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MINIREVIEW

Hormonal Aspects of the Muscle-Bone Unit

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Summary

Osteoporotic fractures are the result of low density and especially inferior bone quality (microarchitecture) caused by both internal (genes, hormones) and external (life style) influences. Bone mechanosensors are extremely important for the overall integrity of the skeleton, because in response to mechanical load they activate its modeling, resulting in an increase in bone density and strength. The largest physiological loads are caused by muscle contractions. Bone mass in adult men has a closer relationship to muscle mass than is case in women. The sexual differences in the relationship between bone and muscle mass are also apparent in children. Based on the mechanostatic theory, the muscle-bone unit has been defined as a functional system whose components are under the common control of the hormones of the somatotropin-IGF-I axis, sexual steroids, certain adipose tissue hormones and vitamin D. The osteogenic effects of somatotropin-IGF-I system are based on the stimulation of bone formation, as well as increase in muscle mass. Moreover, somatotropin decreases the bone mechanostat threshold and reinforces the effect of physical stress on bone formation. The system, via the muscle-bone unit, plays a significant role in the development of the childhood skeleton as well as in its stability during adulthood. The muscle and bone are also the targets of androgens, which increase bone formation and the growth of muscle mass in men and women, independently of IGF-I. The role of further above-mentioned hormones in regulation of this unified functional complex is also discussed.

Key words

Muscle • Bone • Hormones • Bone density • Bone quality

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The fact that bone density and quality depend on body weight is well-known (Wapniarz *et al*. 1997). The isolated effect of fat and muscle components of soft tissue on bone mass and strength has not yet been unabiguously defined. Sun *et al.* (2006) showed in large groups of men and women that there is a more significant relationship between the geometrical parameters of the skeleton (cross section, sub-periosteum dimensions and thickness of the corticalis) and muscle mass than weight. The physiological basis of the relationship between muscle function and the skeleton is illustrated by the mechanostatic theory, which presumes that muscle contractions induce tension in the bone, which in turn activates bone modeling on the internal and external side of the cortex via osteocyte mechano-receptors (Frost 2003). This adaptation of the skeleton to stress leads not only to an increase in bone mass, but also to an improvement in bone geometrical parameters and to an increase in bone strength (Fricke and Schoenau 2007). The theory of a functionally unified muscle-bone system is supported by the common embryogenesis of both components signalled via the Wnt pathway and by the fact that they are regulated and controlled by the same hormones and genes (Matsuoka *et al*. 2005).

The role of muscle in skeletal development during childhood and in its integrity in adulthood, including the effect of gender

Before reaching adulthood, the total bone mass increases approximately fifty times. The relationship of bone mass to muscle mass is comparable in young boys and girls. By age seven, we can already note bone mass

values in boys greater by 4.5 % than that of girls of the same age. Non-adipose soft tissue is already, at this age, a strong predictor of bone mass (Hasselstrom *et al.* 2006). The sexual differences in bone mass increase and its qualitative differences are thus already apparent in pre-puberty. Clear sexual differentiation occurs later, in early puberty, whereby bone density and parameters of bone strength measured in the tibia using pQCT show values in boys that are greater by 6-15 % than those in girls (MacDonald *et al.* 2006). In this period of rapid bone mass increase, the functions of the individual soft tissue components that play a role in skeleton development markedly differentiate, based on the gender. In boys, the increase in muscle mass due to rising secretion of testosterone, somatotropin and IGF-I plays a key role. Progressive increase in muscle strength is associated with increasing dimensions of the skeleton (Schoenau *et al*. 2000). In boys, the skeleton responds to changes in muscle mass almost immediately, so that in their case the peak of muscle mass increase precedes the peak of bone mass increase by only 0.36 years (Rauch *et al.* 2004). The cross-section of muscle mass in boys at puberty correlates with bone density, independent of adipose tissue, and contributes to the variability of bone mass by 6-12 % (Arabi *et al.* 2004).

