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ORIGINAL PAPER

Bone and Muscle Pleiotropy: The Genetics of Associated Traits

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Abstract Bone and muscle mass are highly correlated. In part, this is a consequence of both tissues sharing common genetic determinants. In addition, both tissues are responsive to their mechanical environments. New genetic tools in mice will allow genes of interest to be inactivated in experimentally defined contexts, thus allowing investigators to distinguish direct effects on each tissue from physiological responses to a primary phenotype in the other.

Keywords Conditional knockout mice · Mechanobiology · Bone modeling · Exercise

Introduction

In this brief review, I aim to introduce the genetic aspects of the association between bone mass and muscle mass. First, an overview of the adaptation of bone and, to a lesser extent, muscle to the mechanical environment will be provided. Next, the bone phenotypes of a few genetic muscle disorders will be summarized. Last, the prospect of using genetically engineered mice to dissect the mechanisms by which the correlation between bone and muscle mass is achieved will be considered.

The Bone Mechanostat

Readers of *Clinical Reviews in Bone and Mineral Metabolism* are likely to be familiar with the mechanostat model of bone physiology [1, 2]. The model postulates that just as the body seeks to maintain extracellular Ca and PO_4 within narrow physiological ranges, it similarly seeks to maintain bone stiffness, perceived as mechanical strain (strain is the fractional change in length, with tension resulting in positive strain, i.e., lengthening, and compression resulting in negative strain, i.e., shortening), within similarly narrow bounds. The mechanostat model can account for changes in skeletal mass that arise from changes in the habitual loading environment. Thus, prolonged bed rest, paralysis, or space flight all lead to reduction in bone mass because the skeleton is underloaded [3–5], while skeletal overloading, as occurs in the dominant arms of elite tennis players, leads to an increase in bone mass [6]. Experimental systems that allow the effects of mechanical loading on the skeleton to be studied systematically [7, 8] are now well-established investigative tools. Clinical application of the skeleton's mechanical physiology is being actively pursued, most visibly in developing passive vibration as a therapeutic modality, though no validated protocols have yet been established [9].

The mechanostat model represents the systematic development of Wolff's law, which states that bone adapts to the loads to which it is subjected, first published in 1892 as Ueber die Innere Architectur der Knochen und ihre Bedeutung für die Frage vom Knochenwachstum and recently reprinted in translation [10]. The model is predicated on the concept that bone has the ability to sense its mechanical state, that bone responds to that state by growth, and that the system is governed by feedback control in order to establish and maintain homeostasis.

Current thinking holds that strain, or fractional change in length, rather than load, or applied force, is the whole-bone level stimulus to modeling. The critical evidence supporting this view comes from experimental loading in living model

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organisms. In these experiments, a defined load is applied to one limb, while the contralateral limb serves as an unloaded control. By administering tetracycline labels, dynamic histomorphometry can be used to quantify the modeling response to the experimental load [7]. This approach demonstrates that the mineral apposition rate is greatest at the bone sites farthest from the neutral axis and least near the neutral axis. In mice, the response is linear between ~300 and ~5,000 με, the thresholds for bone resorption and a damage response, respectively (Fig. 1) [11].

The past decade has been marked by notable progress in defining the molecular components of the skeletal mechanotransduction system. Mutations of *LRP5*, leading to decreased bone mass [12] and a decreased modeling response to mechanical loading [13, 14] with loss of function and the converse with gain of function [13, 15, 16], have led to the realization that the Wnt-β catenin signaling pathway plays a central role in skeletal mechanotransduction. Recent evidence has also demonstrated that the β-adrenergic system also regulates the modeling response to mechanical loading, with β1 signaling promoting bone growth and β2 signaling promoting bone resorption [17].

However, there are other factors that contribute to the robustness of loading-induced skeletal modeling; for example, PTH can potentiate the response [18, 19]. Moreover, the magnitude of the modeling response to loading is controlled in part by allelic variation, as has been demonstrated by experiments in mice (e.g., [20, 21]) and an *LRP5* genotype × exercise interaction in BMD has been found in humans [22].

Equally striking, and of great importance in understanding the physiology of skeletal adaptation to the mechanical environment, is the observation that a bone's cross-sectional size and its Young's modulus, or tissue-level stiffness, are inversely correlated (Fig. 2) [23]. Young's modulus and cross-sectional size each contribute to the whole-bone stiffness and can therefore compensate for each other in satisfying the physiological goal of maintaining whole-bone stiffness [24].

Much of the mechanical load borne by the bones arises from muscle contraction, and for this reason, it is unsurprising that bone mass and muscle mass are highly correlated [25]. Like bone mass, muscle mass is highly heritable [26] and responsive to the loading environment [27]. Moreover, as in bone, genetic constitution determines the hypertrophic response to a specified loading regimen (reviewed by [28]). It is therefore natural to ask whether, to what extent, and by which mechanisms individual genes control both skeletal and muscular mass and strength.

