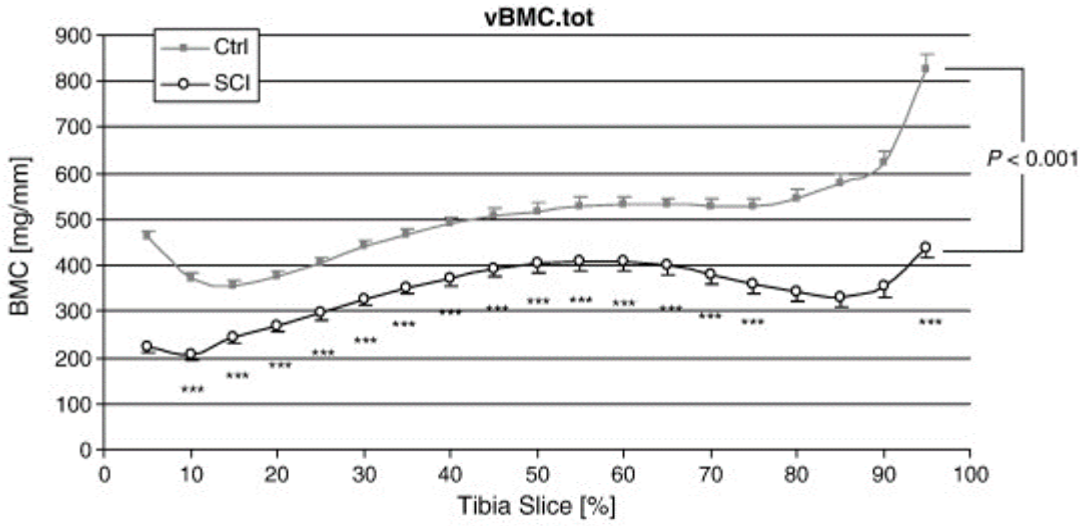


Figure 7. Total tibial bone mineral content (vBMC.tot) in spinal cord injured patients (SCI) and age, height and mass-matched controls (Ctrl). Asterisk indicate significant site*group interactions relative to the 5% site - at * - $P < 0.05$, ** - $P < 0.01$, *** - $P < 0.001$ (Rittweger et al., 2010). Reproduced with permission.

1.3.4 Exercise/physical activity

Increased levels of physical activity (assessed by questionnaire or accelerometry) are associated with greater bone strength in both children (Deere et al., 2012b, Deere et al., 2012c, Deere et al., 2012a, Sayers et al., 2011, Tobias et al., 2007) and older adults (Cooper et al., 1995, Johansson et al., 2015). A number of studies have examined differences in bone strength between athletes (representing prolonged exposure to high physical activity levels) and sedentary controls – with bone strength generally higher in athletes (Heinonen et al., 2002, Nikander et al., 2006, Nikander et al., 2005). However, modality of exercise is important – athletes involved in high-impact sports display far greater bone strength than non-athletic

controls, whereas athletes in low-impact sports have bone strength similar to (or even lower than) controls (Nikander et al., 2005, Fehling et al., 1995, Taaffe et al., 1995). The importance of applied stress and subsequent bone strain is displayed nicely in a study of master athletes, whereby bone strength in athletes in different locomotory disciplines (distance walking, long/middle distance running and sprinting) increased as the locomotory speed (and hence forces applied to the bone) increased (Wilks et al., 2009a) (Figure 8). Whilst comparisons between physically active and sedentary individuals may introduce a self-selection bias, between-limb comparisons of athletes participating in unilateral sports allow assessment of the effects of exercise whilst ensuring that genetic, nutritional and other inter-individual factors do not influence results. Accordingly, gymnasts are found to have greater BMD in their self-selected take-off leg, whereas these differences are not evident in sedentary controls (Wu et al., 1998).