In girls, an increase in muscle mass stimulates linear growth of the skeleton, but the role of muscle in the development of bone mass is less apparent, so that pubertal peak muscle mass values in girls precedes peak bone mass values by 0.50 years (Rauch *et al.* 2004). Muscle cross-section in girls contributes only 4-10 % to the variability of bone mass (Arabi *et al*. 2004). In girls, in the period immediately before puberty, the rapid increase in adipose tissue plays a key role (Parfitt 2004, Wang *et al.* 2005). Leptin released from fat activates the synthesis of estrogen and with the onset of menarche stimulates the development of the female skeleton. The increase of bone in proportion to muscles mass in girls is thus faster than in boys, so that in the third stage of puberty the value of cortical tissue in girls is relatively higher than in boys (Schoenau 2006). The set-point of the mechanostate in girls is lower; in other words, bone modeling is activated by lower mechanical tension than in boys (Frost 1999, Fricke and Schoenau 2007). Rapid accumulation of calcium in the skeleton of girls is perceived as preparation of the female skeleton for the demands of reproduction (Kontulainen *et al.* 2006).

Familial factors play a role in the function of the muscle-bone unit. A study conducted on twin pairs showed that in monozygotes the relationship between muscle mass and density of the femoral neck was twice as significant compared to heterozygotes (Seeman *et al.*1996). The relationship between muscle (but not fat) to bone was also demonstrated in father-son, mother-daughter and daughter-son studies (Krall *et al.* 1995). The effect of muscle on the quantitative and qualitative development of the skeleton is thus determined not only by gender, but also probably by genes.

The sexual differences in the relationship between bone and muscle mass are also apparent in adulthood. A certain predictive significance of muscle mass for the vertebral column and forearm skeleton has been demonstrated in pre- and post-menopausal women (Khosla *et al.* 1996), but the ratio of bone density to muscle cross-section (measured using pQCT) was significantly higher in women compared to men (Sumnik *et al.* 2006). In other words, bone mass in adult men has a closer relationship to muscle than is the case in women.

The significance of muscle strength *vs* muscle mass for the integrity of the adult skeleton is often discussed. Some qualitative parameters in the region of the distal radius have been found to be more closely related to grip pressure than to muscle cross-section, in both men and women and over a wide age range (Hasegawa *et al*. 2001). According to the results of the aforementioned study, muscle strength appears to be a stronger determinant of skeleton quality than muscle mass itself. Another study also showed that the effect of grip strength on the volumetric value of bone density is stronger in women than in men (Kaji *et al.* 2005). The predictive significance of muscle strength for the quality and density of individual sections of the adult skeleton in relation to gender will have to be confirmed by further studies.

The effect of physical stress on the skeleton

The close relationship between muscle mass (respectively muscle strength) and bone density and quality implies that physical stress plays a significant role in skeletal homeostasis. Physical stress has the strongest positive effect on the skeleton during the periods of growth and puberty. In children undergoing regular exercise, there was an increase in bone density of up to 5 % per year, while in adults the increase was only 1-3 % (Suominen 2006). It is clear that in cases of hormonal and nutritional imbalance, restriction of movement in childhood may slow down the development of peak bone

mass and thus increase the risk of osteoporotic fractures in later life. Nonetheless, physical stress positively affects the skeleton throughout life, in both genders. It is known that in adolescent men physical stress speeds up the increase in trabecular bone and increases the dimensions of the cortex, not only in the regions of the skeleton undergoing stress. A significantly positive effect of exercise may be noted under conditions of adequate caloric intake and calcium supply even in younger adult women (Borer 2005). In pre-menopausal women exposed to endurance training, 77 % of bone density variability could be explained by the increase in non-adipose soft tissue (Jurimae *et al.* 2005). After menopause, the capacity of the skeleton to adapt to mechanical stress induced by physical exertion falls due to hormonal changes and insufficient calcium intake. Decreased physical stress tolerance in this period of life also undoubtedly plays a role. Nonetheless, vibration training in post-menopausal women improved isometric and dynamic parameters of muscle strength and significantly increased bone density in the hip (Verschueren *et al.* 2004). Physical activity may to some extent compensate for the weakening of the aging skeleton and thus reduce the risk of fractures even after menopause (Borer 2005).

