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Perspective Article

Hylonome

The effect of exercise and nutrition on the mechanostat

S.L. Bass, P. Eser, R. Daly

Centre for Physical Activity and Nutrition Research, Deakin University, Burwood, Australia

Abstract

In this review, we discuss the effect of increased and decreased loading and nutrition deficiency on muscle and bone mass and strength (and bone length and architecture) independently and combined. Both exercise and nutrition are integral components of the mechanostat model but both have distinctly different roles. Mechanical strain imparted by muscle action is responsible for the development of the external size and shape of the bone and subsequently the bone strength. In contrast, immobilization during growth results in reduced growth in bone length and a loss of bone strength due to large losses in bone mass (a result of endosteal resorption in cortical bone and trabecular thinning) and changes in geometry (bone shafts do not develop their characteristic shape but rather develop a rounded default shape). The use of surrogate measures for peak muscle forces acting on bone (muscle strength, size, or mass) limits our ability to confirm a cause-and-effect relationship between peak muscle force acting on bone and changes in bone strength. However, the examples presented in this review support the notion that under adequate nutrition, exercise has the potential to increase peak muscle forces acting on bone and thus can lead to a proportional increase in bone strength. In contrast, nutrition alone does not influence muscle or bone in a dose-dependent manner. Muscle and bone are only influenced when there is nutritional deficiency – and in this case the effect is profound. Similar to immobilization, the immediate effect of malnutrition is a reduction in longitudinal growth. More specifically, protein and energy malnutrition results in massive bone loss due to endosteal resorption in cortical bone and trabecular thinning. Unlike loading however, there is indirect evidence that severe malnutrition when associated with menstrual dysfunction can shift the mechanostat set point upward, thus leading to less bone accrual for a given amount of bone strain.

Keywords: Mechanostat, Immobilization, Loading, Energy, Protein

Introduction: The effect of loading and nutrition on the mechanostat

The development of muscle and bone during growth is influenced by forces associated with gravity and physical activity[1-3]. It is the muscle forces that create the peak forces acting on bone. These forces are generally greater than the external forces acting on the body (e.g., ground reaction forces) because of the body's poor muscle leverage. Thus growth in the presence of unloading results in both a muscle that lacks functional capacity, and a bone that lacks the specific shape that is unique for its function[4]. This intrinsic relationship between muscle and bone is encapsulated by the mechanostat theory, which postulates that increasing maximal muscle force during growth or in response to increased loading will affect bone mass, size and strength predictably and correspondingly[4]. Similarly, unloading (disuse or immobilization) will lead to reduced muscle development (and muscle force) and invariably have a negative effect on the mass, size, and strength of bone.

The proper functioning of the mechanostat depends on the normal state of all its cells (osteocytes, -blasts and -clasts), the customary mechanical usage of the skeleton, and the endocrine-metabolic environment[5]. In the normal "healthy" situation, the mechanostat postulates that bone strength is adapted to keep typical peak strains within a safe physiological range to prevent microdamage and fracture, and to optimize bone structure to best suit its functional needs. The fine tuning of the mechanostat is achieved by physiological set points that act as thresholds for the initiation or inhibition of bone modelling and remodelling.

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Corresponding author: Associate Professor Shona Bass, Centre of Physical Activity and Nutrition Research, Deakin University, 221 Burwood Hwy, Burwood Australia 3125
E-mail: shonab@deakin.edu.au

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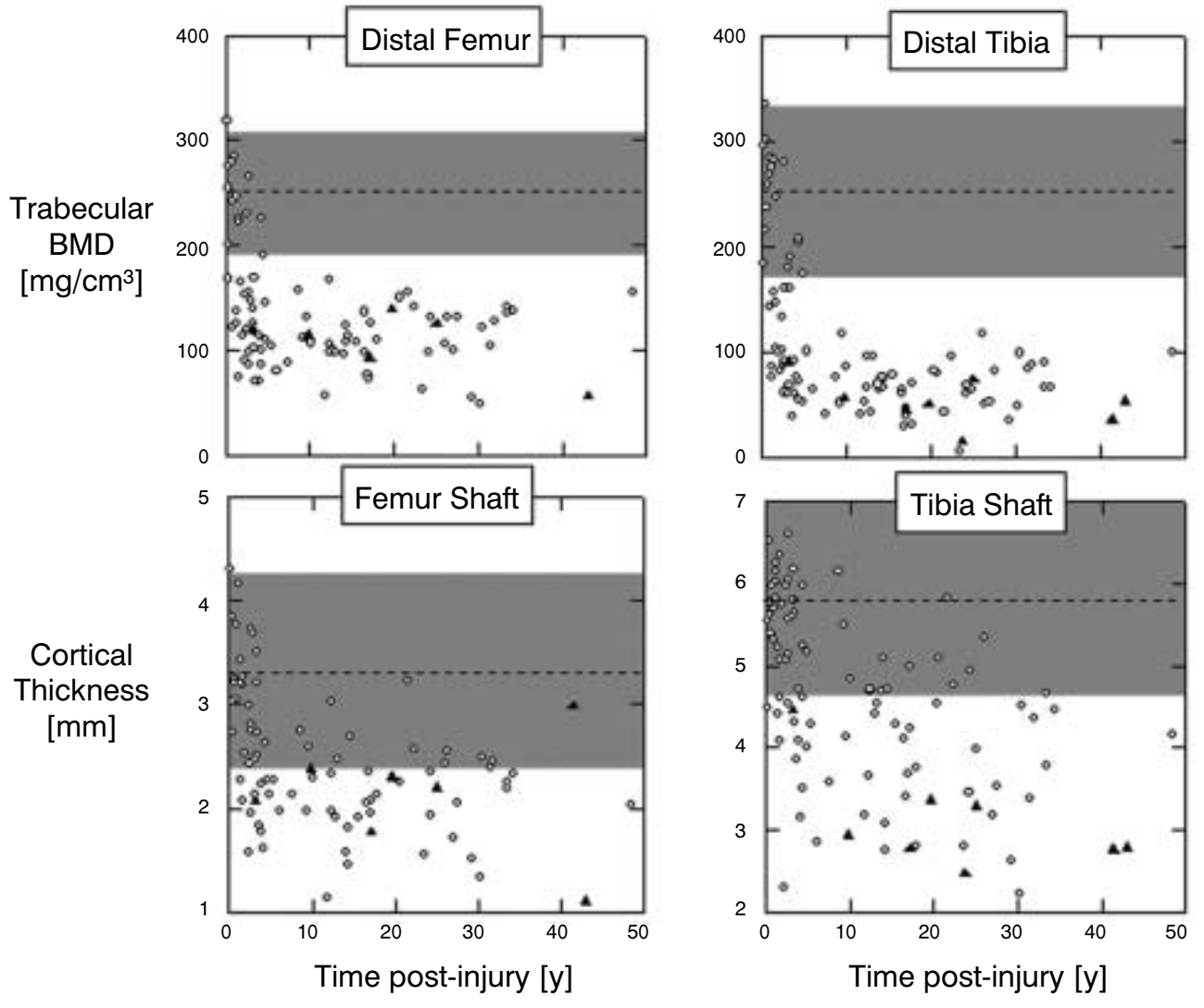

Figure 1. Bone loss with time after spinal cord injury measured cross-sectionally in a population of 99 subjects. Trabecular vBMD of the distal epiphyses of the femur and the tibia (top), and cortical thickness of the femur and tibia shaft (bottom). The shaded area shows the mean ± 2 standard deviations of a control group including 25 able-bodied persons. Circles depict subjects with SCI at age >18 years and filled triangles depict subjects with SCI at age < 18 years [Adapted from Eser et al.[19]].

