

MOLECULAR MECHANISMS OF EXERCISE IN BONE AND MUSCLE: THE SEARCH FOR AN EXERCISE PILL

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Exercise has numerous beneficial effects on the musculoskeletal system. Aerobic exercise improves the oxygen delivery to skeletal muscle and the ability of muscle to burn carbohydrates and fatty acids. Strength training increases skeletal muscle mass and strength. In addition, exercise strengthens ligaments, tendons and bones. Exercise helps to prevent many chronic conditions, including diabetes, obesity, arthritis, osteoporosis, atherosclerosis, high blood pressure and coronary heart disease. Imagine the numerous medical uses for a pill that simulates the effects of exercise. The exercise pill is not as farfetched as it might seem. Recent advances in our understanding of the underlying biology of exercise are making pharmaceutical replacement for exercise a real possibility. Myostatin is expressed almost exclusively in skeletal muscle and is a negative regulator of muscle size. One of the ways that strength training increases muscle bulk and strength is by suppressing myostatin. Experimental drugs that suppress myostatin have been shown to increase muscle size and strength, and to reverse muscle wasting caused by muscular dystrophy. Exercise increases muscle endurance by increasing the amounts of mitochondria and myoglobin in muscle cells. These responses can be replicated by genetic engineering in mice. A key gene involved is peroxisome proliferator-activated receptor- δ (PPAR δ). Drugs that activate PPAR δ increase mitochondria in muscle and make mice more resistant to weight gain when fed a high-fat diet. A major regulator of bone

strength is Wnt signaling through the LRP5 receptor. If this receptor is inactivated, exercise cannot stimulate bone formation. A gene mutation that activates LRP5 increases bone mass similar to what would be expected with rigorous exercise.

INTRODUCTION

Exercise has multiple beneficial effects on overall health and well-being. Exercise makes the heart pump more efficiently thus improving oxygen delivery to skeletal muscles. Skeletal muscles in turn increase their ability to utilize oxygen and metabolize fat and carbohydrates more efficiently. In addition to its effects on muscle, exercise strengthens ligaments and tendons and bones. If exercise were a drug, it would be widely prescribed for chronic conditions like diabetes, obesity, arthritis, osteoporosis, atherosclerosis, high blood pressure and coronary heart disease. But exercise cannot be packaged into a pill, or can it? Recent advances in our understanding of the molecular biology of exercise may allow the development of medical treatments that provide some of the beneficial effects of exercise. Here I focus on key molecular pathways activated by exercise in the musculoskeletal system and I highlight experimental medical treatments that simulate exercise effects.

HOW DOES EXERCISE AFFECT BONE AND SKELETAL MUSCLE?

Exercise has multiple effects on skeletal muscle. Strength training, e.g. weight lifting, increases muscle size and strength largely through



Figure 1: Belgian Blue bull showing the double muscling phenotype. Reprinted from McPherron and Lee. Proc Natl Acad Sci USA. 1997; 94: 12457-61. Copyright © 1997 by The National Academy of Sciences of the United States of America, all rights reserved.

hypertrophy of muscle fibers. This form of exercise involves forceful muscle contractions but with few repetitions and targets type II or fast-twitch muscle fibers. Aerobic exercises like running or biking increase muscle endurance and oxygen utilization. These exercises typically involve less forceful contractions with large numbers of repetitions. Aerobic exercise mainly targets the type I or slow-twitch muscle fibers. These fibers increase myoglobin content, which increases the amount of oxygen stored in muscle, and make more mitochondria, thus increasing the capacity of the muscle to metabolize fats and carbohydrates. In addition, aerobic exercise improves the vascularization of the muscle so more oxygen can be delivered to the muscle fibers.

Muscles are connected to bones through tendons, so muscle contraction causes force to be transmitted through the tendon to the bone. Exercise not only improves muscle strength but also tendon strength and the strength of the tendon-bone interface. In addition, bone strength is improved by exercise. Bone is under constant attack by osteoclasts that dissolve bone and this is balanced by osteoblasts that make new bone. Exercise stimulates osteoblasts, particularly at the periosteal (outside) surface of the bone and suppresses osteoclasts. Exercise augments bone structure during growth and slows bone loss in the

aging skeleton. At the joints, bones are attached to each other by ligaments. Like other musculo-skeletal structures, the ligaments are strengthened by exercise albeit to a lesser degree than muscle or tendons.

