The rabbit (*Oryctolagus cuniculus*) as a model in the study of human muscular dystrophies

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Abstract. In this paper we propose to discuss the role that the rabbit (*Oryctolagus cuniculus*) has recently had as a human model in furthering research on muscular dystrophies. Several mutant lines of rabbits have been successfully used to mimic some types of muscular dystrophies known in humans. Although muscular dystrophies found in humans and animals cannot yet be completely cured, the development of molecular biology techniques opens new horizons for their treatment.
Key Words: Cas9, Duchenne, dystrophy, IGF-1, *Oryctolagus cuniculus*, MGF, sgRNA,

Introduction. Muscular dystrophies are diseases varied in type and difficult to cure. In this regard, numerous studies are being conducted for a better understanding of these diseases. In this paper we propose to discuss the role that the rabbit (*Oryctolagus cuniculus*) has recently had as a human model in furthering research on muscular dystrophies.

Muscle development. With the exception of muscle development in the early ontogenetic stages, muscle development is the result of physical stress, which initiates a cascade of reactions for the synthesis of signal peptides (peptide hormones) (Figure 1). Basically, physical effort is the trigger for muscle recovery and growth through overcompensation. Basically, increasing or preserving muscle mass is a process of adapting over time to the individual's living conditions. Therefore, muscle atrophy and decreased muscle strength is often caused by a sedentary lifestyle.

As we said, a number of peptide hormones are involved in the process of muscle recovery and growth. Growth hormone–releasing hormone (abbreviated GHRH, see Figure 1), also known as somatocrinin or by several other names in its endogenous forms and as somatorelin (INN) in its pharmaceutical form, is a releasing hormone of growth hormone (GH) (Aguiar-Oliveira & Salvatori 2021). It is a 44-amino acid peptide hormone produced in the arcuate nucleus of the hypothalamus (wikipedia.org).

GHRH has a synthetic analogue, called GHRP (with two known variants, GHRP-6 and GHRP-2). Growth hormone-releasing peptide 6 (GHRP-6) (developmental code name SKF-110679), also known as growth hormone-releasing hexapeptide (Dawood et al 2022), is one of several synthetic met-enkephalin analogues that includes non-natural D-amino acids. It was developed for its growth hormone-releasing activity and is considered a growth hormone secretagogue (Dawood et al 2022). These GH secretagougues lack opioid activity but they are potent stimulators of GH release (Dawood et al 2022). These peptides are distinct from GHRH in that they share no sequence relation and derive their function through activation of a completely different receptor (wikipedia.org). This receptor was originally called the GH secretagogue receptor (GHSR), but due to subsequent research, the hormone ghrelin is now considered the receptor's natural endogenous ligand, and it has been renamed as the ghrelin receptor (wikipedia.org; Dawood et al 2022).

Growth hormone (GH) also known as somatotropin (STH) is a peptide hormone that stimulates the body growth, cell reproduction, and cell regeneration in humans, but also in other animals (Lu et al 2019). It is thus important in human body development (Melmed 2019; Brinkman et al 2021). GH also stimulates production of insulin-like growth factor-1 (IGF-1, produced in the liver, see Figure 1) (Ipsa et al 2019) and increases the concentration of glucose and free fatty acids (Greenwood & Landon 1966; Ranabir & Reetu 2011). It is a type of mitogen which is specific only to the receptors on certain types of cells. GH is a 191-amino acid, single-chain polypeptide that is synthesized, stored and secreted by somatotropic cells within the lateral wings of the anterior pituitary gland (Brinkman et al 2021; wikipedia.org).

Mechano-growth factor (MGF) is an anabolic peptide found in muscle, bone, tendon, neural, and cardiac tissue following periods of increased stress (see Figure 1). It is a split variant or isoform of IGF-1, also known as IGF-1Ec (Pennuto et al 2020). MGF acts by increasing stem cell proliferation in given tissues and thus allowing for a faster recovery (Kelly et al 2020; focalpointvitality.com).