The determination of multiple phenotypes by a single gene is called pleiotropy, and several genetic mapping studies have reported quantitative trait loci affecting both

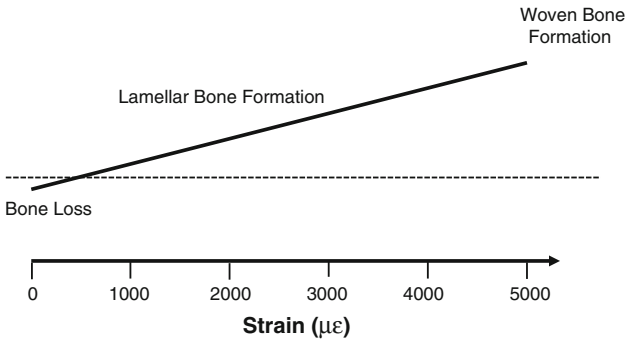

Fig. 1 Conceptual summary of the mechanostat. At low strain, as in microgravity or disuse, bone is resorbed. A higher strain, modeling results in the accretion of lamellar bone. At very high strain, a damage response, characterized by formation of woven bone, occurs. The thresholds for these responses are ~300 με for bone loss and ~5,000 με for woven bone formation. The *dashed line* represents the "adapted" bone mass

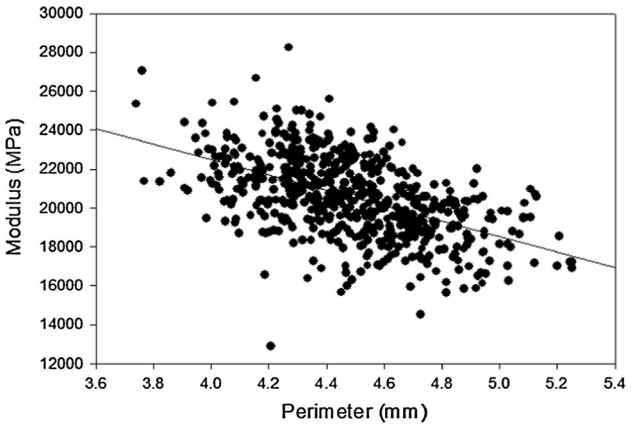

Fig. 2 Regression of Young's modulus on femoral mid-diaphyseal perimeter in HcB-8 × HcB-23 F2 Intercross Mice. Three point bending tests were performed on femora from 603 mice. *Each point* represents a single mouse. Reproduced with permission from [23]

bone and muscle phenotypes (e.g., [29, 30]). Mice in which the melanocortin receptor MC4R has been knocked-out display increases in bone, muscle, and adipose tissue mass [31]. Yet, while studies such as these provide evidence that bone and muscle share genetic determinants, they do not provide insight regarding the mechanisms by which the observed pleiotropy arises.

Bone Phenotypes in Selected Genetic Muscle Disorders

Genetic disorders of muscle provide an opportunity to learn how muscle and bone interact. Duchenne's and Becker's muscular dystrophy arises from loss of function mutations of the dystrophin (*DMD*) gene [32, 33]. Decreased bone mechanical performance is a well-known feature of DMD,

with fractures occurring in about 20 % of patients [34]. Consistent with the increased risk of fracture, BMD is also decreased in muscular dystrophy patients (e.g., [35]). The fracture rate appears to increase with age, consistent with progressive loss of muscle strength [36]. These observations are easily reconciled with the mechanostat model—decreased muscle contractile force leads to decreased skeletal loading and consequent loss of skeletal integrity. Consistent with the human findings, tibial mechanical performance and cross-sectional dimensions were reduced in male Dmd^{mdx} mice, which harbor a spontaneous mutation of *Dmd* and mimic many features of human muscular dystrophy [37], at both 7 and 24 weeks of age [38]. Furthermore, these authors found that the deficit in muscle performance was proportionally greater than the deficit in bone strength at 7 weeks, suggesting that muscle weakness contributes to impaired bone biomechanics. However, 4-month-old female $Dmd^{mdx/mdx}$ mice displayed increased femoral cross-sections and increased 3rd trochanter size, anatomical features generally considered to be characteristic of muscle contraction-induced modeling [39]. These authors interpreted their findings as suggesting that muscle mass rather than the force of muscle contraction is the principal determinant of bone modeling. Such muscle mass-dependent skeletal growth might be mediated by secreted factors by which allow communication between the two tissues. These disparate findings can be reconciled in part by appreciating some limitations of the Dmd^{mdx} model. Unlike human Duchenne muscular dystrophy, Dmd^{mdx} mice suffer only a modest decrease in lifespan and the regenerative capacity of their muscles is greater than in the human disease. Thus, Dmd^{mdx} mice display profound, prolonged muscle hypertrophy, resulting from the muscle's attempt to compensate for weakness. This hypertrophy is sufficient to drive overgrowth of entheses, but the deficient strength is insufficient to stimulate normal modeling.