THE SECRET TO BIG MUSCLES

Strength training builds muscle bulk and strength but some animals have massive muscles naturally even without exercise. One example is the Belgian Blue bull (Figure 1), which has about 20% more muscle mass than normal. The Belgian Blue has an inactivating mutation of the myostatin gene [1]. Myostatin (or growth and differentiation factor 8) is expressed almost exclusively in skeletal muscle and is a negative regulator of muscle size. Mice lacking myostatin have about twice as much skeletal muscle mass as normal [2] and these enlarged muscles continue to be maintained for the life of the animal [3]. In addition to larger muscles, the myostatin-null mice have increased bone strength where the muscles insert into the bone [4]. In the only report of a myostatin mutation in humans, the affected boy had twice the normal skeletal muscle size at seven months of age [5]. Strength training suppresses myostatin expression demonstrating that down regulation of myostatin signaling is one mechanism by which resistance exercise increases muscle mass [6].

A pharmacological treatment that suppresses myostatin should replicate the muscle building effect of strength training. To design

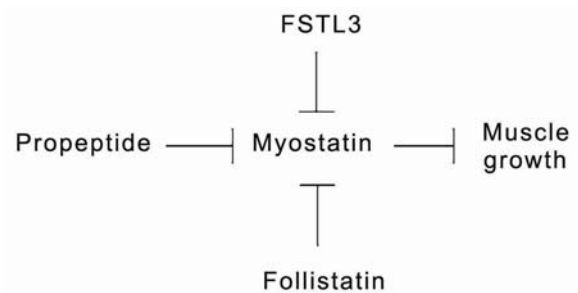


Figure 2: Myostatin activity is inhibited by extracellular binding proteins, including its propeptide, follistatin, and follistatin-like protein 3 (FSTL3).

such a treatment one must understand the molecular pathway through which myostatin works. Myostatin is a member of the TGF- β superfamily of peptides. It is synthesized as a precursor protein that undergoes two proteolytic events before it becomes biologically active. The first cleavage allows the peptide to be secreted and the second cleavage separates the active C-

common in *mdx* mice (Figure 3). These findings demonstrate that myostatin blockade can simulate the effects of strength training. In addition to muscle wasting conditions like DMD, this type of therapy could be used to reverse sarcopenia in the elderly and possibly to prevent muscle loss that occurs in astronauts.

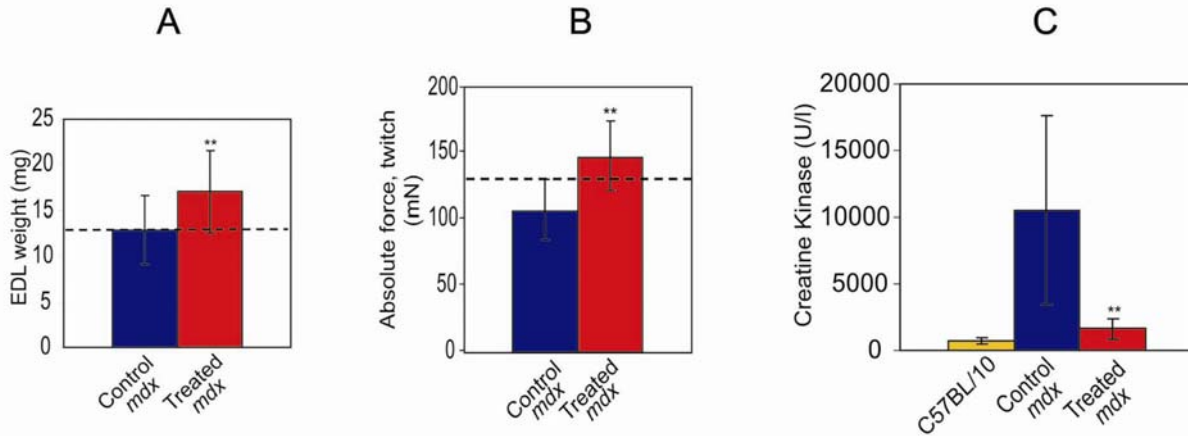


Figure 3: Results of propeptide treatment to suppress myostatin in *mdx* mice. Treated *mdx* mice had larger muscles, for instance the weight of the extensor digitorum longus (EDL) was improved by 32% (A). Strength of the EDL in *mdx* mice was improved 38% after treatment (B). Muscle wasting measured by creatine kinase (CK) activity was reduced to near control levels by propeptide treatment (C). Reprinted from Bogdanovich et al. FASEB J. 2005; 19:543-49.

terminal fragment (myostatin) from the N-terminal propeptide. The propeptide acts as an inhibitor of the active myostatin and thus is one means by which myostatin can be suppressed. Other peptides that suppress myostatin activity include follistatin and follistatin-like protein 3 (FSTL3) [3] (Figure 2). Myostatin binds to an activin type II receptor, most likely ACVR2B, and initiates a signaling cascade that involves the activation of Smad proteins.