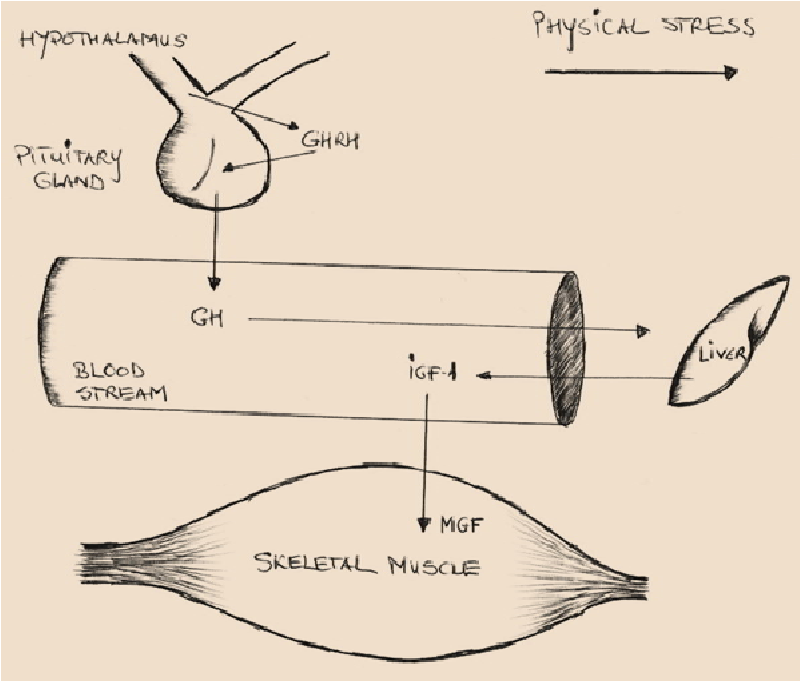

Figure 1. The cascade reaction for the synthesis of signal peptides, induced by physical effort (original drawing).

Muscular dystrophies. Unlike muscular atrophy, muscular dystrophy refers to a group of conditions involving the progressive loss of muscle mass and associated reduction in muscle strength, which are caused by a hereditary disease. The main forms of muscular dystrophy can affect up to 1 in 5000 men (reginamaria.ro). The most common type is Duchenne muscular dystrophy (Duan et al 2021). This type of distrophy generally affects young boys, but some forms of the dystrophy can also occur in adults (reginamaria.ro).

Muscular dystrophy is caused by genetic mutations that interfere with the production of muscle proteins needed to build and maintain healthy muscles (Duan et al 2021). Having a family history of muscular dystrophy increases the likelihood that the person will be affected (reginamaria.ro).

The rabbit as a model in the study of human muscular dystrophies. Duchenne muscular dystrophy is an X-linked muscle-wasting disorder caused by mutations occurred in the nucleotide sequence of dystrophin gene (Abreu & Waldrop 2021). It has an incidence of 1 in 3500 new male births (Sui et al 2018a). Mdx mice (*Mus musculus*) are widely used as a human model for Duchenne muscular dystrophy (van Putten et al 2019; Sun et al 2020). However, such mice do not faithfully recapitulate Duchenne muscular

dystrophy patients in many regards, rendering the preclinical data in this model questionable (Sui et al 2018a). Although bigger animal models of Duchenne muscular dystrophy, such as pigs (*Sus scrofa*) and dogs (*Canis familiaris*), have been created, use of these animals is costly and only limited to several facilities in the world. Sui et al (2018a) announced the creation of a rabbit organism (*O. cuniculus*) as a model of Duchenne muscular dystrophy by co-injection of Cas9 mRNA and sgRNA targeting exon 51 into rabbit zygotes. The Duchenne muscular dystrophy knockout (KO) rabbits (*O. cuniculus*) exhibit the typical phenotypes of Duchenne muscular dystrophy, including severely impaired physical activity, elevated levels of serum creatine kinase, and progressive muscle fibrosis or/and necrosis. They observed clear pathology in the cardium and in the diaphragm in 5 months old rabbits (*O. cuniculus*), similar to the pathology of Duchenne muscular dystrophy patients. Echocardiography recording showed that the Duchenne muscular dystrophy KO rabbits (*O. cuniculus*) had chamber dilation with decreased ejection fraction and fraction shortening (Sui et al 2018a). This new model rabbit (*O. cuniculus*), generated with the CRISPR/Cas9 system, mimics the histopathological and functional defects in Duchenne muscular dystrophy patients, and could be very useful for preclinical studies (Sui et al 2018a).