The significance of muscle mass measurement in the diagnosis of diseases of the skeleton

Concurrent measurement of bone density and non-adipose soft tissue has important diagnostic significance, especially in paediatric osteology. This differentiates between patients with primary bone defects and with primary muscle atrophy. While the former defects are characterised by disorders of skeleton adaptation to bio-mechanical stress, in the latter - this adaptation mechanism remains intact. The diagnostic criterion is the index of *bone/non-adipose soft tissue*. In primary bone defects, this index is low while in secondary defects the index values are higher (Crabtree *et al.* 2004, Pludowski *et al.* 2006, Fricke and Schoenau 2007). Classic examples of primary bone defects include osteogenesis imperfecta, juvenile osteoporosis and osteoporosis in children with a kidney transplant (Ruth *et al*. 2004). Examples of primary muscle lesions include Duchen´s dystrophy and poliomyelitis. Mixed defects may also be observed, with low values of both bone and muscle mass.

For clinical purposes, the proportion of muscle, fat and bone mass in children and adults may be measured using dual energy X-ray absorptiometry (DXA) and a three-compartment model according to which BW (body weight)=BF (body fat)+BMC (bone mineral content) + FFM (fat free mass). The disadvantage of DXA lies in the fact that it does not take into consideration bone geometry and thus evaluates the relationship between the muscle and the skeleton only approximately. More precise methods include the measurement of total body nitrogen using neutron activation, computer assisted tomography and magnetic resonance imaging, or measurements with the aid of bioelectric impedance. All these methods enable the prediction of the risk of later osteoporosis already in childhood.

Muscle and skeleton – common targets of the same hormones

A number of hormones affect the remodeling of the skeleton via a direct osteogenic effect and concurrently affect its modeling by muscle mass. From the aspect of hormonal regulation and function, muscle and skeleton represent a unified complex (Frost and Schoenau 2000).

Hormones of the somatotropin-IGF-I axis and the muscle-bone system

Somatotropin is a hormone that modulates the function of the muscle-bone unit on several levels. The osteogenic effect of this hormone is based on the activation of osteoblasts and the stimulation of bone formation on the endostal surface as well as inside the periosteum. Moreover, somatotropin stimulates proteosynthesis and increase in muscle mass. It decreases the bone mechanostat threshold and reinforces the effect of physical stress on bone formation (Forwood *et al.* 2001). Somatotropin plays a significant role in the development of muscles and the skeleton in puberty as well as in the stability of the adult skeleton. Men with a deficit of somatotropin (GHD syndrome) have significantly lower muscle and bone mass values than healthy men. They suffer from muscle weakness and have a high risk of fractures (Mukherjee *et al.* 2004). A weaker relationship between somatotropin and bone mass was discovered in healthy men and in GHD women who required higher doses of the hormone in order to achieve measurable responses to the treatment (Hitz *et al*. 2006). The response of the muscle-bone unit to somatotropin

thus depends on the secretion of the hormone and on gender.

The muscle-bone system is also under the control of IGF-I, a product of somatotropin. This peptide is synthesised in the liver as well as directly in bone, whose formation it stimulates upon binding to specific receptors (Chihara *et al*. 1997). Muscle is also the target tissue of IGF-I. The peptide activates the cell cycle by suppressing $p27^{Kip1}$, stimulates the proliferation of muscle fibril progenitor cells and their fusion with pre-existent myofibrils (Machida and Booth 2004). Mice with over-expression of IGF-I had, apart from higher cortex density, higher values of non-adipose soft tissue (Banu *et al.* 2003). Moreover, IGF-I activates phosphatidylinositol 3-kinase, Akt/protein kinase and the calcium signal of the muscle cell (by releasing Ca^{++} from inositol triphosphate) and thus increases muscle fibril contractility.

The physiological rise in IGF-I levels, together with the activation of sexual hormone production, induces growth of bone mass in puberty. Furthermore, the proteo-anabolic effect of IGF-I slows down aging in tissues, including bone and muscle tissue. Inhibition of IGF-I production together with consistently high levels of inflammatory cytokines (IL-6) in old age leads to the development of sarcopenia and to a decrease in muscle function, whereby muscles stop responding to mechanical stress (Barbieri *et al.* 2003, Hameed *et al*. 2003).