Mechanostat set points are genetically determined but are regulated by the endocrine environment. For instance, it is proposed that reduced estrogen concentrations increase the set points for bone modelling and remodelling. Therefore, a deformation of 1,000 με may induce bone formation in an estrogen replete but not in an estrogen deplete state. Thus, it is proposed that it is the interaction of the endocrine environment with bone cell function that affects the sensitivity with which bone adapts its mass, geometry, or structural properties to bone deformations caused by loading[5].

Exercise and nutrition are key environmental factors known to affect muscle and bone development. Exercise acts directly through muscle action and indirectly through endocrine regulation; during growth exercise is thought to influence bone modelling and thus bone geometry. The role of exercise in the mechanostat has been extensively discussed, however, little is known about the action of nutrition in regulating the mechanostat. Nutrition acts indirectly through endocrine factors that act on muscle and bone metabolism (modelling and remodelling). Nutritional deficiency has the most profound effect on the mechanostat; not only through the large losses in muscle and body mass but also through the associated hormonal imbalances. These hormonal imbalances have been hypothesized to alter the mechanostat set points. Adequate nutrition is also critical for the optimal expression of the genetic template for bone length, which may interact (via lever arms) to influence the loads imparted to bones.

Proponents of the mechanostat maintain that the peak forces imparted by muscles drive the attainment of bone strength. Is this the case, however, for all levels of exercise and nutrition? Are there some human scenarios where the level of exercise and/or nutrition challenge the integrity of the mechanostat? In this review, we discuss the effect of increased and decreased loading and nutrition deficiency on muscle and bone mass and strength (and bone length and architecture) independently and combined. We hypothesize that as long as nutrition is sufficient to allow anabolic conditions and replete hormone levels, the effect of exercise (in terms of peak forces acting on bone, not training volume) on bone strength will be proportional. We also hypothesize that hormonal imbalances associated with nutritional deficiency has the potential to alter the mechanostat set points. To address this, we present several scenarios to elucidate the effect of exercise and nutrition deficiency on muscle and bone and the mechanostat. The first scenario focuses on the effect of immobilization and additional loading in the presence of adequate nutrition. In the second scenario, we investigate the effect of inadequate nutrition with normal loading and additional loading.

The effect of immobilization and loading on the muscle-bone relationship

According to Frost's mechanostat theory, the switches to turn bone modelling and remodelling on and off are regulated by bone deformation. Mechanical forces are needed to deform bone, and these forces are predominantly created by muscle contractions, and in weight-bearing bones, gravitational forces associated with body weight are added. During growth, bones are continually challenged to adapt to increases in bone length and muscle force. Longitudinal growth increases lever arms and bending moments, which create greater loads on bone[6]. Body weight also increases and muscle forces parallel these changes in weight in order to allow effective movement. Thus, growing bone has to continually adjust its strength to keep strains (bone deformation) within the threshold range for modelling and remodelling.

The magnitude of deformation is determined by the characteristics of the deformed object (e.g., material properties, size, architecture) and the force acting on it (mass times acceleration). Exercise training can increase muscle force and subsequently subject the skeleton to higher loads. Exercise may also increase muscle mass, thus further increasing gravitational forces acting on the weight-bearing bones. While it is recognised that bone deformation is the critical input driving the mechanostat, strain magnitude or rate are seldom measured directly (due to the invasive nature of such measurements)[7]. Instead, several surrogates are used to estimate the forces acting on bone. Maximal muscle force, which is often measured statically (isometrically), is often used as a surrogate for the maximal forces exerted on bone. However, statically measured muscle forces are always much smaller than the maximal dynamic forces encountered in real every- day movements or exercise. Thus, measuring external muscle forces provides only a surrogate for the actual internal maximal forces acting on bones. Others have taken this one step further and used muscle mass or cross-sectional area as a *surrogate* for muscle force; in this case it is a *surrogate* (i.e., muscle mass or area) being used as an estimate of another surrogate (external muscle force) for the actual measure of maximal forces likely to be acting on bone[8-12]. While muscle mass and size correlate well with isometric and isokinetic muscle force[13-15], and can consequently be used as a surrogate for muscle force, there are other contributing factors, such as fiber type, fiber angle, and muscle lever arm length that contribute to the development of muscle force[16].

In the absence of studies that have tested the real essence of the mechanostat theory, we present an overview of studies that have investigated the influence of muscle on bone by using surrogate measures for bone deforming forces. We present both ranges of the spectrum, reduced or absent muscle forces as well as increased muscle forces as a result of exercise training.

Bone loss as a response to immobilization

Bone loss associated with immobilization has been identified in several conditions such as spinal cord injury (SCI), stroke, peripheral nerve damage, space flight, bed rest, and hind limb unloading in animal studies. Amongst these conditions, the pattern of bone loss as a consequence of SCI and hind limb unloading in animals has been studied in most detail. The reason for choosing SCI as a study population is the large number of available subjects and the high degree of immobilization in the studied populations with little variation between subjects. From a scientific perspective, the population of SCI individuals with a motor complete paralysis provide a model that can be equally well controlled as an animal model using hind limb unloading. It is thus not surprising that similar findings have been derived from the two models.

In SCI individuals, muscle forces are completely absent in the first weeks to months during the spinal shock phase. Thereafter, involutional muscle contractions in the form of spasms return in those patients with a lesion above T12, restoring at least some minimal loading to the lower extremities. In the following paragraphs, we present data from a study on adult individuals with SCI. There are very few studies investigating pediatric SCI or other immobilizing conditions on the growing skeleton. In addition to the typical bone atrophy observed during immobilization in adults, the growing skeleton is also at risk of scoliosis, subluxation of the hip, heterotopic ossifications and hypotrophy when appropriate mechanical loading is missing[17,18].

We have recently published a cross-sectional study on 89 individuals with motor complete SCI[19]. The results from

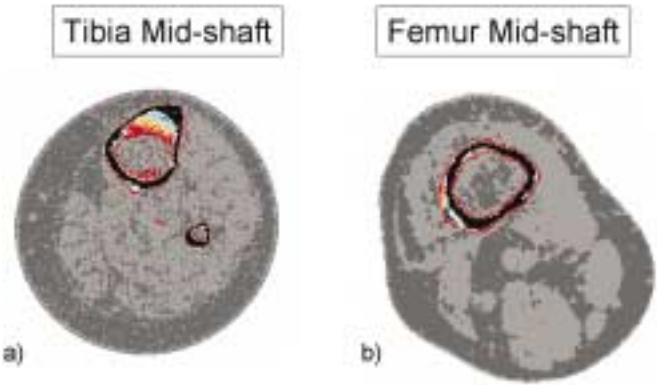

Figure 2. Typical examples of bone loss at the shaft in pQCT scans at the mid-shaft of the tibia (a) and femur (b) in two males with SCI both at 3 years post-injury. The predominant process of bone loss found at the bone shaft was endosteal resorption (a), but sometimes, periosteal resorption (b) was also detected. The black colour represents cortical bone. Blue and red represents bone of lower vBMD, indicating that cortical bone is more porous and probably in the process of resorption. Muscle and connective tissue are shown in light grey, while fat is shown in dark grey.