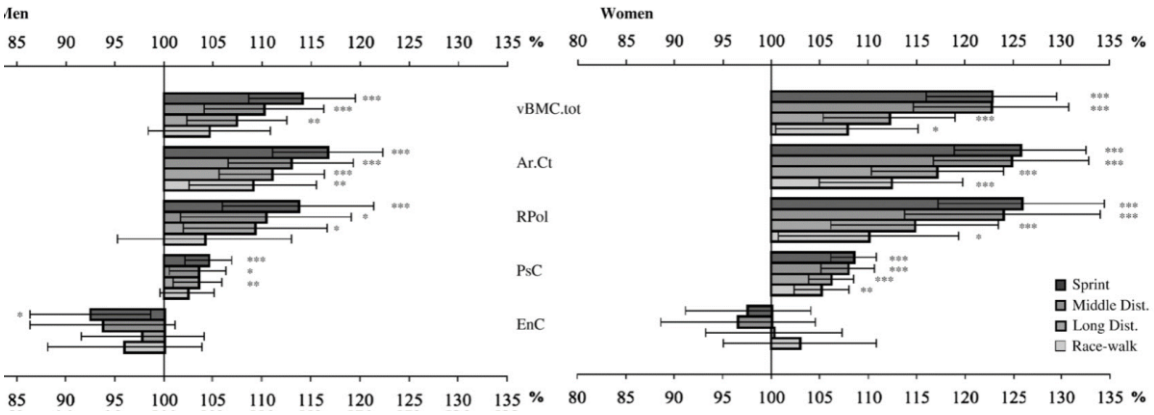


Figure 8. Tibial bone strength indicators at 38% distal-proximal tibia length in male and female master athletes of different disciplines (as percentage relative to age, height, mass and sex-matched sedentary controls). Note increasing advantage in bone strength as speed of events (and hence likely reaction and muscle forces) increases. Asterisks indicate significant difference from control group at * - $P < 0.05$, ** - $P < 0.01$, *** - $P < 0.001$. vBMC.tot – total bone mineral content, Ar.Ct – cortical bone area, RPol – polar moment of resistance, PsC – periosteal circumference, EnC – endocortical circumference. (Wilks et al., 2009a). Reproduced under a Creative Commons Licence.

Whilst the majority of studies investigating effects of exercise and disuse on bone strength focus on the lower limbs, some studies have investigated responses in upper limb bone strength. Upper limb-based studies permit focus on the effects of muscular loading on bone strength, as gravitational loading does not occur regularly at this site. Sedentary individuals display some small side differences in bone strength in favour of their self-selected dominant limb (Haapasalo et al., 2000, Kontulainen et al., 2002) (*e.g.* 2.1%-5.1% in bone mass, and 3.1-3.4% in total/cortical bone area) – likely due to the preference for performing unilateral

everyday tasks with the dominant arm. This is supported by the findings of side differences in bone mass in children developing up until age 6, with side differences then remaining stable through to adolescence (Siminoski et al., 2013). In comparison, large side-to-side differences of up to 27% in bone mass and 67% in bone torsional strength – in favour of the racquet arm - have been found in tennis players (Haapasalo et al., 2000, Kontulainen et al., 2002, Bass et al., 2002) with similar results observed in the humerii of baseball players (Warden et al., 2014, Warden et al., 2009). These adaptations appear to be site-specific and related to the prevailing modality of stress at each site. For instance, at epiphyseal sites bone adapts by increasing by bone area and particularly trabecular density to best resist mainly compressive stress. In comparison, at diaphyseal sites where torsional and bending forces prevail bones experiencing regular exercise are certainly larger although bone density does not differ greatly. Bone geometry is altered; both periosteal and endocortical expansion moving bone away from the centre of bone mass and increasing torsional and bending strength (Haapasalo et al., 2000, Wilks et al., 2009a, Heinonen et al., 2002). Exercise-induced bone gains also seem to be enduring, with good maintenance of side differences in bone strength in tennis players found after 5 years of reduced training (Kontulainen, 1999). Recent work has demonstrated a persisting advantage in bone outer geometry (and hence torsional strength) in retired baseball players in their throwing arm several decades after cessation of regular play (Warden et al., 2014).

Whilst cross-sectional athlete-control studies and investigations of inter-limb differences in athletes suggest a sizeable potential for exercise to improve bone, the results of interventional exercise studies have mainly been unimpressive (Nikander et al., 2010b). In addition, there has been no controlled trial to investigate whether exercise interventions aimed at bone influence fracture risk (Seeman, 2005). Generally, the most positive interventional effects have been observed in growing children – particularly in adolescence (Hind and Burrows, 2007). Compared to controls, high-impact jumping-based interventions have resulted in 5-10% increase in BMD in less than one year (Fuchs et al., 2001, Morris et al., 1997). This has been attributed to the rapid increase in body size and concomitant bone mass accrual thereby representing a 'window of opportunity' to improve bone (Khan et al., 2000, Bass, 2000).

