



저작자표시-비영리-변경금지 2.0 대한민국

이용자는 아래의 조건을 따르는 경우에 한하여 자유롭게

- 이 저작물을 복제, 배포, 전송, 전시, 공연 및 방송할 수 있습니다.

다음과 같은 조건을 따라야 합니다:



저작자표시. 귀하는 원저작자를 표시하여야 합니다.



비영리. 귀하는 이 저작물을 영리 목적으로 이용할 수 없습니다.



변경금지. 귀하는 이 저작물을 개작, 변형 또는 가공할 수 없습니다.

- 귀하는, 이 저작물의 재이용이나 배포의 경우, 이 저작물에 적용된 이용허락조건을 명확하게 나타내어야 합니다.
- 저작권자로부터 별도의 허가를 받으면 이러한 조건들은 적용되지 않습니다.

저작권법에 따른 이용자의 권리는 위의 내용에 의하여 영향을 받지 않습니다.

이것은 [이용허락규약\(Legal Code\)](#)을 이해하기 쉽게 요약한 것입니다.

[Disclaimer](#)

Thesis of Master of Science

**The Effect of Resistance Training on Muscle
and Bone Quality in C57BL/6N mice: Related
to Fibroblast Growth Factor Signaling**

저항성 운동이 C57BL/6N 쥐의 근육과 뼈의 질에
미치는 영향: Fibroblast Growth Factor Signaling에 관하여

By

Suhan Cho

February 2017

Department of Physical Education

College of Education

Graduate School of Seoul National University

**The Effect of Resistance Training on Muscle
and Bone Quality in C57BL/6N mice: Related
to Fibroblast Growth Factor Signaling**

By Suhan Cho

A Thesis submitted in partial fulfillment of
The requirement for the degree of
MASTER OF SCIENCE

Supervisor: Wook Song, Ph.D.

February 2017

Approved by

Chairman : Lee, Yongho

Vice chairman : Kim, Yeon Soo

Committee : Song, Wook

**College of Education
Seoul National University**

ABSTRACT

Many studies identified that muscle and bone are functionally interlinked to one another and maintain the quality and strength of muscle and bone are important for quality of human life. Here, I examine the relationship linking Fibroblast growth factors, potential candidates for the role player in muscle and bone cross talk, to bone and muscle quality during growth and resistance exercise. To elucidate the relationship and potential role of FGFs and its signaling, FGFR inhibitor was used while 8 weeks of resistance training was conducted.

Administration of FGFR inhibitor decreased grip strength, reduced muscular endurance and running capacity. 8 weeks of inhibitor treatment did not affect muscle mass, body weight or body composition. Inhibitor treatment showed no difference with resistance trained group while the group without inhibitor treatment with exercise showed significant increase in grip strength, muscular and aerobic endurance capacity and maximal load lifted during session. RT-Sham group solely showed hind limb muscle mass increments. FGF2 gene expression responded to resistance training in

gastrocnemius muscle but, did not respond much in tibia. However, FGF2 gene expression in tibia exhibited the change in much larger scale than gastrocnemius muscle when inhibitor were treated. Myostatin gene expression in bone and muscle responded opposite to each other with 8 weeks of resistance training.

FGFR inhibitor treatment in normal and exercise condition both attenuated muscular strength, endurance and aerobic endurance capacity. 8 weeks of resistance training could not restore the detrimental effects, however seemed to affect FGF-2 mRNA expression in bone. Myostatin responded to resistance training and inhibitors in gastrocnemius muscle but one responded to resistance training in bone tissue. Further analysis is needed to elaborate the definite correlation between gene expressions and resistance training with or without inhibitor treatment.

Keywords: FGF-2, FGFR signaling, Muscle-Bone crosstalk, Resistance training

Student number: 2014-20986

CONTENTS

I. INTRODUCTION	- 1 -
1. Significance of the Study.....	- 1 -
2. Purpose of the Study.....	- 3 -
3. Research Hypothesis	- 3 -
II. LITERATURE REVIEW	- 4 -
1. Resistance Exercise Models of Animal	- 4 -
2. Resistance Exercise and Bone Quality	- 5 -
3. Fibroblast Growth Factor-2 and Bone	- 6 -
4. Fibroblast Growth Factor Signaling in Bone Formation	- 7 -
III.METHODS.....	- 8 -
1. Animal Model.....	- 8 -
2. Experimental Design	- 8 -
3. Exercise Apparatus and Protocol.....	- 10 -
4. Grip Strength Test.....	- 12 -
5. Muscular Endurance Capacity Test	- 13 -
6. Aerobic Endurance Capacity Test.....	- 14 -
7. Skeletal Muscle Dissection.....	- 15 -
8. Molecular Biological Analysis	- 16 -
9. MicroCT and PetCT analysis.....	- 18 -

IV.RESULT	- 19 -
1. Effect of FGFR Inhibitor (NVP-BGJ398) on Growth Adaptation	- 19 -
2. Effect of FGFR Inhibitor (NVP-BGJ398) on Resistance Training Adaptation....	- 22 -
3. FGF-2 and Myostatin Expression on Muscle and Bone	- 24 -
4. Effect of Resistance training and FGFR Inhibitor on Bone Quality	- 26 -
V. DISCUSSION.....	- 28 -
VI.CONCLUSION	- 33 -
VII. REFERENCE.....	- 34 -

LIST OF FIGURES

Figure 1. Experimental Design of Study	- 9 -
Figure 2. Resistance Training Protocol.....	- 11 -
Figure 3. Exercise Apparatus	- 11 -
Figure 4. Grip Strength Test	- 12 -
Figure 5. Muscular Endurance Capacity Test	- 13 -
Figure 6. Aerobic Endurance Capacity Test	- 14 -
Figure 7. In vivo PET/CT machine.....	- 18 -
Figure 8. FGFR inhibitor affects normal adaptation to gain strength	- 21 -
Figure 9. FGFR signaling is necessary for adaptation to resistance exercise ..	- 23 -
Figure 10. FGF2 and myostatin gene expressions on bone and muscle	- 25 -
Figure 11. 8 weeks of FGFR inhibitor treatment decreases bone quality	- 27 -

LIST OF TABLES

Table 1. Primers for Real-time PCR using SensiFAST SYBR Lo-ROX Mix protocol	- 16 -
--	--------

I. INTRODUCTION

1. Significance of the Study

The ability to maintain muscle and bone quality is important for life quality and longevity of human lifespan. Regular activity is considered as a great tool to provide many health benefits (Warburton, Nicol, & Bredin, 2006). Especially resistance exercise, regular basis resistance training promotes a prominent adaptation on individuals, such as increased strength and mass in muscle, increased bone density, and reduced risk of all-cause mortality (Christianson & Shen, 2013). Although enhanced muscle strength and bone quality are well defined beneficial effect on resistance training adaption, it is difficult to elaborate the close relationship of muscle and bone interlink as exercise effect on health benefits are achieved by multiple mechanisms.