Figure 3. Change in bone shape at the shaft after long-term SCI. Peripheral QCT scan at the lower leg (38 % proximal from distal end of tibia) of a 24-year-old able-bodied active male (left), and a 24 year old male with paraplegia since age 14 (right).

this study show a pattern of bone loss very similar to the animal studies using hind limb unloading[20], where there is a rapid decrease in volumetric bone mineral density (vBMD) of the trabecular bone compartments, and a decrease in cortical thickness due to endocortical resorption. We have found a loss in bone mass of approximately 50% at the epiphyses of the femur and tibia during the first 5 years after injury[19]. Thereafter, bone mass appears to remain stable. Loss of bone mass is smaller in the femur and tibia shafts, where endosteal resorption decreases the cortical thickness at a rate of approximately 0.25 mm per year during the first few years. This corresponds to a loss in shaft bone mass of approximately 30% during the first 7 years after injury. Figure 1 shows the decrease in trabecular vBMD of the epiphyses and cortical thickness with time after injury. In the (almost) complete absence of loading, the epiphyses become almost devoid of trabeculae and the shaft cortical thickness can be reduced down to 1 mm in the femur and 2 mm in the tibia (Figure 1). Subjects who sustained their injury during adolescence are marked with different symbols. Those with pediatric SCI are amongst subjects with the poorest bone status in the tibia, but not in the femur (where spasticity preserves some bone mass). In the distal tibia, trabecular vBMD was lower than 20 mg/cm^3 in some subjects, which means that the central part of the epiphysis was filled with predominantly fat marrow (fat and red marrow have a vBMD of approximately 0 and 60 mg/cm^3, respectively). No changes in size or shape were found at the epiphyses. More absolute as well as relative mass was lost at the epiphyseal sites compared to the diaphyseal sites. The reason for this remains an interesting topic for further research.

Figure 2a shows an example of endosteal resorption associated with SCI, which is the bone loss process typically found at the femur and tibia shaft in the first few years after SCI. In rare cases (Figure 2b), increased periosteal porosity (which is likely to be a precursor of resorption) may also occur. In individuals with long-standing SCI, the sharp outer contours of the tibia shaft were often lost so that the bone shape was round rather than triangular. This was particularly evident when the SCI was acquired during adolescence (Figure 3).

In order to assess whether the reduced muscle activity that is present in subjects with involuntary muscle spasms is effective at reducing this large loss in bone mass, we have correlated the bone status found in 48 chronic (time post-injury >5 years) patients with the clinically measured degree of spasticity[21]. We found that in the thigh, a significant correlation exists between the measured spasticity and trabecular vBMD at the distal femur ($r=0.35$, $p=0.01$) and cortical thickness at the shaft ($r=0.42$, $p=0.003$). This indicates that subjects with stronger muscle spasms had less atrophied bones than those with weaker or absent spasms. This finding suggests that even in the state of almost complete disuse, muscle forces control bone strength in a proportional manner. Our findings are consistent with the results of earlier studies which have found a correlation between bone status and completeness of motor paralysis[21-23], however, in our study all subjects had a complete motor paralysis.

In SCI individuals, extensive muscle loss is initiated shortly after the injury, and most of this loss is complete within a few months. Thereafter, fat mass often continues to increase[24]. Hence it could be argued that bone loss is purely caused by a decrease in muscle mass, force, and activity.

Figure 4 shows the relationship between muscle cross-sectional area (CSA) and bone mass at the femur and tibia epiphyses as well as between muscle CSA and cortical thickness at the shaft of the femur and tibia. From these data, it is apparent that the linear relationship found in the able-bodied population is maintained at lower levels in most of the spastic subjects, and in the femur also in the flaccid subjects. In the tibia, it appears that some paralysed subjects have lost less bone mass relative to the loss in muscle CSA. Additionally, in the flaccid subjects muscle is completely denervated and inactive, which would suggest that they should lose even more bone for a given muscle CSA. Instead, rather the opposite is found in the tibia of the flaccid subjects. Despite the fact that the number of flaccid subjects included in this study was small, they do not confirm that the mechanostat is maintained down to the most extreme forms of disuse, such as denervation.

In the tibia shaft, the variability in cortical thickness at a given calf muscle CSA is much larger than the same comparison in the thigh, implying that other factors gain importance in controlling bone loss at the tibial shaft. Other factors appearing secondary to paralysis have been suggested to be responsible for bone loss, such as neurological and vascular changes[25]. Diameter and blood flow of the femoral artery was reduced by over 25% within 6 weeks after SCI[26].

This vascular atrophy has been found to parallel the atrophy in muscle mass[27]. The decrease of arterial wall diameter and blood flow in the paralysed legs at a concomitantly unchanged blood pressure indicates that leg vascular resistance is increased after SCI[28]. Because shear forces at the arterial wall are increased when arterial diameter is reduced, flow-mediated dilation after venous occlusion was found to be greater in subjects with chronic SCI than able-bodied subjects[29]. Vasorelaxants such as nitric oxide (NO) are responsible for flow-mediated dilation. There is a large body of evidence on the importance of NO in bone turnover processes, whether it be fracture healing[30], bone resorption[31], or bone formation[32]. The origin of systemically measured NO in the different *in vivo* animal studies can not be determined with any confidence since several cell types release NO (such as endothelial cells, osteocytes and osteoblasts). Despite the unknown origin, it could be hypothesized that endothelial NO release of blood vessels by increased shear forces caused by a small vessel diameter may have a role in maintaining bone status at a higher level than expected from muscle status in flaccid patients.

Nutrition has received little attention in immobilization-induced bone loss of SCI individuals. Because of hypercalciuria[33,34], calcium is not supplemented in the early phase after SCI. However, most patients in primary rehabilitation do not receive any special diet. In terms of hormonal status, the situation is more complicated. Hind limb unloading in animal studies has been found to cause hypogonadism[35]. Depending on the lesion level, hypogonadism in men may occur[36]. Women often become dysmenorrheic as a response to SCI, however, they often resume menses within one year. Hypogonadism cannot be excluded to play a role in the rapid and extensive bone loss after SCI, however, extensive bone loss was found in all male and female SCI individuals with a motor complete lesion, while hypogonadism was only found in some[37].

The effect of loading on the muscle-bone relationship

Consistent with the unloading model, Frost's mechanostat theory also predicts that increased loading in the presence of adequate nutrition and hormonal status will enhance muscle mass, size, and strength which should impart greater forces on the tendon-bone junction leading to a positive skeletal response. It is difficult, however, to test whether the osteogenic stimuli created by increased forces acting on bones due to larger, stronger or more powerful muscles lead to a proportional increment in bone because a number of factors are known to influence both muscle and bone development. This includes common genes regulating both muscle and bone size, and external or intrinsic stimuli such as nutritional or hormonal factors[38,39]. While there are cross-sectional studies showing that muscle and bone are highly correlated and that young athletes have both greater muscle and bone mass than controls[10,40,41], this does little to prove causation. Similarly, there have been few longitudinal exercise trials which have specifically shown that an increase in muscle size and strength translates to a proportional increment in bone size, mass and strength.

