Perspective Article

Krogh's principle for musculoskeletal physiology and pathology

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Abstract

August Krogh was a comparative physiologist who used frogs, guinea pigs, cats, dogs, and horses in his research that led to his Nobel Prize on muscle physiology. His idea to choose the most relevant organism to study problems in physiology has become known as Krogh's principle. Indeed, many important discoveries in physiology have been made using naturally occurring animal models. However, the majority of research today utilizes laboratory mouse and rat models to study problems in physiology. This paper discusses how Krogh's principle can be invoked in musculoskeletal research as a complementary approach to using standard laboratory rodent models for solving problems in musculoskeletal physiology. This approach may increase our ability to treat musculoskeletal diseases clinically. For example, it has been noted that progress in osteogenesis imperfecta research has been limited by the absence of a naturally occurring animal model. Several examples of naturally occurring animal models are discussed including osteoarthritis and osteosarcoma in dogs, resistance to disuse induced bone and skeletal muscle loss in mammalian hibernators, and bone phenotypic plasticity in fish lacking osteocytes. Many musculoskeletal diseases (e.g., osteoarthritis) occur naturally in companion animals, which may provide clues on etiology and progression of musculoskeletal diseases and accelerate the development of pharmaceutical therapies for humans.

Keywords: Animal Models, Comparative Physiology, Companion Animals, Evolutionary Physiology, Musculoskeletal

August Krogh: his research and his principle

August Krogh was a Danish scientist who was awarded the Nobel Prize in Physiology or Medicine in 1920 for his discoveries on the mechanisms that regulate capillaries in skeletal muscle[1]. He was a comparative physiologist who used frogs, guinea pigs, cats, dogs, and horses in his research that led to his Nobel Prize. He co-founded the Nordisk Insulin Laboratory, which became Novo Nordisk. He continued to work on comparative physiology until his death at age 74. In comparative physiology circles he is best known for his ability to choose the most relevant organism (e.g.,

plant, insect, amphibian, bird, mammal) to study problems in physiology. In 1929 he stated, "For a large number of [physiological] problems there will be some animal of choice, or a few such animals, on which it can be most conveniently studied"[2]. Krogh's mentor remarked that the tortoise was "expressly 'created' for special physiology purposes, but I am afraid that most of them are unknown to the men for whom they are 'created'[2]. Hans Krebs, also a recipient of the Nobel Prize for his work on the "Kreb's cycle", provided a few examples of the value of using Krogh's principle to solve problems in physiology[3]. Krebs himself used skeletal muscle from pigeons for his research on the tricarboxylic acid cycle because they were more suitable for answering his research question than rat muscles. The remainder of this article deals with the use of Krogh's principle - studying animals with unique physiological mechanisms - for solving problems in musculoskeletal physiology and disease. The use of naturally occurring animal models, which include spontaneous models of pathology as well as models of healthy but unique physiological mechanisms, may enhance the ability to solve problems in musculoskeletal research. For example, it has been noted that "progress in OI [osteogenesis imperfecta]

research has been limited because of dependence on human fibroblast and osteoblast specimens and the absence of a naturally occurring animal model for this genetic disorder[4]." More recently a canine model of naturally occurring OI has been identified[5].

There is great interest in solving problems in human musculoskeletal physiology in order to improve the clinical treatment of musculoskeletal diseases. Musculoskeletal tissues evolved and adapted for numerous biological, physiology, and mechanical functions for hundreds of millions of years prior to the appearance of humans. In many circumstances the adaptions were in response to extreme environmental conditions or for performing unique functions[6]. Bone, for example, appeared in animals about 430 million years ago (Mya), but homo sapiens appeared only about 0.2 Mya. Even bipedalism occurred possibly only about 7 Mya. Thus, there is a lot to learn from the biological and physiological processes of animals that have capitalized on hundreds of millions of years of evolution for performing musculoskeletal functions.

Of mice and rats

There is no doubt that laboratory mice and rats continue to be extremely valuable in solving problems in human physiology and clinical medicine. For example, they have elucidated molecular mechanisms that regulate bone formation[7], increased our understanding and ability to treat arthritis[8], and increased our ability to develop therapies for osteoporosis[9,10]. However, mice and rats do have several limitations as research models that should be recognized. For example, the standard housing conditions for wild type mice and rats which include small cages and *ad libitum* food produce sedentary and overweight "control" animals relative to food restricted and exercised animals[11]. Thus, the so called control animals do not necessarily represent a healthy physiological state. In fact, they more closely model disease states like obesity, diabetes, and chronic inflammation. Rodents housed in standard conditions have high body fat, blood pressure, plasma concentrations of glucose, insulin, and cholesterol, as well as high levels of pro-inflammatory cytokines and hormones like leptin compared to exercised and diet restricted animals[11].