1.4 Factors influencing bone mechanoadaptation

1.4.1 Growth and maturation

Although a number of studies have investigated exercise and physical activity effects on bone in older children and adolescents, the effects of habitual loading in early childhood are little known. Even during foetal development, impaired movement as a result of neuromuscular disease is associated with narrower long bones with thinner cortices although bone length is unaffected (Rodríguez et al., 1988a, Rodríguez et al., 1988b). As discussed earlier, muscle and reaction forces during locomotion are large – for example, even during walking they are estimated at around four times bodyweight (Hardt, 1978). Attainment of independent movement milestones such as standing, walking and running represent the first postnatal exposure of the skeleton to these forces. Radiographic study has shown a peak in bone strength velocity at around 1-2 years *i.e.* around onset of walking which could not be explained by changes in body size (Ruff, 2003) (Figure 9). The substantial increase in bone length during puberty is associated with sizeable bone accrual. However, a far greater stature velocity is evident during early life (Ruff, 2003) and may represent an opportune time for bone strength accrual via loading. Despite this, the effects of early life movement on bone strength remain poorly explored. Walking age was not found to be a predictor of bone strength at 3-5 years old, or explain differences in bone strength between pre and term children (Samra and Specker, 2007). However, there was a large variation in outcome assessment age and small

variation in walking age (standard deviation of 2 months) in this sample which may have limited the ability to detect an effect. Therefore a study investigating bone strength in children of similar age may have greater power to detect effects of early life mobility on bone strength. Such effects would have strong clinical relevance – a number of population groups associated with low bone strength (*e.g.* low birthweight children, and those with Down syndrome) also have delayed attainment of locomotor skills. Therefore any effects of early life movement on bone strength may help explain bone deficits in this group.

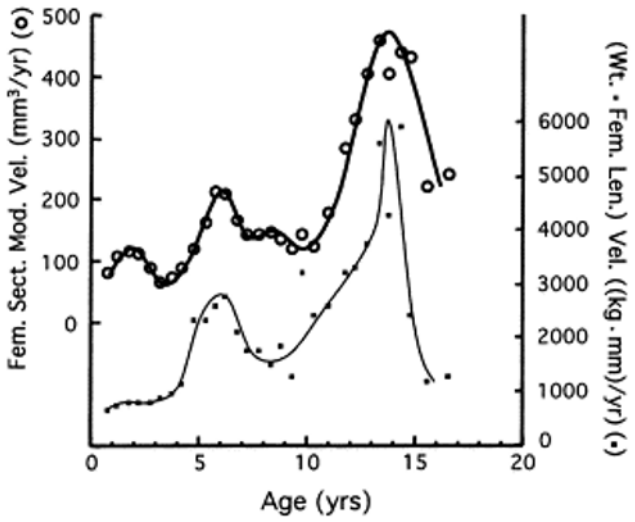


Figure 9. Longitudinal growth in body size (mass * femur length / year, black dots) and bone strength (section modulus / year, open dots) throughout childhood (Ruff, 2003). Reproduced with permission.

In Figure 9 it can be seen that whilst bone strength velocity peaks around 6 and 14 years accompany similar peaks in body size velocity, no such body size peak accompanies bone strength peak around 18-24 months.

1.4.2 Ageing

Muscular forces are the greatest stressor of bone, hence any change in maximal force will influence the capacity of an individual to load the bone during physical activity and exercise. With increasing age, maximal force and power decrease substantially particularly in the lower limbs (Goodpaster et al., 2006). Whilst in the general population this can be attributed – at least in part – to reduced physical activity (Caspersen et al., 2000), substantial age-related loss of muscular output is observed even in master athletes *i.e.* those maintaining a high level of vigorous physical activity (Michaelis et al., 2008, Grassi et al., 1991, Pearson et al., 2002). Reduced mechanosensitivity and osteogenic response to loading in older age (Kohrt, 2001, Rubin et al., 1992, Klein-Nulend et al., 2002) may further impair the ability to improve bone strength in older age but this topic has not been well-explored. Results of high-impact interventional exercise studies in older adults are generally less impressive than those in children; with the most effective studies obtaining results of <2.5% bone area and mass increases in men (Allison et al., 2013) and women (Bailey and Brooke-Wavell, 2010). Few studies compare effects of the same intervention in participants of differing age on bone strength. However, in one such study employing a jumping exercise intervention greater bone mineral

density increases were observed in premenopausal than postmenopausal women despite greater ground reaction forces relative to bodyweight during exercise in the latter (Bassey et al., 1998).