As muscle and bone are neighboring organs, many scientists have speculated that they may have close relationship between each other in functional way; it is still covered with vague concepts. However, recent evidences suggested that muscle serves a role of a secreting organ which is named as secretome in some reviews (Pedersen, 2011; Hamrick, 2012). Muscle secreted protein called myokine plays autocrine, paracrine or endocrine role in other organs and may provide the evidence in muscle-and-bone-crosstalk (Kawao & Kaji, 2015 ; Karsenty & Olson, 2016). Novotny, S. et al reported that myostatin-induced muscle hypertrophy leads to increase in bone strength (2015). Myostatin knock-out mice was resulted in showing

significantly higher muscle mass on triceps brachii than wild type, suggesting that lean body mass somewhat correlated with bone strength.

This study focuses on the recently reevaluated exercise-induced growth factor, fibroblast growth factor 2 (FGF-2) and its receptor, fibroblast growth factor receptor (FGFR) signaling (Hamrick, 2011). Basic fibroblast growth factor (FGF-2) has a prominent role in proliferation and differentiation in many different cell types in vivo (Nagai, et al., 1995) and vitro (Canalis, et al., 1988). It is stored in extracellular matrix (ECM) and released during muscle contraction (Clarke, 1996; Hamrick, 2011). Binding FGF-2 on its receptor FGFRs, propagate the signaling pathway to enhance osteoblast differentiation.

There are some evidences suggesting that exercise has close linked to FGF-2 expression after acute exercise were taken (Clarke, Khakee, & McNeil, 1993; Hamrick, 2011). Many reviews declared that FGF-2, as a myokine, is secreted during muscle contraction, and this may have a potential role in muscle-bone cross talk (Pedersen, 2011; Hamrick, 2012 ; Karsenty & Olson, 2016). However, the physiological role of FGF-2 and its signaling during exercise remain to be determined.

From this study, we elucidate that resistance exercise, ladder climbing activity induced FGF-2 to enhance FGFR signaling in muscle and bone, and stimulated bone formation by exercise is paralleled to FGFR signaling in bone. Disturbing normal FGF-2 activity by using FGFR inhibitor, NVP-BGJ398 may result in reduced bone quality and increased bone resorption.

2. Purpose of the Study

The purpose of this study is to illustrate that exercise-induced FGF-2 and FGFR signaling are paralleled to increase in muscle and bone quality which is achieved by resistance training. To demonstrate the relationship, inhibiting FGFR signaling was used.

3. Research Hypothesis

- 1). 8 weeks of FGFR inhibitor (NVP-BGJ398) administration would decrease muscle and bone quality and attenuate the increase of size and strength of skeletal muscle which were induced by growth and resistance training
- 2). 8 weeks of FGFR inhibitor (NVP-BGJ398) administration would reduce FGF-2 expression and FGFR signaling in muscle and bone, and this may affect the muscle and bone quality changes adapted by resistance training
- 3). Inhibitor treated group would show the lowest exercise performance than the other groups

II. LITERATURE REVIEW

1. Resistance Exercise Models of Animal

The use of animal models for exercise have been widely accepted to mimetic human condition of hypertrophy (Cholewa et al., 2013). Many guidelines have been developed for human, but due to the difficulty of instruction, it is still difficult to provide the proper resistance training guideline for rodents.

Many research studies have used resistance exercise as an intervention for the effect of exercise, however not many studies were able to represent or derive the main adaptation of resistance exercise, hypertrophy. Previous study was done in 1990, progressive resistance exercise had successfully induced enlargement of rat muscle (Yarasheski et al., 1990). In contrast to the aforementioned study, Duncan et al. (1998) investigated the exercise effect on male Wistar rats (3 weeks old) that trained rats were able to lift up to 800g or 140% of their body weight after 26 weeks of training that involves climbing a 40cm vertical ladder with application of external loads attached on their tails. However, there were no difference in body weight or muscle wet weight of extensor digitorum longus (EDL) and soleus muscles observed between training group and sedentary control group.

During resistance training, maximum weight lifting ability of the rodents were improved while there was no improvement in force production nor muscle hypertrophy. Ladder climbing exercise with 3 days per week frequency is known to be the most effective on the flexor hallucis longus (FHL) muscles as 8 weeks of training increased 23% of absolute weight and 24% increases in myofibrillar protein (Hornberger and Farrar 2004). Lee et al. had conducted study with ladder climbing

exercise (1-m ladder with 2-cm grid steps and inclined at 85°), 8 weeks of resistance training showed 23.3% increase in muscle mass in FHL muscle.

2. Resistance Exercise and Bone Quality

The responses of bone to external loading is well known; increased weight bearing induced bone formation, but under extreme condition of microgravity or prolonged bed rest, reduces bone mineral density and increases bone resorption. Decreased bone density is critical for old age that increases mortality of aged individuals by increasing risk of fracture. (Consensus, 1993).

Exercise, especially resistance exercise, has great advantage on age-related bone loss without causing side effects (Shackelford et al., 2004; Gómez-Cabello, et al., 2012). Regular physical activity showed protective effects on age-related bone loss (Wolff, et al., 1999). In addition, resistance exercise group showed enhanced muscle and bone mass did not only happen in aged individuals but in young and adolescent youth group as well (Gunter et al, 2012). This suggested that effect of resistance training on bone mass increase is universal for all age and genders.

Exercise during growth can significantly increase the peak bone mass reached at young adulthood (Gunter et al., 2012). Resistance exercise increases bone formation and modulates mineral composition without adding new bone (Kohn, et al., 2008).