Myostatin deficiency causes approximate doubling of muscle mass and has been observed in multiple species including humans [40], mice [41], cattle [42], and dogs [43]. Myostatin is a member of the TGF-β family and acts via binding to the activin A type 2b receptor (ACVR2B). Myostatin administration can induce cachexia [44], while inhibition of myostatin is being pursued as a strategy to increase muscle mass (reviewed by [45]). In addition to having increased muscle mass, individuals deficient in myostatin also demonstrate increased skeletal mass [46] and decreased fat mass [47]. The increased bone mass is due in part to a greater response to the load environment [48], but myostatin deficiency also favors differentiation of mesenchymal stem cells toward the osteoblast lineage rather than the adipocyte lineage, but in a mechanical loading-dependent fashion [49]. These findings unequivocally identify myostatin as a key regulator of body composition and provide molecular mechanisms by which exercise and genotype can interact. Recent work suggests that common polymorphisms of myostatin and other key muscle regulatory genes are associated with fracture and BMD [50]. Finally, femora and facial bones of myostatin deficient mice display anatomical features that are characteristic of loading-induced modeling [51, 52]. Taken together, the findings demonstrate that some of the skeletal features that accompany muscle disease are related to the mechanical loading environment.

Directions for Future Research

Pleiotropy may arise by any of several mechanisms, and it is worth considering how bone–muscle pleiotropy might arise. Most simply, a gene might encode a protein expressed in both tissues whose function is parallel in each. Thus, a gene that regulates mechanosensitivity might do so in both bone and muscle. The pleiotropy is simply a consequence of the gene performing a single, well-defined function in multiple cell types. Alternatively, the gene might affect only one tissue and affect the other tissue as a consequence of the expected physiological response to the effect of allelic variation on the first tissue (Fig. 3).

Modern mouse genetics methods, particularly tissue-specific gene ablation, provide the tools needed to distinguish among the mechanisms driving pleiotropy. Unlike the mechanostat model, these may be unfamiliar to clinically oriented readers. It is now technically possible to generate mice in which specific genes have been inactivated in many cases in a restricted set of tissues (reviewed by [53]). The process is illustrated in Fig. 4 and relies on the tissue specificity of the promoter driving CRE recombinase expression. By using CRE strains that are limited to specific lineages and developmental stages, it will be possible to test the role of proteins participating in putative signaling cascades separately in myocytes, osteoblasts, and osteoclasts. Moreover, in osteoblasts, maturation-dependent CRE drivers [54–59] will allow us to identify the stage at which osteoblasts are most sensitive to signals of muscle origin. The same is true of myoblast CRE drivers [60–66]. Several of these now allow induction of CRE activity, so

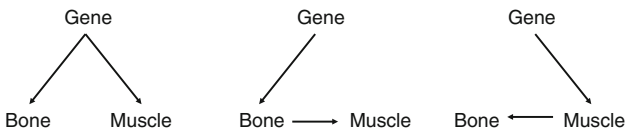

Fig. 3 Different paths to pleiotropy. *Left* a gene exerts parallel, direct effects on both bone and muscle. *Center* a gene exerts an effect on bone, and a physiological response to this effect occurs in muscle. *Right* a gene exerts an effect on muscle, and a physiological response to this effect occurs in bone. Combinations of any pair, or even all three of these mechanisms may occur in fact

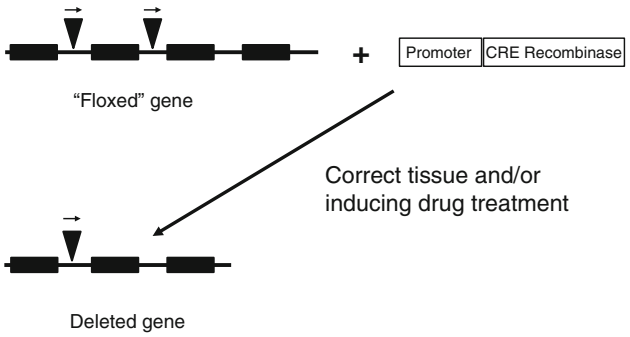

Fig. 4 Schematic of tissue-specific gene ablation. The target gene is modified by the introduction of loxP sites, short DNA sequences that are recognized by CRE recombinase, illustrated by triangles with arrows above them. Such a modified gene is said to be "floxed" (flanked by loxP). In the presence of an engineered CRE recombinase, the DNA between the loxP elements is deleted, leaving only a single loxP site. The loxP sites are chosen so that they remove an essential element of the gene. The promoter controlling CRE recombinase expression determines the tissue and/or drug exposure needed to cause CRE recombinase expression and consequent inactivation of the target gene

that animal age as well as developmental stage of the target cells can be experimentally controlled [59, 61, 65, 66]. Together, these genetic tools will allow investigators to map both genes and their tissues to unravel the mechanisms by which bone and muscle communicate.

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Disclosures

Conflict of interest Robert D. Blank declares that there is no conflict of interest.

Animal/Human Studies This article does not include any studies with human or animal subjects performed by the author.

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