Master athletes display greater bone strength than age-matched peers (Wilks et al., 2009a), although the advantage appears to diminish with increasing age (Wilks et al., 2009b). However, comparison of athletes with sedentary controls may be confounded by a self-selection bias *i.e.* that those who choose to participate in regular sport may have differing nutritional, genetic or other environmental factors. Therefore, observed group differences may not be entirely attributable to effects of exercise. Study of side differences in master tennis players of differing age could help reveal effects of age on the potential of exercise to benefit bone. There is some conjecture that the ability to improve bone strength via exercise may diminish in adulthood (Frost, 2004a, Rittweger, 2008a). It is thought that physeal closure may limit the ability of bone to increase in size in response to increased loading; although current evidence is limited. Greater side differences in bone strength (indicating the exercise advantage) have been observed in adult tennis players who commenced playing tennis during childhood: in comparison with those starting in adulthood (Kontulainen et al., 2002, Kannus et al., 1995). However, in the earlier study DXA was employed for bone imaging which does not permit analysis of bone geometry or analysis of trabecular/cortical bone. In the later study, adult starters were around twenty years older than childhood starters which may have influenced observed results. pQCT study of age-matched tennis players who had begun tennis play in

childhood and adulthood would help elucidate important information of bone's adaptive capacity throughout life. Study of muscle-bone relationships in these groups could also give important information on the role of muscle in exercise-based bone adaptation, and to what extent observed muscle-bone relationships are simply allometric associations.

1.4.3 Sex

Males have greater muscle strength and physical activity than females, which – along with body size – may contribute to greater bone strength. However, increases in muscle strength following exercise are similar in both sexes suggesting no sizeable effect of sex on the ability to increase the loading stimulus to the bone via exercise. Accordingly school-based exercise interventions result in similar bone strength increases in boys and girls (MacKelvie et al., 2004, MacKelvie et al., 2003). However, estrogen is thought to affect bone mechanosensitivity (Sievänen, 2005, Lanyon and Skerry, 2001); hence exercise benefits to bone in postmenopausal women may be less than in males of the same age. In contrast to this proposal, increases in bone strength in older men and women following a hopping intervention were similar (Allison et al., 2013, Bailey and Brooke-Wavell, 2010); however, the small magnitude of changes relative to measurement error may have reduced the ability to detect a gender effect. Study of bone strength in master athletes found no interaction of gender and sports participation (Wilks et al., 2009a) – although self-selection may also confound

these comparisons. Side differences in bone strength in tennis players (*i.e.* controlling for self-selection effects) were similar in young adult male and female (estrogen-replete) players (Ashizawa et al., 1999). Examination of side differences in bone strength in older male and female tennis players would provide interesting information pertaining to the effect of sex on exercise benefits to bone in older age.

1.5 Summary

Bone strength is largely determined by the habitual level of physical activity, and cross-sectional studies suggest an impressive potential for exercise to improve bone strength particularly in childhood. However, the influence of early movement (*e.g.* from onset of walking), and physical activity in later life are unknown. In addition, the influence of muscular action on bone throughout life and the importance of stress modality for bone mechanoadaptation remain poorly explored.