The importance of IGF-I for skeleton integrity is demonstrated by clinical studies. In adult men, total bone mass correlated positively with serum IGF-I levels (Gillberg *et al*. 2002). Similarly, circulating IGF-I in men was a significant predictor of bone density in the region of the femoral neck (Szulc *et al*. 2004). The age-associated fall in IGF-I production is considered to be one of the causes of male osteoporosis in old age. On the other hand, activation of IGF-I partially explains the positive effect of physical stress on the skeleton. Under acute stress, there is very early release of IGF-I from the binding protein IGFBP3 and there is a concurrent increase in the synthesis of the peptide *de novo,* both independently of somatotropin levels. Activation of IGF-I was also demonstrated during long-term training (Berg and Bang 2004).

IGF-I thus, via the muscle-bone system, plays a significant role not only in the development of the childhood skeleton, but also in its stability during adulthood. Similarly to somatotropin, IGF-I is a candidate molecule for the treatment of muscle atrophy and associated osteoporosis in men with the GHD syndrome and in old age.

Sexual steroids and the muscle-bone system

Androgens

Androgens increase the expression of osteoprotegrin which neutralises the RANKL osteo-clastogenic effect via their own osteoblastic receptors as well as via estrogen receptors (they are aromatised to oestrogen directly in the bone). Remodeling of the skeleton is thus directed towards bone formation (Chen *et al.* 2004). Androgens stimulate trabecular and cortical bone modeling, speed up the radial growth of bones and increase bone dimensions (Venken *et al*. 2007). By stimulating calcium re-absorption in the distal renal tubules, they maintain a positive calcium balance and thus decrease the risk of bone loss due to secondary hyperparathyroidism (Couchourel *et al.* 2004).

The muscle is also a target tissue of androgens which bind to myocyte membrane receptors. Androgens stimulate the growth of muscle mass independently of IGF-I production (Vanderschueren *et al*. 2004) and by activating the calcium signal they also increase muscle contractions (Estrada *et al*. 2003). Muscle growth during protracted physical stress in men is potentiated, apart from IGF-I, also by an increase in testosterone production (Baker *et al*. 2006). Discussion is currently taking place as to the possible direct myogennic effect of the testosterone precursor – dehydroepiandrosteron (DHEA), whose levels correlate with muscle mass (measured using qCT) and strength (Valenti *et al.* 2004). However, administration of DHEA for a period of one year increased bone density in the hip in the elderly, but did not affect muscle parameters (Jankowski *et al*. 2006).

A steep rise in testosterone levels in boys and girls precedes the pubertal increase in bone mass (Yilmaz *et al*. 2005). Androgens chiefly play a significant role in the male skeleton, though. It is well known that an androgen deficit in boys leads to a significant slowing of pubertal muscle and bone mass increase. In adult men, insufficient androgen production speeds up the physiological loss of bone and leads to osteoporosis. In contrast, substitution of testosterone increased bone density in hypogonadal (Amory *et al.* 2004) as well as eugonadal men by as much as 5 % (Anderson *et al*. 1995). Androgens may also play some role in the regulation of the muscle-bone system in adult women. Low levels of testosterone were associated with a more rapid decrease in bone mass in pre-, peri- and post-menopausal women (Slemenda *et al.* 1996).

Administration of androgens to post-menopausal women slowed down bone loss (Tok *et al*. 2004).

Estrogens

Estrogens play a key role in the development and integrity of both the female and male skeleton. Via ERα and ERβ receptors, they suppress the production of the pro-resorption cytokines IL-1, TNFα, RANKL (receptor activator of nuclear factor kappa B ligand) and GM-CSF (granulocyte-macrophage colony-stimulating factor) by bone megakaryocytes and T cells and inhibits bone resorption (Compston 2001). However, in hypo-estrinism loss of bone mass is accelerated not only by the direct effect on bone remodeling, but also by the increase in the set-point of the mechano-stat on the endostal surface and the decreased effectiveness of mechanical stress on the skeleton through inhibition of estrogen receptors (Lee *et al*. 2004).