Data from a case report of a 26-year-old healthy, physically active woman who sustained an anterior cruciate ligament injury revealed that the loss of muscle strength preceded the decline in bone mass. Similarly, muscle strength recovered prior to bone mass following high intensity training[42] (Figure 5). In pre-menarcheal girls, site-specific associations between gains in lean mass and bone accrual measured by dual-energy X-ray absorptiometry (DXA) have been reported following a 10-month high-impact, resistance training intervention[43]. The authors speculated that the increased rate of bone accrual may have been due to the higher mechanical loading generated by the greater lean mass, but it only accounted for, on average, 20% of the variance in bone mineral acquisition. Two additional resistance training studies in adolescent girls reported little change in either DXA-derived lean mass or bone mass following the intervention, despite large increases in muscle strength[44,45]. However, interpretation of these data is difficult because of the short duration of the intervention[44], the high rate of attrition[45], and the use of lean mass as a surrogate for the maximal force producing capacity of muscle. Furthermore, changes in the fat to lean mass ratio as a result of training and change in pubertal status may produce additional errors in DXA-derived changes in bone density. The paucity of data supporting a strong association between changes in muscle and bone in response to increased loading could also

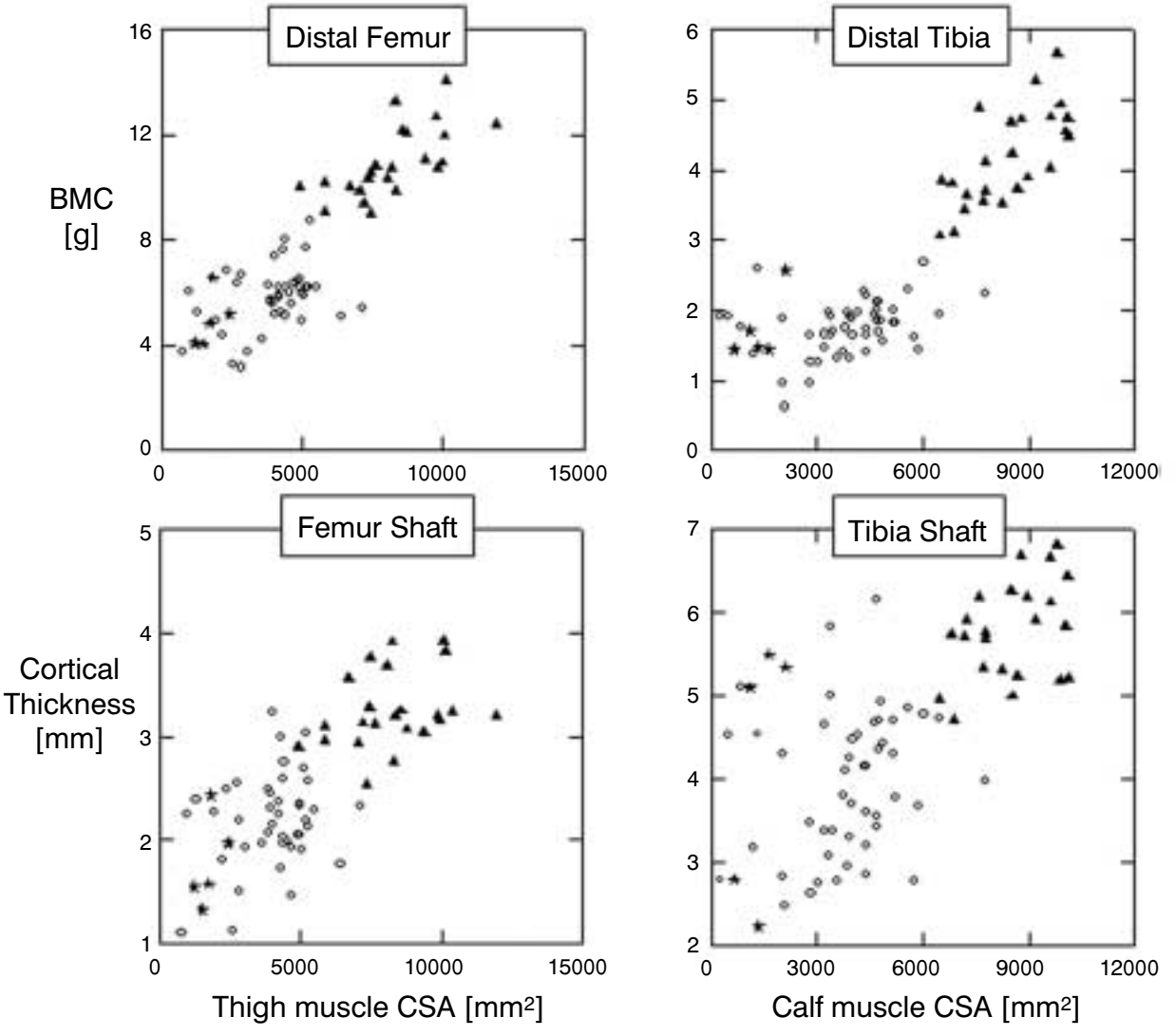

Figure 4. Muscle-bone relationship in able-bodied and SCI individuals. There is a closer relationship of muscle cross-sectional area of the thigh (left) and calf (right) with BMC at the distal epiphyses (top), than with cortical shaft thickness (bottom). Filled triangles depict able-bodied subjects, circles show subjects with a spastic paralysis (lesion at or above T12), and filled stars subjects with a flaccid paralysis (lesion at or below L1). Note that particularly in the tibia, subjects with a flaccid paralysis deviate from the linear muscle-bone relationship in that they appear to lose less bone mass than what would be expected from their muscle loss and level of inactivity [Adapted from Eser et al.[21]].

be attributed to differences in the time course for adaptation of muscle and bone to training. For instance, changes in muscle (strength and mass) can occur as early as 4 to 6 weeks in response to increased loading (e.g., resistance training), whereas skeletal adaptations to loading take much longer. This is because the typical bone remodelling cycle (bone resorption, formation and mineralization) takes 3-4 months, and thus a new steady state that is measurable may not be attained for 6-8 months.

Unilateral sports, such tennis and squash, provide a unique model to examine the effects of loading on muscle and bone because any differences between the playing and non-playing arm are independent of the effects of genes, nutrition and hormones. We recently reported that in pre-, peri-, and post- pubertal female tennis players, muscle and bone traits measured by magnetic resonance imaging were significantly greater (6 to 13%) in the playing than non-playing arm[8]. However, the side-to-side differences in muscle area only accounted for approximately 14% of the variance of the differences in the bone traits (Figure 6). This suggests that muscle size alone was not a good indicator of the strains (deformation) on bones that stimulated an adaptive skeletal response. It is likely that the greater bone size and strength in the playing arm was associated with increased forces at the tennis racket-hand interface associated with the high speed acceleration and deceleration with the racket-ball impact.

Consistent with these findings, there are data showing that high level female volley-ballers have greater bone mass than controls, despite comparable lean/muscle mass[12]. This indicates that training for some young athletes may lead to neuromuscular adaptations and/or improvements in the intrinsic force production capacity of muscle that influences muscle strength (and thus force development) independent of muscle size[46,47]. It has also been suggested that external mechanical loads applied through weight-bearing activities are necessary to create sufficient muscular forces to stimulate an adaptive skeletal response. For instance, in swimmers and cyclists, the mechanical loading from muscle pull at insertion sites appears to be ineffective at enhancing bone accrual[48-50], and astronauts typically experience a reduction in bone mass, despite physical training[51]. In swimming, forces acting on bones are small because accelerations are small and the accelerated mass is less than body weight. Clearly, the large forces imparted to the lower limbs during the landing phase of volleyball (due to the large eccentric forces developed during deceleration) are much greater than during a revolution in cycling.