Furthermore, rodents housed in standard conditions have a high incidence of spontaneous tumors. In contrast, a naturally occurring rodent model (naked mole rat) has remarkable cancer resistance despite exceptional longevity (approximately 30 years) relative to standard research rodents[12]. Thus, clues from the molecular mechanisms that prevent cancer in naked mole rats may be used for comparative studies with laboratory rodent models of cancer[13], which may increase the understanding of and ability to treat cancer. Gene expression is also significantly different in the tissues of *ad libitum* fed versus diet restricted rodents[11]. Thus, standard housing conditions for control animals may confound the interpretation of genetic and physiological

responses in drug and disease etiology studies. Additionally, even though genetically modified animals provide insight on musculsoskeletal diseases like osteoarthritis, as it is possible to cause osteoarthritis by knocking out or overexpressing specific genes in animal models, it does not confirm these same genes are involved in the etiology of human osteoarthritis, which may be related to subtle changes in development, growth, and decades of physical activity[14]. Ultimately, findings from studies on animals that have developed unique musculoskeletal physiological characteristics, due to evolutionary pressures, increase our understanding of the form, function, and biological regulation of musculoskeletal tissues and may help develop novel clinical therapies for musculoskeletal diseases and injuries. For example, the role parathyroid hormone (PTH) plays in regulating bone metabolism in hibernating bears to prevent disuse osteoporosis[15] may lead to the development of a new PTH drug that could be used to treat patients with low bone density and at risk for fracture[16].

The purpose of this paper is to encourage researchers to ask themselves if there are naturally occurring animal models that may be better models and provide deeper insight for answering specific questions in musculoskeletal physiology than lab mice and rats, or if naturally occurring animal models may be complementary or synergistic to standard laboratory animal models for solving problems in musculoskeletal physiology. One of the best and most accessible animal models for comparative physiology and pathology are pedigree dogs. There are over 300 breeds of dogs and many are predisposed to musculoskeletal disorders such as osteoarthritis (OA), osteosarcoma, anterior cruciate ligament (ACL) failure, and hip dysplasia[17]. Elucidation of canine genomes enables the ability to assess the role of genetics in canine musculoskeletal disorders[18]. For example, genetic studies in Dachshunds with naturally occurring osteogenesis imperfecta are helping understand the roles of post-translational modifications and cross-linking of collagen in bone[5]. In addition to providing models for understanding the etiology of musculoskeletal diseases, companion canines provide pre-clinical animal models for clinical procedures to treat musculoskeletal diseases. For example, approximately 1 million dogs are treated for ACL injury annually[19]. Canine and equine athletes have also helped us understand how fatigue microdamage and targeted bone remodeling contribute to the etiological and clinical aspects of stress fractures[20-22].

Skeletal muscle

Skeletal muscles are remarkable organs with respect to their ability to generate large tensile forces to produce motions of the skeletal system ranging from organismal locomotion to mastication. For example, the tension in human leg extensor muscles can exceed 1,100 pounds of force for a squat exercise[23]. Comparative models of animals particularly well suited for muscular force production have contributed greatly to our understanding of structure-

function relationships of skeletal muscle. For example, crabs are able to exert exceptional bite forces because they are capable of producing muscle stresses much larger than vertebrates[24]. The ability to produce the high muscle stresses results from the longer resting sarcomere length in crabs than in vertebrates. Much of our understanding of muscular power output and the interactions of muscles and tendons comes from studies on animals like insects, kangaroo rats, frogs, and kangaroos[25-28]. Many notable discoveries on skeletal muscle physiology were made with non-rodent models. Krogh studied gas diffusion in frog muscle[29]. Oxygen is essential for providing energy (i.e., ATP) for muscular contraction and force production. Krogh also studied the rate at which oxygen is used in skeletal muscle and the distance an oxygen molecule has to travel from a capillary. In these studies he used muscles from horse, dog, guinea pig, frog, and cod[30]. These were some of the investigations that led to his Nobel Prize. The research of Archibald V. Hill has contributed extensively to our current understanding of muscle physiology. Hill received the Nobel Prize in Physiology or Medicine for his work on skeletal muscle just two years after Krogh did, but his contributions to elucidating the mechanisms of muscular contraction continued long after that[31]. Hill used many animals such as the hummingbird, tortoise, sloth, whale and horse for comparisons with human muscle mechanics[32]. He has had a profound influence on more recent prominent muscle researchers including those trying to find treatments for diseases that affect skeletal muscle such as cerebral palsy[33-36].