In puberty, estrogen is one of the factors that induces the growth spurt and the increase in bone mass in both genders. While bone density in girls correlated only with estrogen levels, in boys it was found to be related to both testosterone and estrogen levels (Yilmaz *et al.* 2005). The importance of estrogens for the male skeleton is also documented in molecular-genetic studies. Very low bone density was recorded in men with congenital estrogen receptor non-sensitivity syndrome and men with aromatase deficiency (mutations in exon 9 of the P450 gene) (Morishima *et al.* 1995). A relationship between polymorphisms of both the aromatase gene and gene for CYP19 and bone mineral density was demonstrated in young men (Riancho 2007). Furthermore, relative hypo-estrinism affects the skeleton of men older than sixty without any clear genetic predisposition, in whom the most relevant determinants of bone mass of the femoral neck were (apart from parathormone) the levels of 17-beta-estradiol (Szulc *et al.* 2004). The importance of estrogens for the male skeleton is also supported by the experience that the physiological level of androgens does not prevent bone loss during concurrent hypo-estrinism (Asteria 1997).

The muscle effect of estrogens is not significant compared to that of androgens. Though estrogen receptors were found in female and male striated muscle (Wiik *et al*. 2003), the effect of the hormone on muscle was not conclusive (Taaffe *et al.* 2005). No relationship between ERα (TA-repeat polymorphism) and muscle mass and strength was demonstrated (Grundberg *et al*. 2005).

Sexual steroids thus significantly modulate the function of the muscle-bone unit in both genders. While homeostasis of the skeleton in women is predominantly under the control of estrogens, in men androgens and estrogens play a comparable role.

Hormones of adipose tissue and the muscle-bone system

The function of the muscle-bone unit is also modulated by hormones produced in adipose tissue. The most important of these- leptin- stimulates the differentiation and proliferation of osteoblasts and suppresses osteoclastogenesis by activating monocyte production of interleukin-1 receptor antagonists (Meier *et al*. 2002). It thus regulates the ratio of osteoprotegrin to RANKL to the advantage of osteoprotegerin (Holloway *et al*. 2002). Leptin affects muscle mass indirectly via the insulin-IGF-I axis whose activity it increases (especially in obese individuals) (McClelland *et al.* 2004). The clinical significance of these mechanisms remains to be verified. The effect of other adipose tissue hormones, such as adiponektin and resistin has not yet been demonstrated.

Vitamin D and the muscle-bone system

The classical target tissues of the active metabolite of vitamin D – $1,25(OH)_2$ vitamin D_3 (D-hormone) are the intestine, the skeleton and the parathyroid gland, followed by the reproductory and immune system, the skin, liver, breast tissue and striated muscle. In bones, D-hormone modulates via specific nuclear receptors (VDR) skeleton differentiation and response to growth factors. It increases the formation of new bone mass. Physiological and lower pharmacological concentrations of D-hormone suppress the excessive production of parathormone and decreases osteoclastic resorption (Shiraishi *et al.* 2000). Vitamin D deficiency thus skews bone remodeling towards resorption.

D-hormone also affects the bone indirectly via muscle mass. Upon binding to the VDR of myocytes, it stimulates proteosynthesis and activates transcription factors (Myf5 and myogenin) that regulate the structure of muscle tissue (Demay 2003). It was demonstrated that mice with blocked vitamin D receptors (VDR-/-) have (independent of mineral metabolism) smaller myofibril dimensions (Endo *et al.* 2003). D-hormone also increases muscle contractility by increasing the calcium pool in myoblasts (Drittanti *et al.* 1989). Expression of VDR decreases with age (Bischoff-Ferrari *et al*. 2004a), which together with other age-related mechanisms leads to

progressive atrophy of muscle mass. The importance of VDR for muscle trophism and function is supported by association studies that demonstrated the relationship between muscle strength and BsmI polymorphism in the VDR gene in pre-menopausal women (Grundberg *et al.* 2004) as well as in women over the age of 70 (Geusens *et al.* 1997). In men over the age of 58, a relationship between sarcopenia and FokI polymorphism in the same gene was demonstrated (Roth *et al.* 2004). The importance of vitamin D for muscle function is also supported by the demonstrated correlation between muscle strength and serum 25(OH)D levels (Bischoff-Ferrari *et al.* 2004b).