3. Fibroblast Growth Factor-2 and Bone

Fibroblast growth factor-2, also known as basic fibroblast growth factor (bFGF), has been identified to be involved in many biological functions, such as growth, cell survival, differentiation and vessel formation (Bikfalvi, et al., 1997). FGF-2 has been known to increase bone morphogenetic protein (BMP-2) to enhance bone formation (Nakamura et al., 2005). FGF-2 interacts with its receptors that situated on cell surface, called fibroblast growth factor receptors (FGFR) to initiate the signaling cascade. Four major receptor families have been discovered; FGFR1, FGFR2, FGFR3 and FGFR4. FGF-2 are involved with various growth related functions in various tissues, especially in muscle and bone (Bikfalvi et al., 1997). Templeton and Hauschka (1992) showed that both growth and differentiation are controlled by the interaction of FGF-2 with FGFR1. Inhibition of this activity may cause a growth retardation and lack of formation of muscle (Amaya, et al., 1993) .

Systemically injection of FGF-2 has an effect on bone formation in ovariectomized rats (Liang, Pun, & Wronski, 1999). Another study represented that FGF-2 stimulated demineralized rat showed 25% greater bone formation than untreated controls (Aspenberg & Lohmander, 1989).

In a recent review, they described that exercise may cause FGF-2 release and delivery to bone, thus exercise induced FGF-2 may have a potential role in muscle and bone cross talk (Hamrick, 2011).

4. Fibroblast Growth Factor Signaling in Bone Formation

FGF/FGFR signaling plays crucial role in skeletal development during prenatal and postnatal period (Ornitz & Marie, 2002; Marie, 2003). Several mice and humans FGFs studies showed chondrogenesis and osteoblastogenesis activities in skeleton by regulating the activity of chondroblast and osteoblast (Dailey et al., 2005; Marie et al., 2005)

FGF2 is known to regulate bone formation and bone mass in vivo (Montero et al., 2000). It was found that FGF2 controls the osteoblast differentiation and anabolic effect of bone by closely modulating parathormone (PTH) (Hurley et al., 2006) and Bone Morphogenetic Protein (BMP-2) in mice (Nakamura et al., 2005).

Receptor for FGF families, FGFR1 and FGR2 have different roles on bone formation (Marie et al., 2005). Recent researches in mice and humans have focused on FGFR1/2 signaling and its potential roles in the control of osteoblast gene expression and bone formation (Wilkie, 2005).

FGF-2 stimulates and activates signaling cascade in osteoblasts which regulate p38 mitogen activated protein kinase (MAPK), protein kinase C (PKC) and extracellular signal regulated kinases (ERKs). Upregulated signaling of such activities enhances osteoblast proliferation, osteoblast related gene expressions and survival (Marie, 2003).

Modulating ERK1/2 signaling was known as an essential signaling to induce cell proliferation in osteoblast precursor cells (Choi et al., 2008) .

III. METHODS

1. Animal Model

CL57BL/6N mice (8-week-old) were managed with the principles established by the college of veterinary medicine animal laboratory in Seoul National University (SNU). All animals were fed with Rodent chow 5057 (Purina Korea, Seoul, Korea) and food intake was recorded in consequence day. All mice were kept at 22 degree Celsius, 40~50% humidity and on a 12:12-h light-dark cycle. All experimental methods were approved by the SNU Institutional Animal Care and Use Committees. The certification number was SNU-150820-4-6

2. Experimental Design

All C57BL/6n mice were purchased from orient bio. (Stock name: C57BL/6NCrljBgi). The animals were randomly assigned to each of the following groups: inhibition control group (Inh-C), Sham control group (Sham-C), Resistance Training with Sham administration group (RT-Sham) and Resistance Training with inhibitor administration group (RT-Inh). At least 6-7 mice were conducted with experiment for each group. FGFR inhibitor NVP-BGJ398 (50mg/kg body weight; Novartis, Switzerland) or sham (PEG-300 [AppliChem]/Glucose 5%, 2:1 mix) were administered by oral gavage. Administration was initiated at 9 weeks old mice over

8 weeks with three treatments per week.

Grip strength and body weight measurement were recorded every week. Resistance training group conducted ladder climbing 3 times per week for 8 weeks. 48 hours after last training session, exercise performance tests were achieved and measured and rested for another 48 hours for recovery from the exhaustion stress.

Animals were then anesthetized and sacrificed to for blood and tissue collection. Following muscles were collected and measured, and other tissues were just collected for further analysis; muscle (Soleus, Gastrocnemius, Tibialis anterior, EDL), heart, kidney, lung, liver, and tibia.

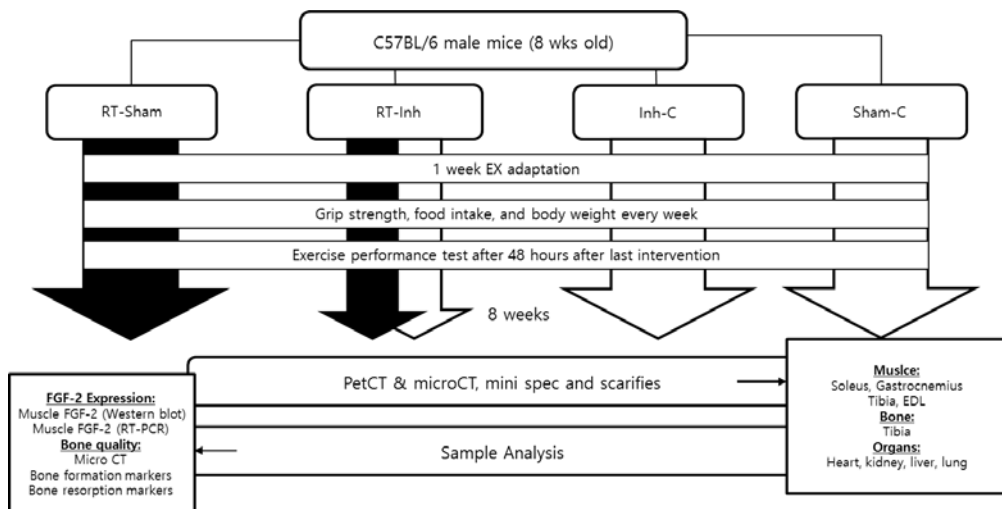


Figure 1. Experimental Design of Study

3. Exercise Apparatus and Protocol

Homberger's ladder training apparatus was adopted in this study for rodent resistance training. Our protocol consist of 8 weeks, 10 sessions a day, and 3days per week. Before the beginning of exercise training period, 1 week of adaptation was done without weight in order to get familiarized with the ladder (1m, 1cm grid, 80 degree incline) for a week. Initial load of 50% of body weight was applied to every individual and added 3g per every successive session. Next exercise trainings were started with 50-75% of maximal weight lifted from previous exercise.