Solutions to debilitating skeletal muscle conditions such as cerebral palsy, sarcopenia, disuse induced atrophy, and Duchenne muscular dystrophy (DMD) may be influenced or inspired by naturally occurring animal models. There is currently no cure DMD, which is caused by a mutation in the dystrophin gene, which leads to progressive muscle degradation and weakness in boys starting at about 4 years of age, loss of ambulation at about 12 years of age, and death at about 26 years of age due to cardiac and respiratory failure. There are dystrophin deficient mouse models, but they display a mild phenotype compared to boys with DMD. However, the golden retriever muscular dystrophy dog (GRMD) faithfully reproduces the human phenotype[37]. Recently, two exceptional GRMD dogs were found to have normal muscle function despite the complete loss of dystrophin[38]. These dogs demonstrated overexpression of the Jagged1 gene which may be able to rescue the dystrophic phenotype, representing a possible new therapy for boys with DMD. Physical inactivity, due to injury or bed rest cause skeletal muscle atrophy[39,40]. While this muscle atrophy can be simulated in rodent models, an alternative approach to developing therapies for disuse induced muscle wasting is to consider animal models that are naturally resistant to disuse induced muscle atrophy. Hibernating mammals are physically inactive for up to 6 months annually, but exhibit relatively little muscle atrophy upon emergence from hibernation[41]. There is evidence suggesting roles for mammalian target of rapamycin (mTOR), PGC-1α (peroxisome proliferator-

activated receptor-γ coactivator-1), and serum and glucocorticoid-inducible kinase 1 (SGK-1) in the regulation of skeletal mass during hibernation, representing possible targets for developing new therapies. Despite whole muscle contracture in cerebral palsy patients, sarcomere lengths are longer than in healthy controls[36]. While there is currently no known naturally occurring animal model were nerve or muscle injury causes longer sarcomere lengths[36], future identification of one or the use of other comparative animal models may increase our understanding of the etiology of and possible treatments for cerebral palsy.

Articular cartilage, ligament, and intervertebral disc

The passive soft tissues are also mechanical organs that are commonly damaged and diseased (e.g., ACL tear or osteoarthritis). There are numerous lab animal models to simulate the human conditions, but there are naturally occurring animals' models (e.g., companion dogs) that may provide greater insight than induced models[42-48]. Osteoarthritis is the progressive degradation and loss of articular cartilage that affects approximately 25% of the adult population[49]. Its etiology is attributed to numerous factors such as genetics, aging, and joint injury. Despite an abundance of research, the molecular mechanisms of osteoarthritis are not well understood. Dogs, cats, horses, and primates are some of the naturally occurring animal models which may help elucidate the mechanisms of osteoarthritis etiology since osteoarthritis occurs spontaneously in these animals[42-45,47]. Macaques spontaneously develop osteoarthritis in the spine that is radiographically similar to human spinal osteoarthritis[42]. Since longitudinal studies with repeated radiographs is not feasible in humans, this model provides the ability to study disease progression over lifetimes. The knee joint of domestic dogs is histologically similar to the human knee joint and some dogs naturally develop knee osteoarthritis[45]. Studying natural osteoarthritis in companion dogs is advantageous because the etiology can be compared to human osteoarthritis and experimentally induced osteoarthritis (e.g., via cranial cruciate ligament transection) in dogs, and may accelerate the development of pharmaceutical therapies through clinical trials[45]. Dogs also naturally develop osteoarthritis in the hip joint[43]. COX-2, 5-LOX, and LTB4 are all upregulated in the synovium of osteoarthritic canine hips and represent possible pharmaceutical targets for treating osteoarthritis in canine and human patients. The only known naturally occurring model of post-traumatic osteoarthritis (PTOA) is race horses and these equine models have shed light on the mechanisms of PTOA etiology and progression[50]. For example, it has been shown that osteoclasts are recruited to subchondral bone by RANKL from chondrocytes during the progression of OA and it was suggested that cathepsin K from osteoclasts contributes to osteochondral degradation[50]. Racehorses with OA have also shown that lubricin, a lubricating glycoprotein,

is upregulated in the synovial fluid, possibly to help protect cartilage from further degradation[47].

Spontaneous ligament and meniscus injuries also occur in dogs[46,48,51], which may be useful models for developing new procedures and implants for repairing human soft tissue injuries[52]. Additionally, there are also several naturally occurring animal models of intervertebral disc regeneration. The sand rat is the most common, but other models include the pin tail mouse, Chinese hamster, baboon, and multiple dog breeds[53]. Several of these naturally occurring models, such as the sand rat and pin tail mouse, demonstrate spontaneous disc degradation similar to that of humans. Thus, these animals could be used to help provide insight on the etiology of disc disease that may not be available in animal models of experimentally induced disc degradation.