Despite the strong biomechanical link between muscle and bone, there remain many unanswered questions regarding the influence of loading on the muscle-bone relationship, particularly during growth. For instance, it is uncertain whether skeletal adaptations to increased loading during growth relate directly to the magnitude of the load from muscle pull or some other aspects of muscle contraction (e.g., rate of force development). The results of animal studies indicate that the rate of loading may be more important than the magnitude in stimulating an osteogenic response, but in these experiments the bone is typically loaded directly rather than through the action of muscle pulling on bone at the site of attachment. These results have not been verified in humans because it is difficult to isolate strain magnitude from strain rate because large strain rates are usually combined with high magnitude loads. However, it has been consistently reported that athletes that experience strains which are high in magnitude and rate have very high BMD (DXA) (e.g., sprinting, triple jump, gymnastics, volleyball)[52,53]. Conversely, endurance athletes (e.g., middle distance runners) who typically experience strains which are low in magnitude and rate are often reported to have low BMD[52,53]. The lower BMD reported in endurance athletes may also be due in part to low body weight and menstrual disturbances.

Future studies examining the influence of growth and/or loading on the muscle-bone relationship need to consider specific muscle properties which contribute to the force (and power) producing capacity of muscles. Similarly, further research is needed to determine the relevant bone traits (mass, geometry, material or microstructural properties) most likely influenced by exercise-induced changes in muscle. This is important because small changes in bone size or shape due to increased loading can lead to large changes in bone strength, sometimes independent of changes in bone

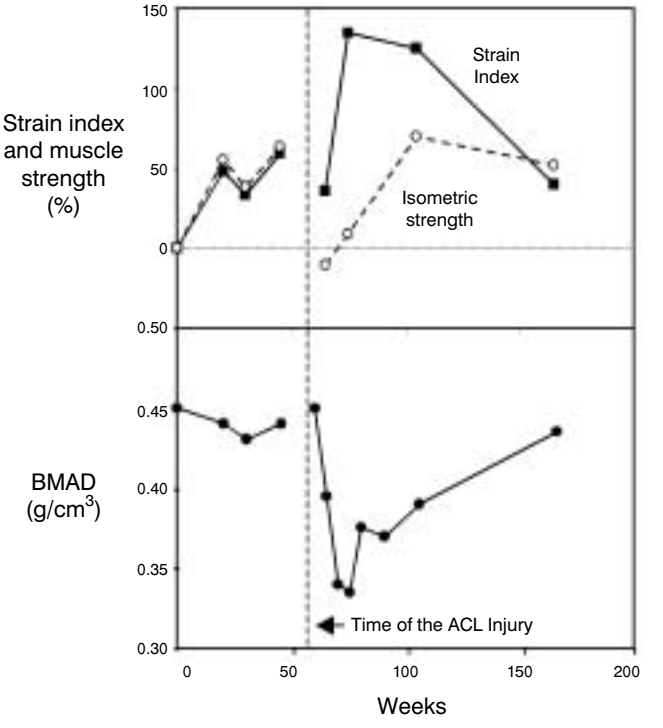

Figure 5. Percentage changes in the strain index in the left patella and isometric extension strength of the lower limb (upper panel) and changes in BMAD of the left (injured) patella (lower panel) over a 3-year training program before and after the anterior cruciate ligament injury at 57 weeks. Patellar strain index is defined as the product of the maximal isometric strength during lower limb extension and the patello-femoral contact area, normalized to bone mineral density [Adapted from Sievanen et al.[42]].

mass. An important area that also requires further investigation is the muscle-tendon-bone relationship. Little is known about whether increased loading during growth leads to changes in tendon properties (e.g., stiffness, length, thickness, strength) that may alter the force-length relation of muscle, independent of change in muscle size or neuromuscular activity. Finally, given the important action of hormones in regulating the mechanostat, investigation of the interaction between loading, hormones (growth hormone [GH], insulin-like growth factor I [IGF-I], testosterone and estrogen) and the muscle-bone relationship is warranted.

Summary

Growth in length and increased muscle mass (and strength) and body mass (only important for weight-bearing bones) all add to the maximal forces to which bones adapt their structure and strength. Exercise has the potential to further increase peak muscle forces acting on bones, which leads to a proportional adaptation of bone strength (predominantly due to periosteal apposition and increase in tra-

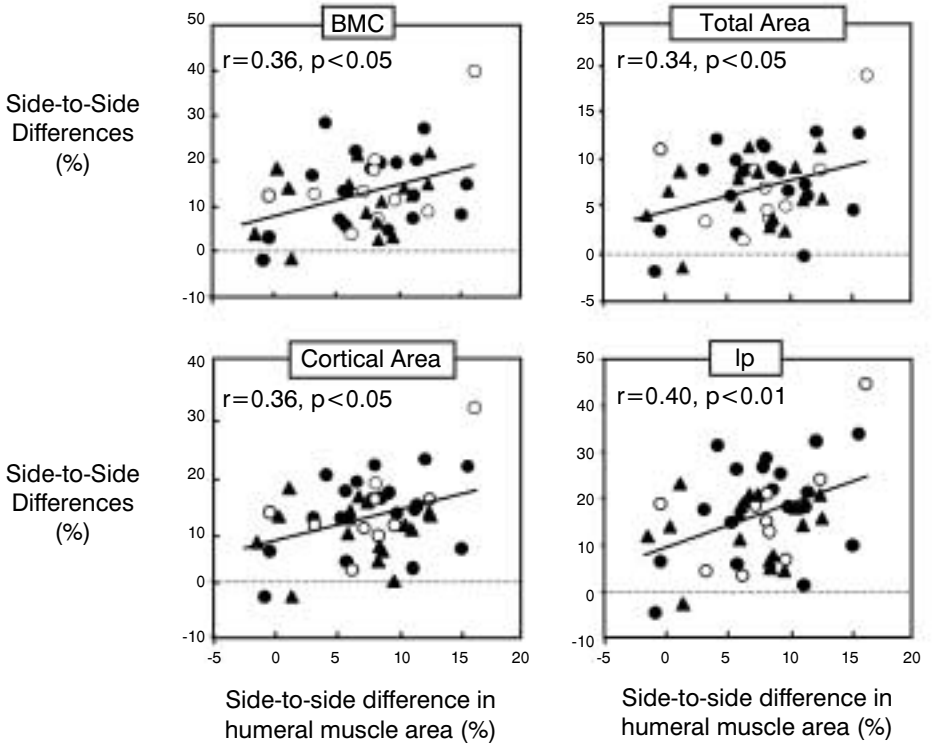

Figure 6. The percentage side-to-side differences in muscle area were positively correlated with the percentage side-to-side differences in BMC, total and cortical area and the polar second moment of area (Ip) in pre- (●), peri- (○) and post-pubertal (▲) female tennis players (n=47). However, the side-to-side differences in muscle size accounted for only 12 to 16% of the variance of the side-to-side differences in the bone traits (Adapted from Daly et al.)[8].

becular thickness). In contrast, immobilization during growth results in reduced growth in bone length and a loss of bone strength due to endosteal resorption in cortical bone and trabecular thinning. Furthermore, long bones may not develop their characteristic shape but develop a more rounded default shape. The finding that bone appears to respond proportionally to increased or reduced loading support Frost's mechanostat. The short time frame within which accrued bone is lost during immobilization illustrates the plasticity of bone, stressing the necessity to continually expose the skeleton to a certain level of strain for bone strength to be preserved.

