学位論文内容の要旨
（Summary of dissertation）

博士の専攻分野の名称　博士（医　学）　氏 名 汪煜民
（Degree conferred: Doctor of Philosophy）　（Name of recipient: Wang　Yumin）

学 位 論 文 題 名
（Title of dissertation）

Passive repetitive stretching is associated with greater muscle mass and cross-sectional area in sarcopenic muscle
(他動的反復ストレッチによるサルコペニア筋の筋量および筋断面積の増加)

Background and Aim: Age-associated loss of muscle mass and function, known as sarcopenia, is characterized by a progressive decline in muscle fiber number and size, a shift in fiber-type composition, and modification of adverse metabolic parameters. It is imperative to identify effective countermeasures that induce hypertrophy of the senescent muscle and combat sarcopenia progression. The regulation of skeletal muscle mass and fiber size is largely determined by the dynamic turnover between protein synthesis and degradation. Muscle hypertrophy occurs when the overall rate of protein synthesis exceeds the rate of protein degradation. Akt stimulates protein synthesis by activating mammalian target of the rapamycin (mTOR) and inhibits protein degradation through Forkhead box O (FoxO)-mediated ubiquitin-ligases including Muscle Atrophy F-box (MAFbx)/atrogin-1, and Muscle Ring Finger 1 (MuRF1) downregulation. Myostatin has been extensively studied as another potential mediator of sarcopenia owing to its potent negative effects on cell growth and intracellular catabolic and anabolic signaling pathways. In addition, skeletal muscle hypertrophy can be induced by the activation of muscle satellite cells. When activated, a surge of myogenic regulatory factors (MRFs), including myoblast determination protein 1 (MyoD), myogenic factor 5 (Myf5), and myogenin, is required owing to the role in driving the differentiation of myoblasts to mature myotubes. Passive stretching, a type of mechanical stimulus, is widely used in rehabilitation medicine to prevent muscle shortening, maintain the range of joints, and muscle flexibility. However, the mechanisms underlying muscle hypertrophy resulting from passive repetitive stretching remain poorly understood. Moreover, aging processes alter mechano-transduction, indicating a perturbed load-induced plasticity for aged muscle hypertrophy. Thus, the aim of this study was to (1) characterize the efficacy of clinically feasible protocols of daily passive repetitive stretching on healthy skeletal muscle mass, myofiber morphology, and then decipher the underlying mechanisms of action in terms of (a) signaling molecules involved in muscle protein synthesis and degradation, and (b) satellite cell content and MRFs expression. (2) further elucidate that whether or not passive repetitive stretching exerts hypertrophic effect against sarcopenic atrophy.

Methods and results: This study mainly consisted of two experiments. The *Experiment 1* using 6-weeks-old Institute of Cancer Research (ICR) mice and the *Experiment 2* using 35-weeks-old Senescence-Accelerated Mouse Prone-8 (SAM-P8) mice. I anesthetized the mice and stretched the right gastrocnemius muscles 15 times/min for 15 min daily, 5 days a week for 2 weeks. Contralateral unstretched muscles were used as controls. Twenty-four hours after the final stretching session, the gastrocnemius muscles of both legs of the mice were removed under deep anesthesia. In *Experiment 1*, I found that the muscle weight and the fiber cross-sectional area (CSA) of the stretched side was

greater than that of the unstretched side, analyzing with hematoxylin and eosin (H&E) staining. The messenger ribonucleic acid (mRNA) expression of MRFs and signaling molecules involved in muscle protein synthesis and degradation were detected by real time polymerase chain reaction (PCR). The mRNA expression of ribosomal protein S6 kinase beta-1 (p70S6K) and myogenin increased while eukaryotic translation initiation factor 4E-binding protein 1 (4E-BP1), MAFbx, MuRF1 and myostatin decreased on the stretched side as compared with the unstretched side. I found there was no significant difference in the number of paired box-7 (Pax7) + cells between the stretched and unstretched sides by immunohistochemistry. The number of myonuclei per fiber determined by H&E staining showed no difference in stretched and unstretched muscles. In *Experiment 2,* I found that the muscle weight and fiber cross-sectional area of the stretched side was greater compared with that of the unstretched side. Passive repetitive stretching increased the mRNA expression level of Akt, p70S6K, 4E-BP1, Myf5, myogenin and MuRF1.The phosphorylation level of p70S6K measured with Western blotting was significantly increased in stretched muscles as compared with unstretched ones. The Pax7+ cells and myonuclei content doesn't differ between stretched and unstretched muscle.

Discussion: Akt signaling enhances protein synthesis primarily via activation of mTORC1. The activation of mTOR1 mediates the phosphorylation of S6 kinase (S6K) and the inhibition of 4E-BP1. In the *Experiment 1*, I found that the mRNA expression of p70S6K significantly increased while 4E-BP1 expression decreased in the stretched side compared to the unstretched contralateral limb. In *Experiment 2*, I provided further evidence that in vivo repetitive stretching strongly increases the phosphorylation level of p70S6K. Overexpression of MuRF1 and MAFbx results in muscle atrophy. In *Expeiment 1*, I observed a decreased expression of MuRF1 and MAFbx after two weeks of passive repetitive stretching. In *Experiment 2*, however, I found that MuRF1 mRNA expression was elevated, indicating the involvement of the cellular degradation pathway in aged skeletal muscle adaptation to passive stretching. Another potential contributor to the increase in muscle mass and myofiber CSA is the significant decrease in myostatin expression as observed in *Experiment 1*. Myostatin inhibits muscle hypertrophy via negative regulation of myogenesis and protein synthesis; therefore, decreased levels of myostatin permit increased protein synthesis and muscle growth. Myostatin expression was not affected by stretching in *Experiment 2*. I also assumed that the hypertrophic or suppressed atrophic observations in passive repetitive stretching may result from satellite cells activation. However, there was no significant difference in the number of Pax7+ cells between the stretched and unstretched muscles in both ICR and SAM-P8 mice. Finally, the number of nuclei per fiber was measured in each muscle section to determine whether or not stretching result in satellite cell activation and incorporation of new nuclei. As a result, the stretched and unstretched muscles did not differ in both ICR and SAM-P8 mice. I am not convinced of the possibility of stretch-induced myogenesis without an increase in MyoD, Pax7 expression levels and new nuclei addition. These findings suggest the hypertrophic or suppressed atrophic observation in stretched muscles are mainly attributable to the protein turnover rather than triggering satellite cell activation and fusion.

Conclusion: Passive repetitive stretching for 2 weeks induces muscle hypertrophy via genetic regulation of Akt/mTOR and MuRF1/MAFbx pathway as well as Myostatin in healthy ICR mice. In addition, passive repetitive stretching proved to be effective in preserving muscle mass and fiber area in sarcopenic muscle in SAM-P8 mice.