Suppression of myostatin activity has been investigated as a means to reverse the muscle wasting caused by Duchene muscular dystrophy (DMD). Studies have been conducted using the *mdx* mouse, a model of DMD. One approach used a monoclonal antibody against myostatin that inhibits binding of myostatin to its receptor [7] and, in another study, stabilized myostatin propeptide was used to suppress myostatin activity [8]. Both therapeutic approaches improved muscle mass and strength, and greatly reduced the muscle damage that is

GENETIC CONTROL OF MUSCLE ENDURANCE

Repetitive contraction of muscle improves the ability of the tissue to utilize oxygen by making more mitochondria and improves delivery of oxygen to muscle cells by increasing vascular perfusion and increasing myoglobin content. Myoglobin binds oxygen and stores it within muscle cells so it can be utilized by the mitochondria to produce energy. The mitochondria are organelles that contain the molecular machinery for the conversion of energy from the breakdown of glucose or fatty acids into adenosine triphosphate (ATP). The energy stored in the high energy phosphate bonds of ATP is then available to power muscle contraction. Biosynthesis of new mitochondria is driven by the intracellular calcium release that occurs with every muscle contraction. Calcium acts as a second messenger to activate certain kinases and phosphatases that in turn activate transcription factors that influence the expression of genes

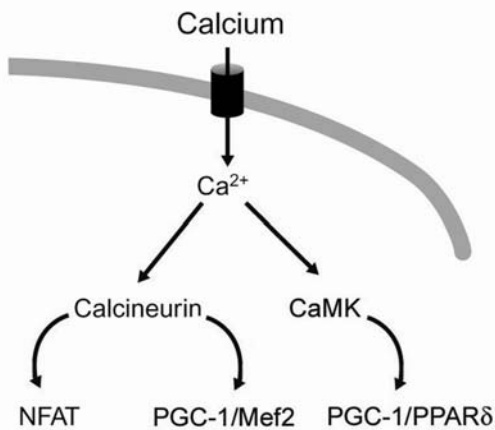


Figure 4: Calcium dependent pathways involved in mitochondrial biosynthesis and fiber type conversion in muscle. CaMK - calcium/calmodulin-dependent protein kinase; NFAT – nuclear factor of activated T-cells; PGC-1 - peroxisome proliferator-activated receptor- γ coactivator-1; Mef2 - myocyte enhancer factor 2; PPAR δ - peroxisome proliferator-activated receptor- δ