The effect of nutrition deficiency on the muscle-bone relationship

Nutrition action is permissive for both muscle and bone development - there is no indication that excess of any macro- or micro-nutrient (e.g., above what is required to regulate normal growth and development) will result in greater muscle or bone development. The only plausible action related to excessive nutrition would be via an indirect effect associated with increased body weight (due to a positive energy balance) leading to increased loads on the weight-bearing muscles and bones. Nutrition may act indirectly or directly on muscle and bone. Nutrition acts predominantly via an indirect mechanism through hormones that regulate musculoskeletal development; before puberty, growth is regulated by GH and IGF-I (provided thyroid function is normal), whereas during and after puberty growth is predominantly regulated by sex steroids (assuming adequate secretions of GH and IGF-I). Total energy, protein and calcium are among the key nutritional factors important for musculoskeletal development. The permissive nature of nutrition means that it has its most profound effect on the muscle and bone unit when there is a state of deficiency. In the following section the effect of energy, protein and calcium deficiency on the development of the muscle-bone unit will be discussed in terms of how these factors influence bone mass and structure and muscle mass.

The impact of poor nutrition on skeletal growth was clearly demonstrated by the increase in long bone growth associated with the introduction of milk into schools in the 1930s[54]. More recently, in Chinese girls with very low dietary calcium intakes, milk supplementation increased both BMD and linear growth – it is unclear, however, if the increase in BMD (DXA) was purely due to a growth-related increase in bone size, or an increase in bone mass relative to bone size, or a

combination of both[55]. It is also unknown whether these changes were related to additional protein, calcium or total energy (or other unknown factors) in the milk that were important in facilitating growth in size and mass in these undernourished children. Calcium supplementation in malnourished children in Gambia resulted in increased BMD (DXA) without any corresponding change in longitudinal growth[56]. These data provide evidence that energy, protein and calcium are important in skeletal growth but models isolating the independent effect of each factor are needed before their influence can be elucidated.

Protein and caloric restriction in young animals results in reduced growth[57-61], whereas protein and caloric restriction in adult animals results in reduced body weight, but naturally has no effect on bone length[62-64]. In growing animals the reduced bone mass accrual appears to be concomitant with the reduced growth in bone length (i.e., failure to grow) because when data are corrected for smaller body size, bone mass and geometry are appropriate for size or weight[57-61]. This indicates that the reduction in bone length fully compensates skeletal requirements in terms of bone strength. In one study there was greater reduction in bone length, relative to reduced bone mass accrual resulting in a stronger skeleton relative to size[60]. Evolution has developed an intricate system of ensuring that resources are used where they are most needed; Miller and German[61] reported that protein restriction in growing rats lead to their skulls being shorter and relatively wider than in control rats in order to accommodate the functional demands of the viscerocranium. The effect of caloric and protein restriction on bone size was found to be similar in magnitude when applied individually, while their interaction was found to be additive rather than synergistic[58]. The severity of protein or caloric restriction appears to influence the effect on the growing and adult skeleton. Osteoporosis (trabecular wasting and cortical thinning) occurs when severe protein and caloric restrictions results in disruption of the sex steroid and the GH-IGF-axes[63,65]. In this case, protein deficiency may only be an indirect cause for osteoporosis by its action on estrogen and IGF-I.

It is uncertain whether the similar magnitude and additive effects of dietary energy and protein restriction found in animals are transferable to humans. These questions are difficult to address in humans as protein restriction rarely occur without energy deficits, and it is unethical to conduct trials in protein or energy restriction in growing children. The work of Stanly Garn in the 1960s, however, provides some unique insights into how poor nutrition influences cortical bone development of long bones[66-68]. Garn used metacarpal morphometry (assessed by radiography) as a template to describe changes that occur at the periosteal and endosteal surfaces of cortical bone. He presents data on skeletal growth and aging from many communities around the world who were uniquely characterized by different dietary intakes. In his work across continents and between communities, he identified groups whose diets contained little animal protein and poor quality vegetable protein (Central America, South America, much of Africa, and the rice-eating parts of Asia) as well as high protein intake communities including the Africa Bushman, the Arctic Eskimo, some cattle-raising communities of South America and the 'well to do' in the United States. Garn compared these contrasting populations and groups of individuals with malabsorption states. Despite many communities having similar nutritional intakes, it was impossible to isolate communities by unique dietary characteristics alone. No two populations were comparable in all respects, such as energy balance, minerals (i.e., fluoride in the drinking water) and level of the communities' automation. Despite the limitations of the use of metacarpal morphometry to make generalised comments about cortical bone and that not all communities were entirely unique, Garn's work offers a detailed and comprehensive account of the effect of nutrition on skeletal growth and development.

In agreement with the findings in the animal studies, Garn provides compelling evidence that in the presence of malnutrition longitudinal growth is retarded and there is significantly less bone accrual. He reported that during growth, simple energy restriction resulted in smaller bones and a reduced cortical mass; a result of reduced periosteal expansion as total cross-sectional area was as much as 15% less in adults where malnutrition was common[66-68]. Interestingly, Garn makes the distinction between the effect of simple energy restriction and protein malnutrition during growth. He reports that in protein malnutrition there was a slight but significant increase in total bone width (periosteal expansion) but a marked reduction at the endosteal surface, and as a consequence, a significantly reduced cortical area[66-68]. This finding is consistent with the result of an animal study where there was greater reduction in bone length, relative to reduced bone mass accrual resulting in a stronger skeleton relative to size[60]. In adult protein malnutrition, there is trabecular thinning and loss in trabecular bone compartments as well as an exaggerated loss of bone at the endosteal surfaces in bone shafts[66-68] which is comparable to the period immediately following ovariectomy or immobilization. While these findings may be attributable to protein deficiency, one cannot discount the contribution from the reduced loading associated with reduced muscle mass, body weight, and physical inactivity (particularly in children with Kwashiorkor).

The biochemical pathway by which protein or caloric malnutrition have their catabolic effect on bone growth and development is most likely by suppressing IGF-I levels or the bone cells' sensitivity to IGF-I[59,65]. Dietary protein controls bone metabolism by influencing both the production and the action of growth factors, particularly those of the GH-IGF-I axis[69-72]. IGF-I may directly influence osteogenic cells; osteoblasts are not only equipped with specific IGF-I receptors but can also be endowed with the ability to produce IGF-I[69]. The local amino acid environment can also influence IGF-I production by bone cells[69]. Low IGF-I levels have also been reported to be responsible for early defective

osteoblastic functioning leading to progressive bone loss[65].

While malnutrition induces a cascade of metabolic steps that result in reduced bone accrual or even bone loss, malnutrition also has a catabolic effect on muscle mass and thus muscle force. Thus, some of the bone loss may be related to decreased forces acting on the bones and the resultant smaller bone deformations. Unfortunately, Garn does not report the concomitant changes in muscle mass in malnutrition and protein malnutrition during growth. However, it is likely that the muscle-bone relationship remains intact in malnutrition up to the point where hormonal balances are changed. In addition to affecting the GH-IGF-I axis, states of energy or protein deficiency are also associated with major hormonal imbalances in the sex steroid axis. For instance, low protein diets have been associated with estrogen deficiency and/or resistance to estrogen in adult female rats[73]. Thus, besides low IGF-I levels, estrogen deficiencies or resistance to estrogen action could be involved in low-protein isocaloric diet-mediated bone loss[73]. The effect of chronic malnutrition therefore, has the potential to affect the mechanostat indirectly by altering the set point when malnutrition and associated leanness leads to menstrual dysfunction and alters estrogen secretion. The effect of protein malnutrition on the bone-muscle unit can be explored by building on Garn's data with what is known about the bone-muscle unit in other subgroups of individuals who experience malnutrition.