1.6 Aims

Within this thesis, I will provide first or further insight into the following questions:

- Does early life movement *e.g.* walking influence bone strength? Evidence presented in Publication 4.1 *'Time since onset of walking predicts tibial bone strength in early childhood'.*
- In adolescence (when exercise for bone appears most effective), what is the role of muscular action in the response of bone to exercise? Evidence presented in Publication 4.2 *'Upper Limb Muscle-Bone Asymmetries and Bone Adaptation in Elite Tennis Players'.*
- Is the effectiveness of exercise on bone affected by older age, and to what extent is this attributable to changes in muscle size and strength? Evidence presented in Publication 4.3 *'Effects of age and starting age upon side-asymmetry in the arms of veteran tennis players - a cross-sectional study'.*
- How are osteogenic effects of exercise affected by age at starting exercise? Are these effects related to the ability to improve bone density or bone geometry? Evidence presented in Publication 4.3 *'Effects of age and starting age upon side-asymmetry in the arms of veteran tennis players - a cross-sectional study'.*
- Does gender influence the effectiveness of exercise for bone? Evidence presented in Publication 4.2 *'Upper Limb Muscle-Bone Asymmetries and Bone Adaptation in Elite Tennis Players'.* and Publication 4.3 *'Effects of age*

and starting age upon side-asymmetry in the arms of veteran tennis players - a cross-sectional study'.

- To what extent does stress modality (compression, bending or torsion) influence bone adaptation to exercise? Evidence presented in Publication 4.2 *'Upper Limb Muscle-Bone Asymmetries and Bone Adaptation in Elite Tennis Players',* Publication 4.4 *'Greater Tibial Bone Strength In Male Tennis Players than Controls in the Absence of Greater Muscle Output'* and Publication 4.5 *'Tennis Service Stroke Benefits Humerus Bone'*.

2. DISCUSSION

The aim of the work within this thesis was to examine bone mechanoadaptation throughout lifespan, and the influence of muscular action on bone over this period. Bone is highly responsive to habitual loading; and the current prevailing Mechanostat theory (Frost, 2004a) proposes that bone adapts its size and shape to keep the habitual strains it experiences within a certain range. Accordingly, pronounced bone mass loss occurs following reduced periods of physical activity as in *e.g.* bed rest (Rittweger et al., 2005) or spaceflight (LeBlanc et al., 2000). Conversely, previous studies have shown sizeable bone adaptation to long-term increased physical activity via exercise in trained athletes (Haapasalo et al., 2000, Wilks et al., 2009a, Nikander et al., 2010a). However, the 42% and 45% greater bone mass observed in the racquet arm humerus and radius in Publication 4.2 (Ireland et al., 2013a) represent the largest exercise-induced bone adaptations observed in humans, alongside similar recent observations in baseball players (Warden et al., 2014). These effects are over 50% greater than in previous observations, and provide a new benchmark for the osteogenic potential of exercise. They highlight the unrealised potential of exercise interventions in substantially improving bone strength, and hence reducing fracture risk.

Whilst the effects of physical activity on bone strength in older children and adults are well-studied, effects of early life locomotion (crawling, walking, *etc*.) are unknown. These activities represent the first exposure of the lower limb bones to

forces far in excess of body mass. In Publication 4.1 (Ireland et al., 2014c) a strong association between time since exposure to these forces and bone strength was observed during a longitudinal cohort study. These results show for the first time that bone is highly influenced by the habitual loading environment even in early childhood. Whilst early life motor ability is associated with bone strength at the same age, it is unclear whether these effects persist into later life. Therefore we have subsequently investigated relationships between gross motor ability at eighteen months of age, and bone strength at seventeen years in a large, longitudinal cohort (Avon Longitudinal Study of Parents and Children or ALSPAC – results presented at 7th International Conference on Children's Bone Health and manuscript under review). In addition, we are currently investigating links between movement in early childhood and bone strength in elderly adults in other existing cohort studies. In the ALSPAC cohort motor ability was associated with bone strength in adolescence with effects more pronounced in males. Although associations were partly attenuated by adjustment for indirect effects of motor ability via lean mass and physical activity, a number of strong associations remained. This may be supportive of a persisting direct effect of early life physical activity on bone strength – it may be that the high stature velocity in early childhood (Ruff, 2003) represents a fertile period for improving bone strength via loading. This proposal could be investigated via longitudinal follow-up of children investigating early life motor ability and bone strength throughout childhood. In addition, the results have potential clinical significance – a number of pediatric populations such as low birthweight, preterm and Down syndrome children begin

walking later than unaffected peers (Jeng et al., 2008) and also have low bone strength and higher fracture incidence (Baptista et al., 2005, Vyhmeister and Linkhart, 1988). Therefore interventions aimed at hastening onset of independent walking may be effective in attenuating this bone strength deficit – such interventions are effective in improving walking onset age by several months (Wu et al., 2007).