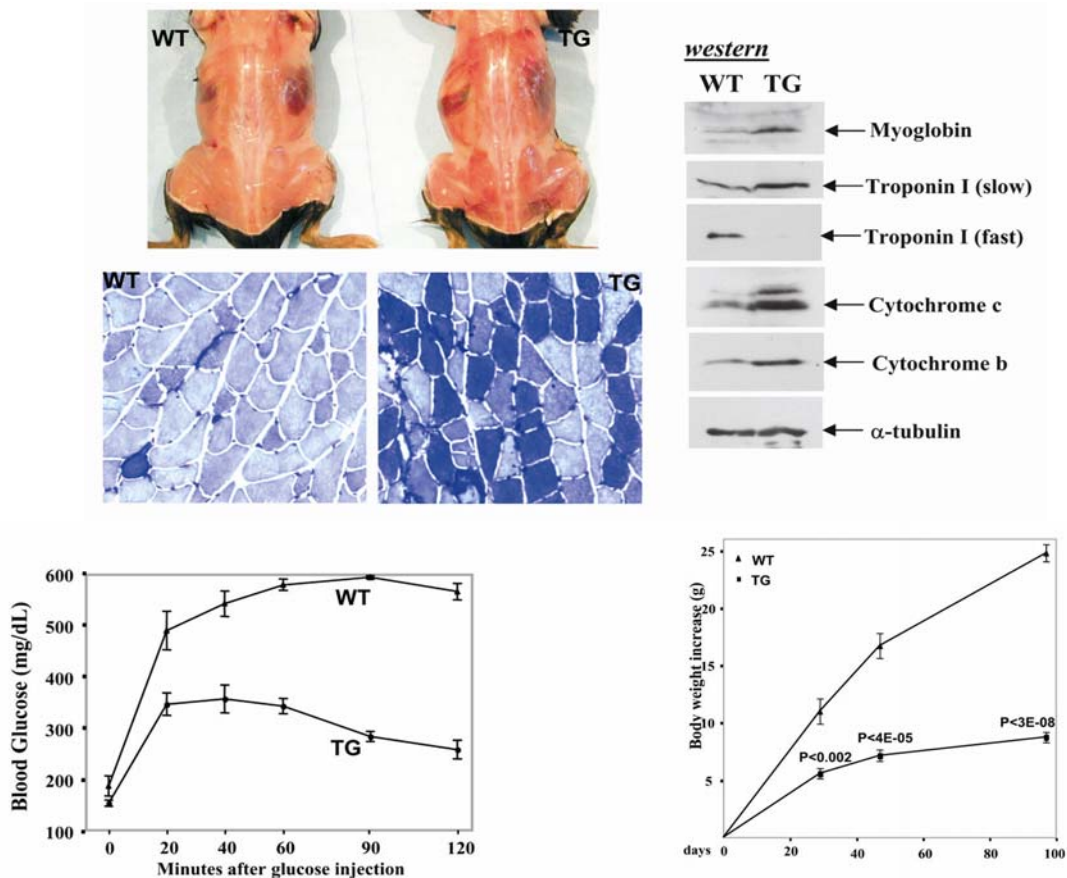


Figure 5: Mice that overexpress an active form of PPAR δ in skeletal muscle (TG) have strikingly more red muscle compared to wild type (WT) controls (top left). In the plantaris muscle, many more type I oxidative fibers (dark blue) are evident in the TG mice (middle left). The skeletal muscle from the TG mice express more myoglobin, more troponin I (slow twitch), less troponin I (fast twitch), and more cytochrome c and b indicating more mitochondria (with α -tubulin serving as a loading control). TG mice maintained lower blood glucose levels after a glucose injection (lower left) and TG mice gained less weight when fed a high-fat diet (lower right). Reprinted from Wang Y-X, Zhang C-L, Yu RT, et al. PLoS Biol 2004; 2:e294.

encoding mitochondrial proteins. The important kinases in this process are the calcium/calmodulin-dependent protein kinases (CaMK) [9] and the key phosphatase is calcineurin [10,11]. These activate several integral transcription factors including nuclear factor of activated T-cells (NFAT) [10], peroxisome proliferator-activated receptor- γ coactivator-1 (PGC-1) [12], myocyte enhancer factor 2 (Mef2) [13], and peroxisome proliferator-activated receptor- δ (PPAR δ) [14] (Figure 4)

Mice that overexpress the active forms of either CaMKIV, PGC-1 α , or PPAR δ display remarkably similar phenotypes, including increased numbers of slow-twitch muscle fibers, increased myoglobin content, more mitochondria in muscle tissue and increased muscle endurance [9, 12, 14]. PPAR δ is expressed at higher levels in type I (slow twitch) muscle than in type II (fast twitch) muscle. This suggests that PPAR δ might regulate muscle endurance since type I muscle has more mitochondria and myoglobin, and is more fatigue resistant compared to type II muscle. Type I muscle can be readily distinguished from type II muscle by its red color. When activated PPAR δ was overexpressed in skeletal muscle of mice, the muscles became redder and many type II muscle fibers were transformed to type I fibers (Figure 5). Mitochondria increased over two-fold in muscle and myoglobin was significantly increased. PPAR δ transgenic mice resemble conditioned endurance athletes. Even though these mice never trained, they were able to run 60% longer and 80% further than normal mice [14]. Skeletal muscle is the major energy consumer of the body so major alterations in muscle fiber type will affect overall metabolism. When fed a high-fat diet, PPAR δ transgenic mice were far more resistant to weight gain than normal mice and they maintained much better control of blood sugar levels (Figure 5).