In healthy boys/girls, men, and pre- and postmenopsaual women, there is a close linear relationship between bone and lean mass[74]. The slope of the curves for the relationship between muscle and bone is similar for all the groups regardless of age and reproductive status; in contrast, the intercepts vary between the groups growing in the order: boys/girls < postmenopausal women < men < pre-menopausal women[5] (i.e., there is more bone per unit of muscle in pre-compared to postmenopausal women). These data reflect the biological control of bones by muscles through the bone mechanostat and that a positive modulation of that control is associated with sex hormones[5]. That is, if there are changes in the endocrine-metabolic environment then bone mass could be disproportionately low with respect to muscle mass. For instance, in the lower limbs of female ballet dances bone mass was reduced relative to muscle mass[5]. In this instance, as was the case with the Argentine sample, the intercept of the muscle-bone curve was lower but the slope was comparable. This suggests that in the ballet dancers the skeleton would require larger forces to reinforce their structure (mass) as a natural response to muscle development after training because of the disturbances associated with impaired estrogen metabolism. This supports the notion of a metabolically-induced shift in the mechanostat set points with no other evident alteration in the system[5].

Malnutrition, leanness and menstrual dysfunction are all characteristics of young women with anorexia nervosa. Consistent with the results reported in ballet dancers, the bone-muscle ratio was also decreased in young women with adolescent onset anorexia nervosa. In this cross-sectional analysis, the bone-muscle mass ratio decreased as the duration of disease (determined by onset of amenorrhea) increased from 0.5 to 3.8 years (Figure 7, R-squared = 0.49, $p=0.008$)[75]. This finding, however, may be biased by the change in body composition with increased duration of disease. Bone mineral density estimated by DXA is influenced by the ratio of fat to lean tissue; that is, a decrease in this ratio (i.e., where there is less fat relative to lean) results in an underestimate of BMD[76]. However, in this sample there was no detectable change in the fat to lean ratio (positive or negative) associated with the duration of disease. This suggests that the decreased bone-muscle ratio was more likely due to an actual change in bone mass relative to muscle mass rather than a result of an artefact in the DXA estimate of BMD.

The large losses in bone mass observed in patients with anorexia nervosa and amenorrheic athletes has traditionally been attributed to disruption of luteinizing hormone (LH) pulsatility and associated chronic hypoestrogenism[77,78]. However, in these women low BMD is not fully recovered with either estrogen therapy or resumption of menses[78]. Thus, chronic undernutrition may also act through an estrogen-independent mechanism to impair bone formation[78]. Consistent with this is the finding that during recovery from anorexia nervosa, nutrition and hormones have independent and additive effects on bone mass[79,80]. To further investigate this estrogen-independent mechanism, Ihle and Loucks[78] determined the dose-response relationship between energy availability (dietary energy intake minus exercise energy expenditure) and bone turnover in regularly menstruating young sedentary women. They report that the degree of energy availability influenced bone turnover in distinctly different ways. For instance, bone resorption (urinary N-terminal telopeptide, NTX) was only affected when energy restriction was extreme, whereas markers of bone formation (serum type I procollagen carboxy-terminal propeptide, PICP and plasma osteocalcin, OC) were significantly suppressed at all levels of energy restriction (Figure 8). Like bone resorption, estradiol was unaffected until energy restriction became severe, whereas the dose-dependent relationship with IGF-I closely resembled the markers of bone formation. This implies that caloric restriction alone only reduces bone formation (hence reduces growth), but once its severity affects the sex hormone axis then increased resorption will eventually lead to osteoporosis.

The effect of energy insufficiency (in the presence of adequate protein intakes) on the muscle-bone unit can be investigated in a group of elite level pre-pubertal gymnasts[41,81]. In this particular group of gymnasts, the daily energy intakes were approximately 2,000 kJ less than controls (6,106 ± 388 kJ vs. 7,949 ± 320 kJ gymnasts vs. controls, respectively) while protein and calcium intakes were comparable. Gymnastics is an ideal training form to optimize the osteogenic response: high magnitude, dynamic loading inducing high strain rates on bone delivered with rest between loading bouts. In the gymnasts, BMD (DXA) was high at all loaded sites, including the arms, which were up to

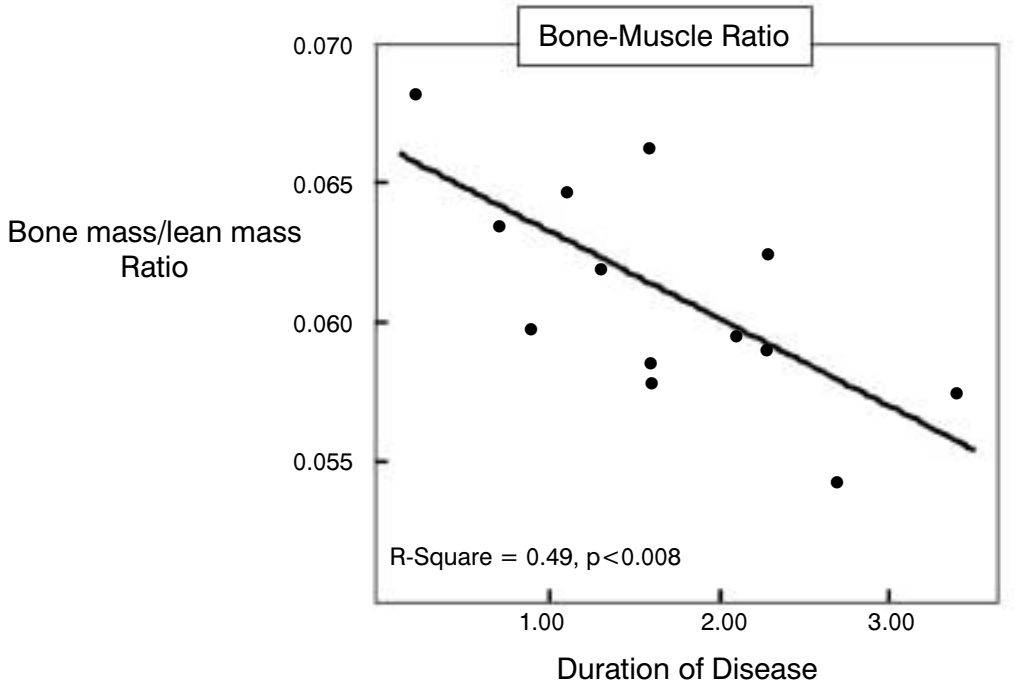

Figure 7. Correlation between duration of disease in females with adolescent onset anorexia nervosa with the BMC to lean mass ratio. The bone-lean mass ratio decreased as the duration of disease (determined by onset of amenorrhea) increased.

4 SD above the mean. Larger bone size has also been reported at the radius in elite female gymnasts[82]. The higher bone mass reported in the gymnasts appeared to be matched by a similar increase in muscle mass, as the ratio for both gymnasts and controls were remarkably similar (0.053 vs. 0.055 gymnasts vs. controls, respectively) and within the normal range for age[41,81,83]. Dietary protein intake was comparable to controls, however this may have been inadequate due to the extensive training undertaken by the gymnasts (25-35 hours per week) as concentrations of IGF-I were reduced in the gymnasts, who also experienced reduced growth and delayed maturation. These results add to the case that when high magnitude loading occurs in a situation of malnutrition, priority is given to ensure the mechanical competence of the skeleton by increasing bone strength. Longitudinal growth has secondary priority and can be reduced when the energy balance is negative.