The effects of age on exercise benefits to bone are relatively unexplored. Whilst exercise benefits from the same intervention were greater in pre- than postmenopausal women (Bassey et al., 1998), use of DXA prevented ability to distinguish effects on bone geometry, and on cortical and trabecular bone. Previous work has shown a diminishing advantage in bone strength in athletes (compared to controls) with increasing age (Wilks et al., 2009b). However, such group differences in athletes and controls could be partly attributable to a self-selection bias (*i.e.* that bigger, stronger individuals choose to participate in competitive sport), rather than being solely attributable to effects of exercise. A cross-sectional study of tennis players allowed control of these inter-individual differences – with the exercised racquet arm compared to the non-exercised arm, negating potential effects of *e.g.* genetic or hormonal influences. Therefore, for the first time Publication 4.3 (Ireland et al., 2014b) investigated age effects in an objective exercise model. Results showed that the racquet arm advantage in bone strength in the shaft was negatively associated with age. These age–associated decreases were similar to those observed in muscle strength, suggesting that the

diminished ability to maintain greater bone mass in older age may in part be related to a reduced ability to substantially increase muscle force via exercise. In contrast, bone strength advantage in epiphyseal bone at the wrist was unaffected by age – this is clinically important as the wrist is a common fracture site in older age (Schuit et al., 2004).

There is conjecture that the potential for exercise benefits to bone may diminish at skeletal maturity *i.e.* closure of the physis – with an inability to increase joint size after this point (Frost, 2000, Rittweger, 2008b). However, this has been little investigated so far – one study found less pronounced exercise benefits in older starters than those who had start in childhood, but the groups were not age-matched (Kontulainen et al., 2002), whilst another was based on DXA limiting the ability to discriminate effects on bone density and geometry (Kannus et al., 1995). In Publication 4.3, we separated players who had begun to play in childhood from those in adulthood. Both groups had similar age, height, mass and national ranking, and side differences in bone (indicating the exercise benefit) were significantly more pronounced in childhood starters. In the bone shaft, these group differences were the result of a greater racquet arm advantage in outer bone circumference in young starters. Most pronounced were group differences at the wrist - whilst adult starters had played for nearly 30 years on average they had no racquet arm advantage in bone size. This is in contrast to childhood starters, and youth and adult players in previous publications (Ireland et al., 2013a, Haapasalo et al., 2000). This suggests that the ability of bone to increase its strength through

increasing its cross-sectional area diminishes with age, particularly at the epiphysis. This has implications for the prescription of exercise interventions, and implicates childhood as a key period for improving bone strength via exercise. Recent work showing that enlargement of cross-sectional area is the only exercise advantage to bone which persists decades after cessation of exercise (Warden et al., 2014) further emphasizes the importance of exercise in youth. However, adult starters still had 20% greater bone mass in their racquet arm. This suggests that whilst most adults will reach a peak bone mass in the third decade (Heaney et al., 2000), the skeleton retains substantial capacity for bone mass accrual via exercise or physical activity after this point.

Due to the short levers that muscles work with, muscular forces are the greatest stressor of bones (Rittweger, 2007). Accordingly, strong relationships between bone strength and muscle size and strength have been observed (Schoenau et al., 2002, Ferretti et al., 1998). However, it is not clear to what extent this is an allometric association. Therefore cross-sectional study of muscle and bone size in tennis players allowed investigation of the role of muscle in exercise adaptation in bone. In Publication 4.2, both muscle size/force and bone strength were greater in the racquet arm; supporting an important role of muscle in bone adaptation. However, we also observed distinctly different muscle-bone relationships in the two humerii (racquet and non-racquet). This result – repeated in observations in veteran players in Publication 4.3, highlighted for the first time the limitation of using local muscle size as a surrogate for the muscular forces experienced by the

bone. It may be that non-local muscles influence bone, or that the type of muscular contraction affects the force experienced by the bone – muscle produces much greater force when lengthening than shortening, for example. Alternatively it may be that certain muscles within a cross-section have a greater influence on the local bone. To this end, we proposed and demonstrated a novel method for segmenting muscle groups in pQCT images in Publication 4.2. This permitted investigation of the effects of particular muscle groups on bone and revealed muscle-specific adaptation to tennis play confirmed by MRI study (Sanchis-Moysi et al., 2012). Results from Publication 4.1 also showed a strong association between muscle and bone size at ~15 months of age. However, muscle and bone were not significantly correlated at birth – suggesting that observed muscle-bone relationships in older humans only occur when muscle stresses the bone mechanically via contraction. Muscle-bone assessment is used increasingly in the clinic, and has been demonstrated to distinguish between primary and secondary osteopenias (Schoenau et al., 2002, Crabtree et al., 2004). Hence, these results can inform more accurate classification of osteopenias.