The only encouragement was a gentle finger tapping on the animal's tail. When the rodents reached the top of the ladder, they were allowed to rest for 1~2 minutes. After the rest period, additional 3g was added on the previous weight, and the rodents were returned to the bottom of the ladder for the next session. If any mice were not able to climb the ladder with attached load, gave one more minutes for further rest then placed at the bottom of ladder. This procedure was repeated until ten climbs were achieved. The every initial loads were set at 50% of the maximal weight lifted of previous exercise training.

	Adaptation	Exercise Period							
	W0	W1	W2	W3	W4	W5	W6	W7	W8
Reps	10 reps	Max 10 reps							
Load	0-50% of BW	• Initial load = 50% BW • Next trial = 50 – 75% maximum weight lifted from previous exercise trial add 3g every successful reps (repeat with previous load if failed)							
Rest	2 min.								
Frequency	3 days/week								

Figure 2. Resistance Training Protocol



Figure 3. Exercise Apparatus

4. Grip Strength Test

Grip Strength test was measured to illustrate the maximal force of grasping object and it is known to represent the strength of an individual. Animals were allowed to grasp a greed which is attached to the force gauge and pulled until they are completely detached from the grid. Measured force was recorded for three times with at least two minutes of rest in between every trial of measurement. Only maximum force record was taken for the further analysis. The grip strength was measured with a grip strength meter (Model GS3, Bioseb), and measurement method was adopted and modified from the one which was reported previously (Meyer et al., 1979).

The grip strength was measured weekly basis after 24-48hours of the last exercise trial of the week. The animal grasped the steel greed with its four-limb then, its tail was gently pulled with the experimenter's fingers. Maximum force (g) was measured and recorded on the instrument



Figure 4. Grip Strength Test

5. Muscular Endurance Capacity Test

Hanging time was measured to represent the muscular endurance capacity of a mouse. Animals were allowed to familiarize on grid without weight less than 1 minute prior to the actual test. Every rodents conducted three trials and the maximum time was taken into result. Animal was placed on the 20 x 30 cm grid with 10g weight attached on tail, and flipped over to measure the time until mouse falls. In case the mouse was fallen within 10 sec, the animal was rested sufficiently and retested afterward. This was conducted 48 hours after last intervention of 8th week of training.



Figure 5. Muscular Endurance Capacity Test

6. Aerobic Endurance Capacity Test

Treadmill exhaustion test was performed to test endurance capacity of animal. Rodents were forced to run until complete exhaustion and time was measured for further analysis. For the analysis, Exer. 3/6 treadmill, (Columbus Instruments, Ohio) was used and no incline was applied throughout the test. Mice were stimulated at tail or posterior part of body when they were reluctant to run. Mice were indicated to run for 5 min. with 5m/min and thereafter speed was increased by 1min/min per every minute until exhaustion. Exhaustion was defined when mice were unable to run although mice were continuously stimulated more than 15 sec.



Figure 6. Aerobic Endurance Capacity Test

7. Skeletal Muscle Dissection

Anesthetized animals were dissected and sacrificed. Then, organs (heart, kidney, liver and lung), muscles (soleus, gastrocnemius, tibialis anterior, extensor digitorum longus), and bone (tibia) were collected and weighed. Collected samples were frozen with liquid nitrogen and stored for further molecular biological analysis. In order to secure the quality of tissues, all procedures were completed within 30 min. per mouse after anesthetic drug was injected.

8. Molecular Biological Analysis

RNA isolation and Real Time-PCR

Total RNA from mouse tissues and organ was extracted by using an RNA purification system (Introgen) following the manufacturer's protocol. mRNA was reverse-transcribed using AccuPower CycleScript RT PreMix (Bioneer, Daejeon, Korea) and RT-PCR was performed using SYBR Green and an ABI StepOne Real Time PCR instrument (Applied Biosystems, Cheshire, UK). Target gene expression was normalized with the control gene (GAPDH), and relative expression was quantified by the comparative Ct method $\Delta\Delta C_t$. The primer used for PCR are listed in table 1.

Table 1. Primers for Real-time PCR using SensiFAST SYBR Lo-ROX Mix protocol

Gene	Forward primer (5'-3')	Reverse primer (3'-5')
MYOSTATIN	AGTGGATCTAAATGAGGGCAGT	GTTTCCAGGCGCAGCTTAC
GAPDH	AGGTCGGTGTGAACGGATTG	TGTAGACCATGTAGTTGAGGTCA
FGF-2	AGGCCACTTCAAGGCC	GAAACAGTATGGCCTTCTGTCC
FGFR1	AACCTCTAACCGCAGAAC	GAGACTCCACTTCCACAG

Western blotting

Total proteins were extracted using PRO-PREP buffer (iNTRON biotechnology Inc., Seoul, Korea) containing phosphatase and protease inhibitors (Roche), separated by SDS-PAGES, transferred to PVDF membranes (Millipore, Billerica, MA, USA), and analyzed by western blotting. Primary antibodies against the following proteins were used:

The antibodies were diluted 1:1000 with TBS containing 0.1% (v/v) Tween-20 (TBST, Biosesang, Seongnam, Korea). The membranes were then incubated with a peroxidase-conjugated secondary antibody (AbClon, Seoul, Korea) and antibody-specific signals were detected by enhanced chemiluminescence and quantified using the microChemi 4.2 system (DNR bio Imaging Systems, Jerusalem, Israel).

9. MicroCT and PetCT analysis

Bone quality was measured with In vivo Na¹⁸F PET/CT (Positron Emission Tomography/ Computed Tomography) by NanoPET/CT in vivo pre-clinical imager (Mediso, Hungary). All procedures were conducted under supervision of Professor Lee, Ho Young, the department of nuclear medicine in Bundang Seoul National University Hospital. All mice were anesthetized during scanning procedure with isoflurane gas inhalation.