Calcium is a major constituent of bone, and dietary calcium intake is commonly thought to be a key determinant for maximizing bone mass during growth. It is well established that calcium is a threshold nutrient (thus, more is not necessarily better) but even at low intakes the growing body appears to make the necessary adaptations so that bone mass is essentially maintained across a broad range of dietary calcium intakes[66]. Despite this, dietary calcium supplementation has been associated with increased bone mass accrual, although the magnitude of the effect appears to be highly variable, being affected by the skeletal sites examined, baseline calcium dietary intakes and the stage of pubertal maturation at the time of the intervention[84]. More recently, a review of all studies related to dietary calcium intake and supplementation during growth has shown that in clinical, longitudinal, retrospective and cross-sectional studies, neither increased consumption of dairy products, specifically, nor total dietary calcium consumption has shown even a modestly consistent benefit for child or your adult bone health[85].

It has been proposed that the effect of calcium supplementation is predominantly the result of a decrease in the rate of bone remodelling rather than an increase in bone modelling[86]. This has been demonstrated in a group of Gambian children whose daily dietary calcium intake was ~ 300 mg/day[56], which is close to the theoretical dietary intake for bone mineral accretion during growth (even without accounting for incomplete absorption)[54]. These pre-pubertal Gambian children had poor growth, delayed puberty and low BMD[56]. However, when supplemented with ~ 700 mg/day of calcium, bone mass increased by 4 to 7%. There was no indication that the calcium supplementation increased bone growth in length or size (periosteal apposition). Supplementation was also associated with a decrease in plasma OC (a marker of bone formation rate) suggesting that the increase in bone mass was due to a transient decrease in bone remodelling. Decreased plasma OC has previously been reported to be associated with calcium supplementation in healthy children[87]. Serum calcium levels also play an

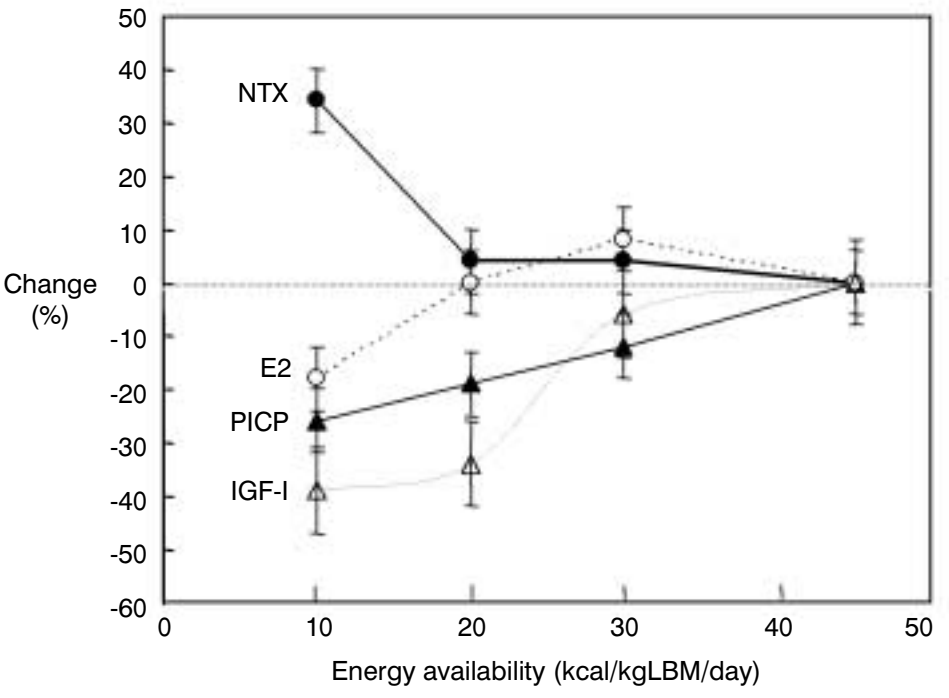

Figure 8. Incremental effects of low energy availability on bone formation (p=PICP) and resorption (~ =NTX), insulin-like growth factor I (r=IGF-I) and estradiol (™=E2). Bone resorption (NTX) was only affected when energy restriction was extreme, whereas markers of bone formation (PICP and OC) were significantly suppressed at all levels of energy restriction. Like bone resorption, estradiol was unaffected until energy restriction became severe, whereas the dose-dependent relationship with IGF-I closely resembled the markers of bone formation. [Adapted from Ihle and Loucks[78]].

important role in muscle and nerve function, however, the tight regulation of circulating calcium levels by parathyroid hormone means that dietary calcium is unlikely to influence muscle function acting on bone. Thus, reduced dietary calcium intake is unlikely to result in the large losses in muscle and bone as observed with protein malnutrition. Therefore, it is unlikely that dietary calcium intake would play a major role in regulating the mechanostat.

In summary

Nutrition has its most profound effect on the muscle and bone unit when in a state of deficiency. Caloric and protein restriction appear to act by retarding growth in length and size, resulting in a proportionally smaller skeleton and reduced cortical mass. Reduced stature as a result of energy deficiency has been a characteristic of the evolution of the human species[88]. Energy and, more so, protein deficiency also reduces body weight and muscle mass, which diminishes the mechanical demands on bone for adaptation in bone strength. There is good evidence that the muscle-bone relationship remains intact in mild malnutrition. However, severe malnutrition influences the GH-IGF-I and sex-steroid axis resulting in a potential shift of the muscle-bone relationship. For instance, menstrual dysfunction associated with estrogen deficiency can lead to a reduction in the bone-muscle ratio. This is consistent with an upward shift of the mechanostat set point; that is, more bone deformation is necessary to stimulate gains in bone mass. Reduced dietary calcium intake has not been found to have effects as severe on bone growth and development as energy and protein malnutrition.

Conclusions

Exercise and nutrition are important for the development of muscle and bone during growth. Both factors are integral to the functional operation of the mechanostat model; but both have distinctly different roles. Mechanical strain imparted by muscle action is responsible for the development of the external size and shape of the bone and subsequently the strength of the bone. In contrast, immobilization during growth results in reduced growth in bone length and a loss of bone strength due to massive losses in bone mass, a result of endosteal resorption in cortical bone and trabecular thinning. Additionally, due to the absent loading that normally directs bone geometry, bone shafts do not develop their characteristic shape but rather develop a rounded default shape. The use of surrogate measures for resulting forces acting on bone (muscle size, mass and strength) limits

our ability to confirm a direct causal relationship between muscle force and bone strength. However, consistent with the mechanostat model, a loss or gain in muscle force is typically matched by a proportional change in bone mass, size and strength (across all levels of immobilization and loading). Unlike exercise, nutritional sufficiency does not influence muscle or bone in a dose-dependent manner. Muscle and bone are only influenced when there is nutritional deficiency - and in this case the effect is profound. Similar to immobilization, the immediate effect of malnutrition in children is reduced longitudinal growth. In adults protein and energy malnutrition can result in large bone losses due to increased endosteal resorption in cortical bone and trabecular thinning, again the same processes as in unloading. In mild malnutrition the muscle-bone relationship may remain intact, however, there is indirect evidence that severe malnutrition when associated with menstrual dysfunction can shift the mechanostat set point upwards, thus leading to less bone accrual for a given amount of bone strain.

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