D-hormone is thus important for balanced bone metabolism homeostasis as well as for the development of the anatomical integrity of striated muscle and its function. Significant vitamin D deficiency leads not only to the development of osteomalacy, but also to severe myopathy resulting in disorders of gait stability, falls and fractures. Clinical manifestations of myopathy occur when as little as 3 % of muscle mass is lost (Visser *et al.* 2003). Secondary hyper-parathyroidism (as a consequence of vitamin D deficiency) also promotes the development of myopathy by increasing the catabolism of muscle protein (independently of vitamin D homeostasis) and reducing the number of muscle fibrils as well as the amount of energy rich phosphates in the myocytes (Sambrook *et al.* 2004). The risk of activating osteo-resorption in hyper-parathyroidism has already been mentioned.

It appears, though, that the muscle-bone system is affected even in cases of much milder vitamin D deficiency. Men and women over the age of 65 are most at risk (Snijder *et al.* 2006). Although vitamin D deficiency and disorders of its metabolism are one of the pathogenetic mechanisms of senile osteoporosis, an increased risk of stress fractures has been demonstrated also in nineteen year old vitamin D deficient men (Ruohola *et al.* 2006). Moreover, vitamin D homeostasis relates to a risk of falls in elerly subjects (Stein *et al.* 1999).

In practice, the severity of vitamin D deficiency is measured with the aid of serum levels of the precursor of the active metabolites of 25-OH vitamin D (25(OH)D) using methods such as CPBA (competitive protein-binding assay) and RIA or the more robust HPLC (Lensmeyer *et al*. 2006). The level of 25(OH)D, which may lead to secondary hyper-parathyroidism and activation of osteo-resorption is 50 nmol/l. Below this threshold, we speak of vitamin D insufficiency (DeLappe *et al.* 2006). The critical value of 25(OH)D, which steeply increases the risk of fractures for both genders, is considered to be 30 nmol/l (or parathormone values above 4.0 pmol/l). In contrast, the safe concentration of 25(OH)D, which represents no risk of hyper-parathyroidism, is 80 nmol/l (Mosekilde 2005). The criteria of balanced vitamin D homeostasis only serve as a general guideline, as they do not take into consideration individual differences in VDR sensitivity to D-hormone.

Is D-hormone the method of choice in the treatment of osteopenia?

Cholecalciferol together with calcium slows the decline in bone mass and significantly decrease the risk of falls (Mosekilde 2005). In healthy individuals, 500 U of cholecalciferol together with 500 mg of calcium per day prevented the activation of bone resorption during winter as well as loss of bone density in the vertebral column and femoral neck in healthy adults (Meier *et al*. 2004). It is becoming apparent, though, that the skeleton is more positively affected by active metabolites of vitamin D than by cholecalciferol (Aloia *et al*. 1988). A meta-analysis of fourteen trials showed that during treatment with 1-alpha OH vitamin D_3 or $1,25(OH)_2D_3$, bone density increases more rapidly than during supplementation with cholecalciferol (Richy *et al.* 2005). Žofková and Hill (2007) demonstrated that post-menopausal women with osteopenia treated over three years with $1,25(OH)_2$ D_3 at doses of 0.40-0.50 ug daily together with calcium achieved a significantly greater increase in bone density in the hip than women treated with cholecalciferol (at a dose of 700 U daily). Similar results in this area have also been reported by Sairanen *et al*. (2000) and Aloia *et al*. (2005). It seems thus, that the administration of D-hormone may be the treatment of choice for osteopenia.

In conclusion, we may summarize that the muscle-bone unit is not only an anatomical, but also a functional term that is significant for the development of pubertal bone as well as for the integrity of the skeleton in adult men and women. The muscle-bone unit can be seen to be a functional unified complex in part due to the regulation of this system by common hormonal circuits. Key regulatory roles are played (apart from physical stress) by the somatotropin-IGF-I axis, sexual steroids, certain adipose tissue hormones and active metabolites of vitamin D. Alteration of the function of the muscle-bone

unit due to the deficiency of any of the aforementioned systems may lead to insufficient development of the skeleton in puberty, and may increase the risk of osteoporosis in old age.

Conflict of Interest

There is no conflict of interest.

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