Figure 7. In vivo PET/CT machine

IV. RESULT

1. Effect of FGFR Inhibitor (NVP-BGJ398) on Growth Adaptation

Administration of FGFR inhibitor for 8 weeks did not affect the body weight growth of mice. Inh-C group (with FGFR inhibition) had no significant difference of their body weight compared to sham-C group (vehicle only) after the treatment as mean body weights of two groups at the end of experiment were 25.04 ± 0.57 g and 26.08 ± 0.47 g respectively (figure 8A). However, inhibitor administration attenuated strength gain during growing period. Sham-C group successfully achieved increase in their grip strength after 8 weeks of vehicle with oral gavage, while inhibitor treated group fail to represent the growth of their strength (figure 8B). Grip strength of Sham-C group was increased from 173 ± 1.96 g to 204.3 ± 2.42 g. 8 week of inhibitor treatment did not increase strength as initial strength (173 ± 3.17 g) and end strength (173 ± 5.34 g) was identical. Four of mice in Inh-C group even showed decreases in their strengths when compared to the baseline value.

Inhibitor treatment seemed to alter the muscular and aerobic endurance as 8 weeks of intervention decreased the physical performance of mice (figure 8G and H). Both the hanging time and time to exhaustion decreased in Inh-C group compared to Sham-C group and showed significance differences ($p = 0.02$ and 0.002 respectively).

There were no significance differences observed in adjusted muscle wet weight between Inh-C and Sham-C group although there were some differences observed in

grip strength and hanging time. Wild type resistance trained (WT-RT) group showed higher grip strength and hanging (data not suggested), and this is correspondence to the adjusted hind limb muscle mass of each group. However, there were no differences in Inh-C and Sham-C group and this might suggest that decreased strength may have been acquired from other mechanism not due to muscle mass decreases.

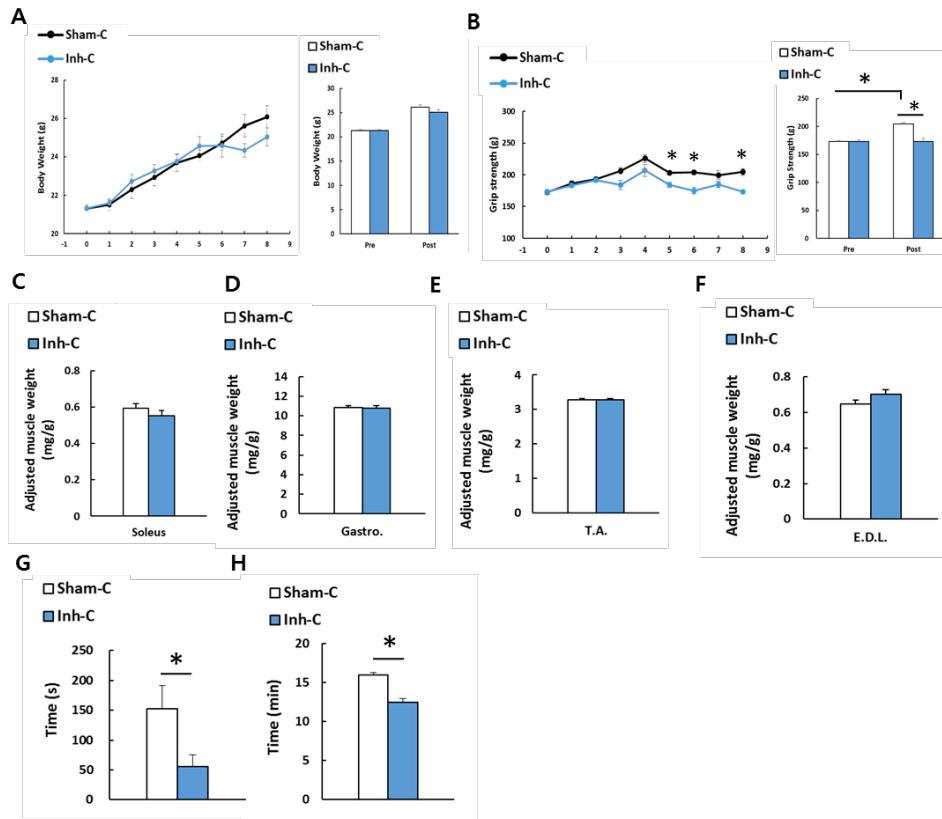


Figure 8| FGFR inhibitor affects normal adaptation to gain strength

(A) Body weight changes during 8 weeks of FGFR inhibitor treatment (Inh-C, n = 7, NVP-BGJ398, 50mg/kg BW) and vehicles (Sham-C, n = 7, PEG 300: glucose 5%, 2:1 mix)

(B) Grip strength changes during 8 weeks of FGFR inhibitor treatment (Inh-C, n = 7, NVP-BGJ398, 50mg/kg BW) and vehicles (Sham-C, n = 7, PEG 300: glucose 5%, 2:1 mix)

(C-F) Muscle wet weight adjusted with body weight E) Soleus, F) Gastrocnemius, G) Tibialis anterior and H) Extensor digitorum longus muscle

(G and H) Physical Performance test after 8 weeks of FGFR inhibitor treatment (Inh-C, n = 7, NVP-BGJ398, 50mg/kg BW) and vehicles (Sham-C, n = 7, PEG 300: glucose 5%, 2:1 mix)

G) Hanging Test, and H) Endurance Test

|All values are presented as mean±SEM, *p<0.05, **p<0.01

2. Effect of FGFR Inhibitor (NVP-BGJ398) on Resistance Training Adaptation

Resistance training increased strength gain, adjusted muscle wet weight of hind limb, hanging time and maximal load lifted from WT control (data not showed). However, inhibition treatment failed to deliver the training adaption of strength, maximal load lifted and hanging time as it suggested on figure 2. Grip strengths of resistance training with sham injection (RT-Sham) group showed increased in grip strength from before training ($204.68 \pm 5.60\text{g}$ to $239.67 \pm 4.25\text{g}$), however resistance training with inhibitor treated (RT-Inh) group maintained their strength as same level as baseline (figure 9B). Maximum load lifted were measured each session to adjust the intensity of training. RT-Inh group showed similar incremental pattern of maximal load lifted to RT-Sham group up to 2nd week of training, however failed to adapt the training overload. RT-Sham group ended up lifting $78 \pm 0.92\text{g}$ while RT-Inh group only lifted $50 \pm 2.45\text{g}$ (figure 9H).