Bone

Mineralized tissues may have first appeared to perform mechanical functions for feeding[54]. Cellular bone (i.e., containing osteocytes, osteoblasts, and osteoclasts) first appeared in fish about 430 Mya[55]. In extant terrestrial vertebrates like humans, bone is known to play numerous physiological and mechanical functions and has the ability to demonstrate phenotypic plasticity in response to altered conditions such as the habitual mechanical environment. The current dogma is that osteocytes mediate changes in bone structure in response to altered mechanical loading[56]. However, acellular fish bone can also adapt to changes in mechanical loading[57]. This is a good example of how naturally occurring animal models can provide insight into musculoskeletal physiology to complement and sometimes challenge research in standard rodent models.

Short bouts (e.g., 1-8 weeks) of physical inactivity in humans and laboratory animals induce substantial bone loss, reduced mechanical properties, and increased fracture risk by uncoupling bone formation from resorption[58-60]. In contrast, hibernating bears that are physically inactive for up to 6 months annually do not have compromised mechanical properties of bone either at the tissue level[61,62] or whole bone level[63-66], and are used as naturally occurring animal models for preventing osteoporosis[67]. Intracortical porosity of bear cortical bone actually decreases with age[68] instead of increasing with age as in human bone[69]. Histological and biochemical studies on the physiology of bone remodeling indicate that bone resorption and formation are suppressed and balanced during hibernation[64,70,71]. The suppressed remodeling is likely driven by the need to conserve metabolic energy and balanced remodeling is likely driven by the need to maintain eucalcemia[70]. The biological and physiological changes that occur during hibernation, and the effects of hibernation on bone have been thoroughly reviewed previously[72-74]. Several studies have been conducted on the biological mechanisms that influence bone metabolism in hibernating bears[15,70,75-81], which have led to the development of new therapies to treat low bone mass and fracture

risk[16]. The unique biological and physiological mechanisms of hibernating bears may also provide insight on how to treat obesity and cardiovascular disease[82]. Hibernating rodents and frogs also provide naturally occurring animal models for understanding the effects of prolonged physical inactivity on bone[83-89]. For example, bone proteomic studies have implicated a role for the endocannabinoid system in regulating bone remodeling in hibernating marmots[90]. Thus, clues from the biological mechanisms that regulate bone metabolism in hibernators may help in the development of novel therapies to treat immobilized human patients such as those affected by stroke[91].

Companion dogs have been extremely valuable in increasing our understanding of spontaneous tumor development and developing novel therapies for treating cancers including osteosarcoma[92]. While there are numerous mouse models of cancer, they often do not faithfully reproduce many of the aspects of human cancer such as long latency periods, recurrence, and metastasis[93]. Canine cancer models on the other hand share many similarities with human cancers. Dogs are excellent models for understanding and treating osteosarcoma in humans because they have remarkably similar cancer biology, clinical presentation, and responses to treatment[92]. Thus, surgical, chemotherapy, and radiotherapy approaches to treating osteosarcoma are being translated from veterinary medicine to human medicine. For example, a chemotherapy protocol developed for dogs improves survival rates for pediatric osteosarcoma patients[94]. Companion dogs may also provide insight on etiology and treatments for other bone diseases such as osteogenesis imperfecta. It was previously noted that progress in osteogenesis imperfecta research has been limited by the absence of a naturally occurring animal model[4]. Subsequently, naturally occurring osteogenesis imperfecta was identified in Dachshunds[95,96]. Dachshunds have been useful for understanding the molecular mechanisms of osteogenesis imperfecta. For example, in Dachshunds with osteogenesis imperfecta, a mutation in the ER chaperone protein HSP47 produced over-hydroxylation and partial intracellular retention of procollagen I[5].

Conclusions

These are just a few of the known examples of animals with unique musculoskeletal physiology and animal models of spontaneous musculoskeletal disease and injury. The next time you are trying to answer a question or solve a problem in basic, clinical, or translational musculoskeletal biology or physiology consider known and yet to be discovered naturally occurring animal models, in addition to lab mice and rats, for your answers and solutions. Capitalizing on the evolutionary physiology of animals with unique adaptions to extreme environments or those prone to spontaneous musculoskeletal injury or disease with similar biology and clinical presentation to humans will increase our ability to understand and treat musculoskeletal disorders. However, the limitations and difficulties of working with naturally occurring animal models

also need to be considered. Field work and housing of naturally occurring animal models is generally considerably more time consuming and expensive compared to ordering traditional lab rodents from a vendor and housing multiple animals in small cages. Consider for example the logistics of studying bone metabolism in hibernating mammals. This may require radio-collaring bears and tracking them in the wild to obtain blood samples for analyses of bone remodeling markers, or trapping marmots in the Rocky Mountains at elevations exceeding 12,000 feet and transporting them to a custom built vivarium and hibernaculum. It may be difficult to get large sample sizes of animals with unique musculoskeletal physiology or spontaneous musculoskeletal disease and injury, and ethical consideration may be more challenging than with conventional rodent models. Clearly not everyone can work with these types of animal models, but certainly everyone can glean insight about musculoskeletal physiology and pathology from studies on them.

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