Genetic engineering of mice to produce more PPAR δ simulated the effects of aerobic athletic training. These mice had improved metabolism and were more resistant to obesity and diabetes. Recent experiments have shown that a drug, that targets PPAR δ and improves its activity, can increase mitochondria in muscle and make mice more resistant to weight gain when fed a high-fat diet. Improvements in glucose

tolerance were also seen [14]. These results demonstrate that it may be possible to design a drug that simulates the effects of aerobic exercise by making muscle that has high endurance and metabolism.

BONE STRENGTHENING GENES

Exercise strengthens bones mainly by improving bone formation on the periosteal bone surface. This is illustrated best by racquet sport players, who have more bone mass in their playing arms. Young female tennis players were shown to have 7-11% greater cortical bone in their playing arms due to more periosteal bone formation at the midhumerus [15]. This bone strengthening effect can be replicated in rodents using the forearm loading model [16] (Figure 6). Axial loading of the forearm causes the ulna to bow laterally so the medial surface is under compression and the lateral surface is under tension. When this form of exercise is applied for 3min/d, 3d/wk, over 16 wks, bone strength was improved by 64% and the energy required to fracture the bone was 94% higher, yet the increase in bone mineral content was only 7% [17]. These results show that exercise is a very efficient means to better bone strength. During exercise, bone formation is greatest in regions of high mechanical stress so bone is formed precisely where it is needed.

A pharmacological treatment that replicates the effect of exercise would effectively strengthen bones. Several potential drug targets are emerging from current research, including membrane ion channels, the P₂X₇ ATP receptor, second messengers such as prostaglandins and nitric oxide, and the LRP5 Wnt receptor. Mechanotransduction in bone involves several cell types, including osteocytes, osteoblasts and marrow stromal cells. Studies using bone explants show that gadolinium, a blocker of the stretch-activated calcium channel, abolished loading-related responses in osteocytes, while a blocker of L-type calcium channels inhibited loading-related responses in osteoblasts [18]. An increase in intracellular calcium, observed in osteoblastic cells seconds after a mechanical stimulus, triggers a mitogen-activated protein kinase (MAPK) signaling pathway [19]. ATP is released from osteoblasts and osteocytes within a minute after a mechanical stimulus. Mice with a