Only a small number of studies have previously examined effects of sex on the osteogenic impact of exercise. Similar benefits to bone in males and females have been observed following similar exercise interventions in adults (Allison et al., 2013, Bailey and Brooke-Wavell, 2010) and children (MacKelvie et al., 2004, MacKelvie et al., 2003). This may be in part attributable to the small magnitude of exercise benefit (particularly in adults) limiting the ability to detect sex effects. Male

and female master athletes were found to have similar advantages in bone strength compared to sedentary controls (Wilks et al., 2009a). However, again the influence of self-selection bias may influence results *i.e.* differences between athletes and controls may be attributable to genetic, nutritional or other factors rather than solely exercise effects. Use of the within-player arm comparison model in tennis players circumvented this problem – in addition, the large magnitude of side difference observed (relative to exercise benefits observed in previous studies) increased the ability to detect sex differences. Men and women in our veteran player study were of similar age, training volume and had started playing tennis at a similar age – suggesting a similar exercise stimulus.

Bone side asymmetries were less pronounced in women, despite similar asymmetries in muscle size and strength; this is supportive of a reduced sensitivity of bone in females to mechanical stimuli. Similar results were also observed in our study of male and female youth tennis players (Publication 4.2). This may have been exacerbated by the high proportion of postmenopausal and premenarcheal women in these cohorts as estrogen is thought to have a positive influence on mechanical sensitivity of bone (Sievänen, 2005). These results suggest that exercise interventions for bone in women may not be highly effective, influencing policy on exercise prescription. However, they also offer a clear route to explore whether hormonal interventions influence the response of bone in females to exercise.

The work submitted in this thesis also offered substantial evidence for the importance of strain modality (*i.e.* compressive, bending or torsional) in bone mechanoadaptation. Whilst the key roles of strain magnitude (Rubin and Lanyon, 1985), rate (Turner et al., 1995) and duration (dynamic *vs*. static) (Lanyon and Rubin, 1984) have been identified, the influence of strain modality on osteogenesis has been little investigated. Our work in tennis players (Publications 4.2 and 4.3) found that the humerus appeared to be highly influenced by torsional strains, as its torsional strength grew more than proportionally as the moment arm for torsional stresses (bone width) increased. Given the important role of muscular force, it is assumed that bouncing movements such as hopping or sprinting are highly osteogenic. This is because they require the muscle to work eccentrically *i.e.* whilst lengthening at a high velocity, and muscle produces greatest force under these conditions. According, exercise effects recorded in athletes in impact sports in both observational (Nikander et al., 2006, Wilks et al., 2009a) and interventional studies (Fuchs et al., 2001) are the largest observed in humans. However, there is evidence in mice that turning movements (thereby producing large torsional stresses) are more osteogenic than linear locomotion (Carlson and Judex, 2007). Tennis players do not regularly complete bouncing movements, but instead perform regular turns. Therefore we compared bone strength in tennis players (representing turning movements) to that of sprinters (exposed to bouncing movements) and controls in Publication 4.4 (Ireland et al., 2015a). In this case-control study, tennis players had ~25% greater bone mass than controls, despite no advantage in muscle size or strength; supporting the osteogenic potential of