8weeks of resistance training were not able to overcome the detrimental effect of FGFR inhibitor as RT-Sham group solely showed resistance training adaptation on muscular endurance (figure 9J). RT-Inh group could not restore in aerobic capacity although resistance training was conducted. Adjusted muscle wet weight of hind limb and body composition did not showed any significant difference in any value. When it compared to Sham-C and Inh-C group, only lean mass showed significance difference between Inh-C and RT-Sham group, however when this was adjusted to percentage lean body mass, difference was not strong enough to derive any significance.

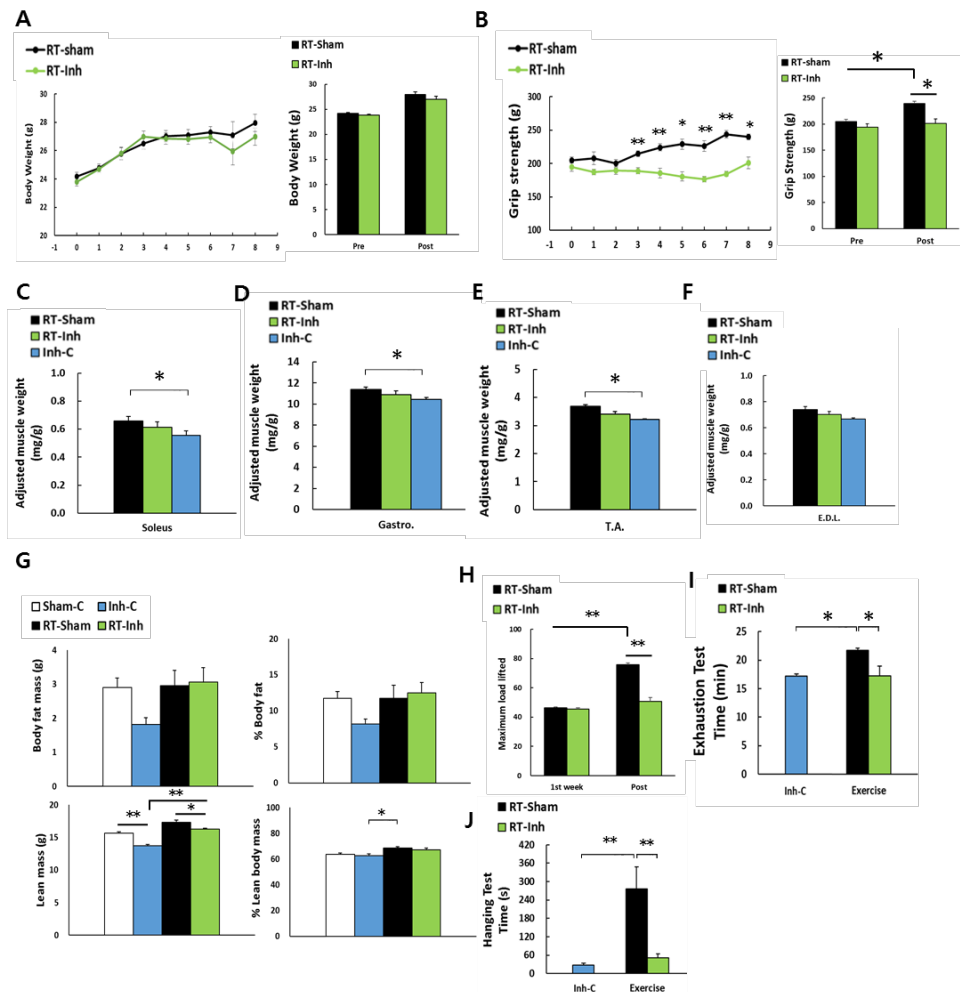


Figure 9| FGFR signaling is necessary for adaptation to resistance exercise

(A) Body weight changes during 8 weeks of resistance training with FGFR inhibitor treatment (RT-Inh, n = 6, NVP-BGJ398, 50mg/kg BW) or vehicles (RT-Sham, n = 6, PEG 300: glucose 5%, 2:1 mix)

(B) Grip strength changes during 8 weeks of resistance training (RT) with FGFR inhibitor treatment (NVP-BGJ398, 50mg/kg BW) or vehicles (PEG 300: glucose 5%, 2:1 mix)

(C-F) Muscle wet weight adjusted with body weight E) Soleus, F) Gastrocnemius, G) Tibialis anterior and H) Extensor digitorum longus muscle

(G) Body composition analysis with mini spec

(H-J) Physical Performance test after 8 weeks of resistance training (RT) with FGFR inhibitor treatment (NVP-BGJ398, 50mg/kg BW) or vehicles (PEG 300: glucose 5%, 2:1 mix), H) Maximum load lifted

I) Endurance Test, and J) Hanging Test

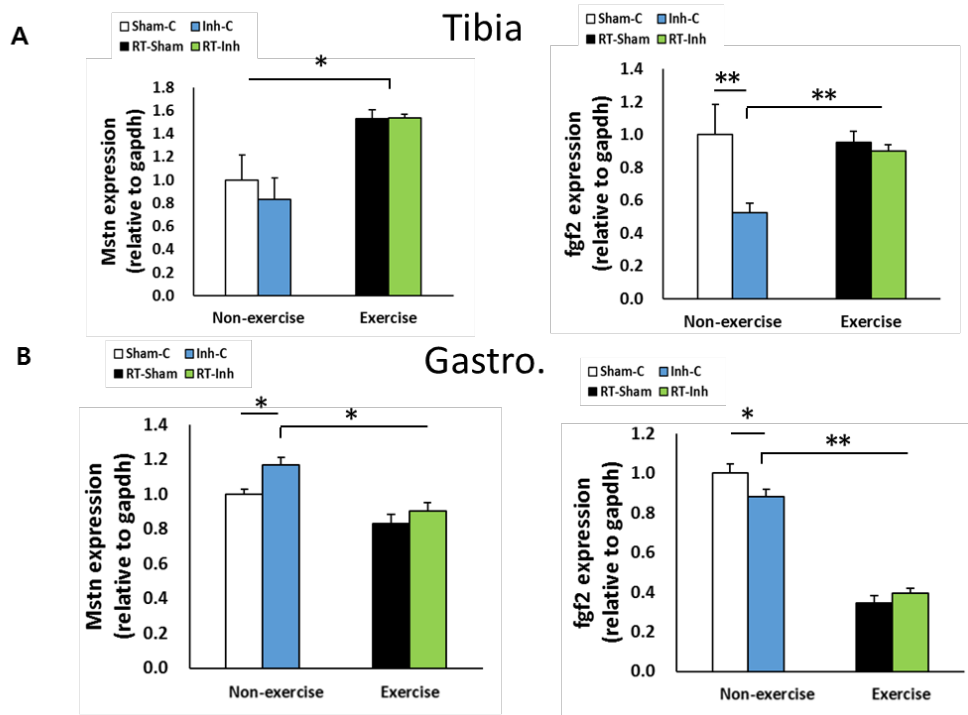
|All values are presented as mean $\pm$ SEM, *p<0.05, **p<0.01