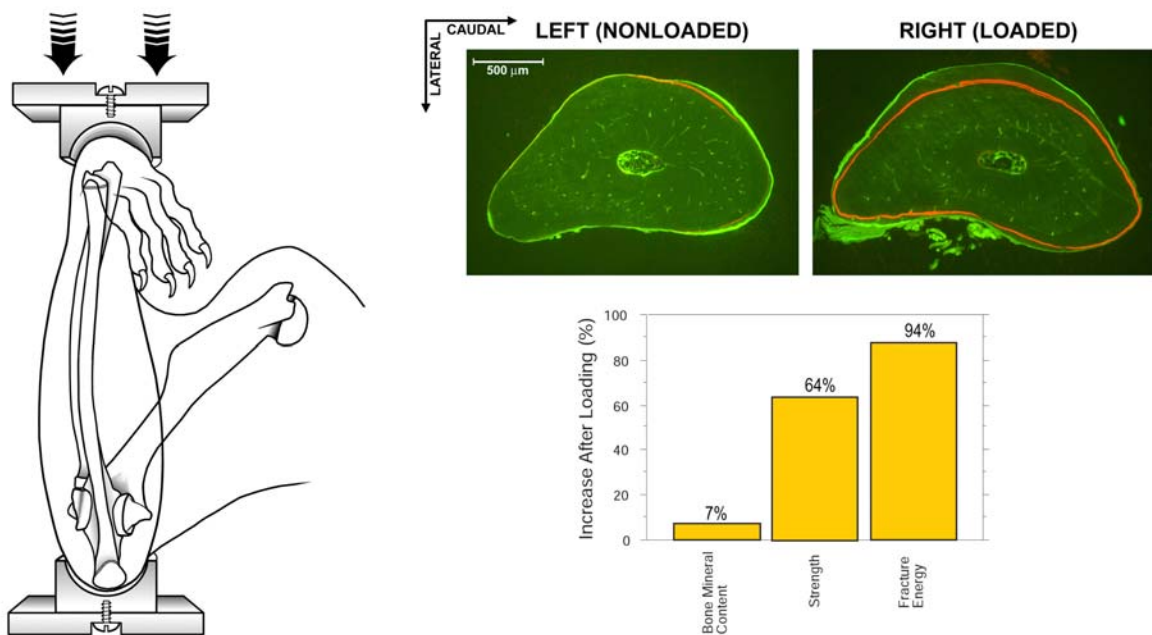


Figure 6: The effect of exercise on the ulna is simulated by applying axial loading on the forelimb of a rat or mouse (left panel). Axial loading causes the ulna to bow laterally so the medial surface is under compression and the lateral surface is under tension. New bone formation is clearly evident on the medial and lateral surfaces (top right). The red label indicates the beginning of the experiment. Loading caused new bone to be formed beyond the red label. Axial loading applied for 3min/d, 3d/wk, for 16 wks improved bone strength by 64% and the energy required to fracture the bone was 94% higher, yet the increase in bone mineral content was only 7% (bottom right). Reprinted with permission from Robling et al. *J Bone Miner Res.* 2002; 17:1545-54. Copyright 2002 by the American Society for Bone and Mineral Research, all rights reserved.

null mutation of the ATP receptor P2X₇ are 60-70% less responsive to mechanical loading of the skeleton, indicating that P2X₇ signaling is critical for proper skeletal mechanotransduction [20]. Prostaglandins and nitric oxide are released from bone cells within minutes after mechanical loading [21, 22]. Prostaglandins probably act as paracrine mediators of bone formation. Prostaglandin E₂ (PGE₂) administered in rats is strongly osteogenic and results in increased recruitment of osteoblasts and increased bone formation [23]. Nitric oxide released from marrow stromal cells is known to be a strong inhibitor of osteoclast activity [24] and probably mediates bone resorption [25]. One key event linking mechanical loading to bone formation is Wnt signaling through the LRP5 receptor. Mice with nonfunctional Lrp5 receptors respond poorly to mechanical loading of the skeleton, with 88-99% reduced bone formation compared to controls [26]. These mice are osteopenic and have reduced diameters of long bones (Figure 7).

People with an activating mutation of LRP5 have high bone mass and increased bone strength similar to what would be expected with rigorous exercise [27]. The mutation in the receptor is thought to inhibit the binding of an antagonist called Dickkopf related protein (DKK). Consequently, pharmacological intervention that targets DKK might simulate the effects of exercise on bone. Another potential drug target is glycogen synthase kinase 3 (GSK3), which must be inactivated before nuclear signaling subsequent to the LRP5 receptor can take place. Drugs that suppress GSK3, and thus simulate the effect of signaling through the LRP5 receptor, increase bone formation [28]. Yet another potential target was identified in a genetic study of people with sclerosteosis, a disease that results in high bone mass and osteosclerosis. The gene responsible for sclerosteosis is SOST [29], which codes for the protein sclerostin, a suppressor of bone formation. Sclerostin could exert its effect by inhibiting bone morphogenic proteins but

recently it has been demonstrated the sclerostin inhibits Wnt signaling through LRP5/6 receptors [30]. Sclerostin may suppress bone formation through a mechanism similar to DKK thus it may also be a candidate for a treatment that replicates the exercise effect on bone.

CONCLUDING REMARKS

In the past few years the genetic basis for exercise responses in skeletal muscle and bone is beginning to emerge. Key genes have been identified that may serve as targets for drugs that simulate the beneficial effects of exercise. The idea of an “exercise pill” has now been transformed from science fiction into a real possibility.

ACKNOWLEDGEMENTS

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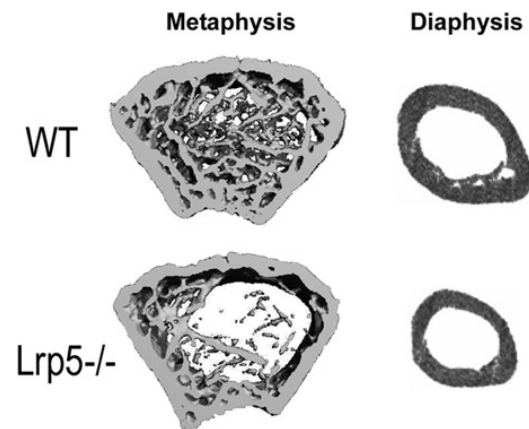


Figure 7. Micro-computed tomography images of the distal femoral metaphysis and the femoral midshaft (diaphysis) from wild type control and *Lrp5* deficient mice. *Lrp5* deficiency caused osteopenia in the metaphysis whereas the femoral diaphysis had reduced thickness and diameter.

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