movements creating torsional stresses. In addition, as large muscle forces are not required to stimulate bone in these exercises, such activities may be more effective than conventional interventions in groups with poor muscle strength or coordination (*e.g.* frail elderly, children and adults with movement disorders, *etc*.). Recent studies have used modelling (Martelli et al., 2014) or *in vivo* (Yang et al., 2015, Yang et al., 2014b) techniques to estimate or measure strain at certain bone sites during different activities. These techniques could be employed to investigate a range of activities to identify those most likely to result in large osteogenic stimulus to the bone at clinically-relevant sites *e.g.* by generating large torsional stresses. One movement which should be explored is the tennis serve, or similar derivatives of that movement. It is well known that regular tennis play is associated with pronounced asymmetries in upper limb bone strength (Jones et al., 1977, Haapasalo et al., 2000, Ireland et al., 2013a). However, the sport consists of a number of strokes (forehand, backhand, serve, *etc.*) and the contribution of each stroke to bone adaptation was previously unknown. Results from our veteran player case-control study Publication 4.5 (Ireland et al., 2015b) suggest that the service stroke is the catalyst for the sizeable hypertrophy observed in the humerus. It is likely to be impractical for patient groups *e.g.* frail elderly to play tennis. However, exercise interventions based on the serve movement will likely be more achievable and could form effective interventions in improving upper limb bone strength.

The invited review articles offer novel summaries of the current state of their respective fields. Publication 4.6 (Ireland et al., 2014a) explained for the first time how different aspects of muscle and bone properties *e.g.* anthropometric, mechanical, dynamometric, *etc.* can be imaged and compared as muscle-bone indices. This is valuable given the increased use of muscle-bone indices in assessing osteopenias, in addition to the research applications of these analyses. In addition, a novel summary of current applications of muscle-bone indices in clinical practice, and clinical and basic research was completed. Furthermore, Publication 4.7 (Ireland et al., 2013b) offered an overview of the mechanical influence of muscular action on bone during exercise. In addition, evidence for the effects of exercise on bone via controlled intervention was summarised. Finally, factors relevant for clinical practice *e.g.* compliance, other factors influencing fracture incidence, nutrition, *etc.* were considered. This summary forms a novel and useful resource for clinicians planning exercise-based interventions in patients.

Limitations

There are a number of limitations to these studies. With the exception of the study in young children, data were collected at a single time point hence causal inference is limited. In addition, examination of age effects may be influenced by secular changes in *e.g.* diet or body size. However, use of the side difference model in the tennis player studies should have minimized this influence, and cross-sectional

and longitudinal examination of bone strength in ageing within the same participants have previously shown similar results (Lauretani et al., 2008). Similarly, the tennis player model prevents intra-individual confounding effects of genetic, nutritional or other lifestyle factors inherent in previous studies examining bone strength in athletes and controls (Wilks et al., 2009b, Wilks et al., 2009a, Nikander et al., 2006, Nikander et al., 2005). However, differences between tennis players, sprinters and controls may be (at least in part) attributable to these factors rather than solely effects of regular sport participation. The tennis player arm studies did not employ a control group – hence we are unable to say definitively that exercise in these groups results in significantly greater bone strength asymmetries than in non-players. However, asymmetries in both youth and old players were greater than in previous studies in which significant differences in asymmetry were observed between tennis players and controls (Haapasalo et al., 2000). In addition, focus of these studies was not on the magnitude of side difference *per se*, but the effects of age, gender, starting age and muscle-bone relationships. Another strength of the side difference model is that these athletes will train much harder (in terms of training intensity and volume) than participants in randomized clinical studies will likely ever do. Therefore they give an indication of the maximal mechanoadaptive capacity of the bone. However, this also limits the ability to generalise these results to the wider population.

Whilst a number of the articles made reference to high magnitude of bone loading and/or to loading modality (*e.g.* compression, bending and torsion), loading was

not measured during activities. This is common amongst such studies in bone, and future studies should employ biomechanical or modelling approaches to assess loading during physical activity or exercise. Nutrition and hormonal factors were not thoroughly investigated – although menopausal status and use of hormone therapy were adjusted for in master tennis player analysis and found not to influence results substantially. There are no current studies investigating nutritional intake in master athletes – such work would be valuable in understanding potential for differences in diet to confound athlete-control studies.

Conclusions

These studies offer new information in a variety of research areas pertaining to bone mechanoadaptation and the influence of muscular action on bone throughout lifespan. A number of these findings *e.g.* links between early life movement and bone, and the importance of torsion for bone mechanoadaptation offer clear routes for future investigations – some of which we are already beginning to explore. Results of the infant walking and tennis player studies highlight the potential for exercise and physical activity to improve bone strength throughout life, although this potential has largely been unfulfilled by current interventional studies.

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