3. FGF-2 and Myostatin Expression on Muscle and Bone

There were no differences found in *fgfr1* gene expression between muscle and bone in wild type (data not shown). However, *fgf2* gene expression showed 2-3 fold higher expression in gastrocnemius muscle when it compared to tibia (figure 10A). During FGFR inhibitor treatment, *fgf2* gene expression significantly dropped in both muscle (gastro.) and bone (tibia) (figure 10C and D). Decreased expression in muscle was only 0.88 fold but much larger scale in bone as expression dropped to 0.5 fold from Sham-C group. This attenuated expression was completely restored to sham control level when exercise training was conducted with inhibitor treatment. Counterintuitively, in gastrocnemius muscle, resistance training decreased expression of *fgf2* by 3-5 fold in both RT-Sham and RT-Inh group. RT-Inh group showed slight increase in expression but was not comparable to training effect.

Myostatin gene expression was not changed with inhibitor in bone, however it was elevated in muscle (figure 10C and D). Resistance training response seemed to happen in both of organs, but in completely different manner. 8 weeks of resistance training elevated myostatin gene expression compared to Sham-C and Inh-C group. No difference was observed between trained groups (figure 10C).

Likewise the expectation, resistance training attenuated myostatin gene expression in gastrocnemius muscle. There were same tendency of inhibitor effect was observed between trained group and non-trained group.



4. Effect of Resistance training and FGFR Inhibitor on Bone Quality

8 weeks of inhibitor treatment reduced bone quality in growing and resistance training condition (figure 12A & B). Using standardized uptake values (SUV) is a perpetual method to display PET/CT imaging and intensity of image reflect the density of bone mineral structure. Sham group showed inhibitor significant difference in averaged SUV (figure 12C) and this is correspondence to the image data of both group (figure 12A).

8 weeks of resistance training could not restore decreased bone quality that was induced by FGFR inhibitor treatment. Resistance training during growing period increases bone quality (Wallace et al., 2007), however, this phenomenon did not occurred in RT-Inh group. RT-Inh group and Inh-C group did not show any difference while RT-sham group showed significantly higher bone quality (figure 12D).

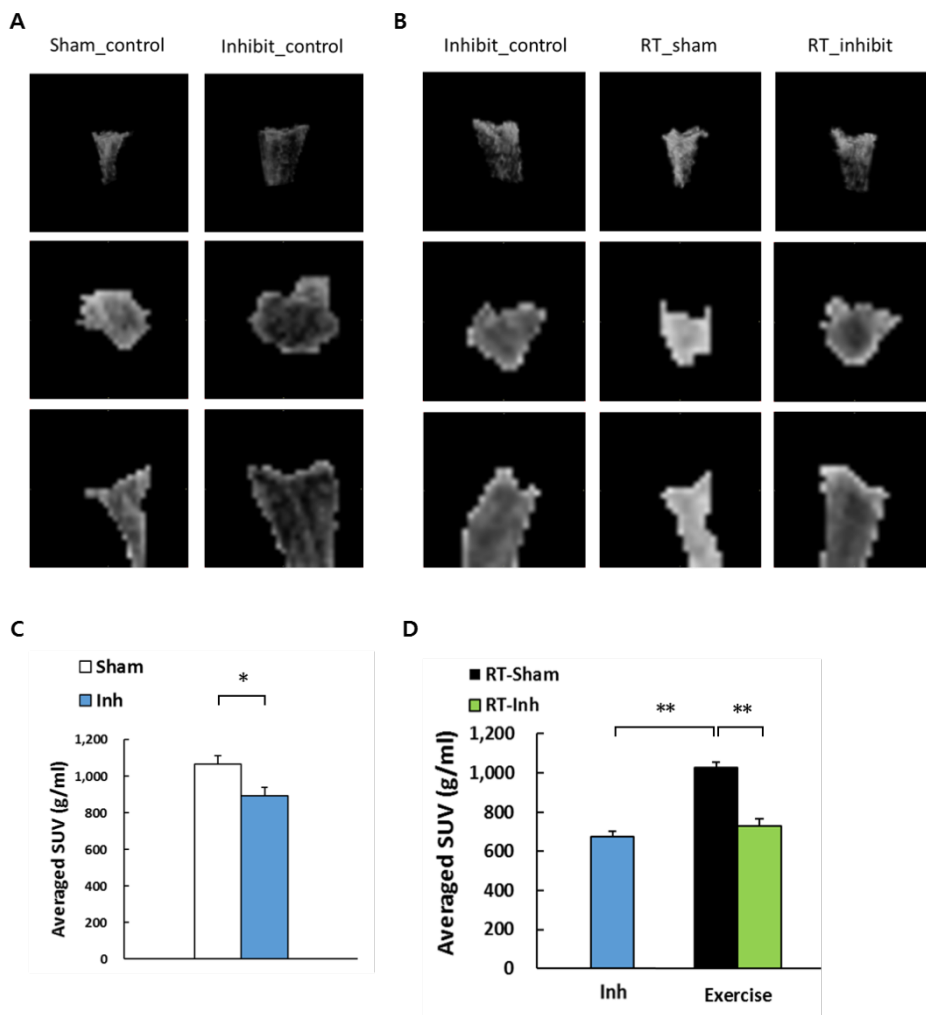


Figure 11 | 8 weeks of FGFR inhibitor treatment decreases bone quality

(A and B) Micro-CT image of representative animals in A) Sham-C and Inh-C, and B) Inh-C, RT-Sham, and RT-Inh group