Another research was developed in the same year, by the same team (Sui et al 2018b). Miyoshi myopathy type 3 (MMD3) and limb girdle muscular dystrophy type 2L (LGMD2L) are cases of autosomal recessive muscular dystrophy generated by mutations in the sequence of the gene encoding anoctamin-5 (ANO5), which are part of anoctamin protein family (Sui et al 2018b; Soontrapa & Liewluck 2022). Two independent mice lines (*M. musculus*) with complete disruption of ANO5 transcripts did not show overt muscular dystrophy phenotypes; instead, one of these animals was observed to express abnormality in sperm motility (Sui et al 2018b). In contrast, a third line of ANO5-knockout (KO) mice with residual expression of truncated ANO5 expression was shown to display defective membrane repair and very mild pathology in the muscle (Sui et al 2018b). Many of the ANO5-related patients carry point mutations or small insertions/ deletions (indels) in the sequence of ANO5 gene (Sui et al 2018b). To more closely simulate the human ANO5 mutations, the researchers engineered mutant ANO5 rabbits (*O. cuniculus*) via co-injection of Cas9 mRNA and sgRNA into the rabbit zygotes (Sui et al 2018b). CRISPR-mediated small indels in the exon 12 and/or 13 in the mutant rabbits (*O. cuniculus*) lead to the development of specific signs of muscle dystrophy with increased serum creatine kinase, muscle necrosis, fatty replacement and fibrosis (Sui et al 2018b). This novel ANO5 mutant rabbit is useful in studying the disease pathogenesis and therapeutic treatments for ANO5-deficient muscular dystrophy, the authors say (Sui et al 2018b; Soontrapa & Liewluck 2022).

The last study we will discuss here is that of the Sui et al (2019) team. The syndromes of premature aging are rare genetic disorders mimicking clinical traits and molecular features of aging (Foo et al 2019; Lessel & Kubisch 2019). Products of the LMNA gene, primarily lamin A and lamin C, are the most important parts of the nuclear lamina (Sengupta & Sengupta 2022). A recently identified number of premature aging syndromes was related to mutations in the sequence of the LMNA gene (Sui et al 2019). Although LMNA disorders have been identified in premature aging syndromes, affect specifically the skeletal muscles, cardiac muscles, and lipodystrophy (Mosbah et al 2020), understanding the pathogenic mechanisms still need to be further elucidated (Sui et al 2019). To establish a rabbit KO model of premature aging syndromes, Sui et al (2019) performed precise LMNA targeting in rabbits via co-injection of Cas9/sgRNA mRNA into rabbit (*O. cuniculus*) zygotes. The LMNA-KO rabbits exhibited low locomotion activity with abnormal stiff walking posture and a shortened stature. All animals in the study died within 22 days. Moreover, muscular dystrophy, cardiomyopathy, joint and bone abnormalities, as well as lipodystrophy were reported by Sui et al (2019) in LMNA-KO rabbits (*O. cuniculus*). They concluded that the novel rabbit LMNA-KO model displayed typical features of histopathological defects that are observed in premature aging syndromes, and may be used as a valuable resource for understanding the pathophysiological mechanisms of syndromes of premature aging and elucidating mysteries of the normal process of aging in humans (Sui et al 2019).

Conclusions. Like other human models, the rabbit has again been used successfully to understand inherited human diseases. Several mutant lines of rabbits have been successfully used to mimic some types of muscular dystrophies known in humans. Although muscular dystrophies found in humans and animals cannot yet be completely cured, the development of molecular biology techniques opens new horizons for their treatment.

Conflict of interest. Authors declare no conflict of interest.

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