(C and D) Averaged SUV (g/ml) value from NaF18-PET/CT scan of C) during 8 weeks of FGFR inhibitor treatment (Inh-C, n = 7, NVP-BGJ398, 50mg/kg BW) and vehicles (Sham-C, n = 7, PEG 300: glucose 5%, 2:1 mix) or D) 8 weeks of resistance training with FGFR inhibitor treatment (RT-Inh, n = 6, NVP-BGJ398, 50mg/kg BW) or vehicles (RT-Sham, n = 6, PEG 300: glucose 5%, 2:1 mix)

[All values are presented as mean±SEM, *p<0.05, **p<0.01

V. DISCUSSION

FGFR signaling is important in normal growth adaptation

Gain strength, muscle and body weight are natural phenomenon can be observed throughout all mice during growing period. Although body weight and muscle mass did not show any significant differences during 8 weeks of administration of FGFR inhibitor (figure 8A, C-F, and figure 9G), there were tendency of lower body weight, muscle mass and lean mass in Inh-C group compared to all other group. With regard to exercise performance such as grip strength, muscular endurance and aerobic endurance capacity, there were distinctive differences between Inh-C and Sham-C group. Inh-C group failed to increase their grip strength while Sham-C group successfully represented increase in strength during normal growth. Related to grip strength value, inhibitor treated mice displayed weaker characteristics compared to Sham-C group when they were conducted hanging and treadmill all-out test (figure 8G and H). These data seem to represent that FGFR signaling is important in growth adaptation. During growing period of all animals, growth factors play critical roles in cell proliferation and differentiation (Baker, et al., 1993; Lupu, et al., 2001). As FGF-2 and FGFR signaling are well determined growth factor and its signaling, inhibition of this signal may lead to growth retardation (Eswarakumar, Lax, & Schlessinger, 2005). FGF-2 knock out mice did not show any distinctive defection in appearance although it has been known to involve many different signaling in developmental, physiological and pathological processes (Okada-Ban, Thiery, &

Jouanneau, 2000). However, other study represented that there were neuronal defection with reduced blood pressure as well as some phenotype abnormalities (Dono, et al., 1998) When FGFR1 and FGFR2 genes are conditionally mutated or knocked out, human and mice exhibited a skeletal growth abnormalities (White et al., 2005; Yu et al., 2003). The result of this study showed correspondence result of the previous literature. Therefore, FGFR signaling may play a potential role in normal growth of strength and endurance when all the evidence were considered into account.

FGFR signaling may have an important role in adaptation to resistance exercise

There were no distinctive changes observed in body weight between groups. Both groups were conducted with 8 weeks of ladder climbing as a resistance training, and while one group treated with FGFR inhibitor the other group was administrated with same volume of vehicle. However, there were strong detrimental effect on RT-Inh group. 8 weeks of resistance training could not restore the effect of inhibitor that was observed in sham and inhibitor control group. With regard to the maximum load lifted during session, up to second weeks inhibitor group showed similar incremental of the load lifted (data not shown). However, since that point, inhibition treated group could not lift anything heavier and barely coped with exercise intensity or fail to continue with training. At the very last stage of training session, maximum load lifted values were significantly different between the groups which is correspondence to the grip strength values.

There were clear effect of resistance training on muscle weight gain when hind limb muscle mass were compared between groups. Resistance training showed significant increase in absolute lean mass, but when these were compared with percentage lean mass, there were only tendency of higher mass in resistance training groups. Likewise inhibition and sham treated group, RT-Sham and RT-Inh group showed similar trend in exercise performance test of muscular and aerobic endurance capacity test. Exercise training could not completely restore the detrimental effect that was occurred in non-exercise group. These physiological represents all sum up to show that lack of FGFR signaling may cause harmful effect on strength and endurance on muscle and it is important in adaptation of resistance training.

During resistance training, different physiological adaptation occurs. FGF-2 is a potential growth factors that induced by exercise and known to play important regulatory role in growth and survival. FGF-2 mRNA expression were relatively unchanged during exercise in bone, however, resistance training decreased FGF-2 mRNA expression by 3 fold in gastrocnemius muscle. In gastrocnemius muscle, myostatin gene expression was decreased after resistance training and increased after inhibitor was treated. As myostatin is one of strong negative regulatory cytokine of muscle growth (Lee & McPherron, 2001), this data seems to represent the tendency of muscle mass and exercise related performance test. Furthermore, FGF-2 and myostatin gene expression have negative correlation to each other (LIU et al., 2006) and this is correspondence to the treatment and exercise intervention. Counterintuitively resistance training decreased fgf2 gene expression in gastrocnemius muscle (figure 3D), and further analysis are needed to interpret this phenomenon. Previous study from our laboratory reported reduction of FGF-2 protein expression in soleus muscle after resistance training in aging mice (Kim, et

al., 2016).

Based on these data, FGF-2 may not seem to respond to resistance training adaptation. However, some studies have shown that FGF-2 responded to muscular damage and inflammation (Dailey et al., 2005; Meyer et al., 1995). In addition, such status like trained individual, decreased inflammation (Beavers, et al., 2010; Botezelli et al., 2016) may be turned out as result in FGF-2 level decreases in muscle. Therefore, further studies are needed to clarify the close relationship between FGF-2 expression and resistance training. FGFR signaling may act independently or differently from FGF-2 mRNA level in muscle while FGF-2 mRNA level changes were different from muscle and bone. As FGF-2 molecules are also produced and stored in bone (Baylink et al., 1993), and this may affect the muscle and bone quality by binding FGFR on muscle and bone.

FGFR signaling is important in bone quality during growth and resistance training

Resistance training is a common method to increase bone quality during normal and aged individuals. There are evidences of performing resistance training during growing period, significantly increases bone quality (Wallace et al., 2007). From the result of this experiment, resistance training fail to restore the attenuated bone quality of NVP-BGJ treatment (figure 12) which inhibits FGFR signaling systemically. While FGFR signaling has an important role in osteogenesis and bone formation (Eswarakumar, Lax, & Schlessinger, 2005), result of this study supported initial hypothesis of adverse effect of FGFR inhibition on bone quality.

Although resistance training elevated FGF-2 expression in bone (tibia) from Inh-C group regardless the inhibitor treatment, elevated FGF-2 expression did not influence on bone quality if you compare the image data of micro CT (figure 12B) and pet/CT SUV level (figure 12D). Elevated expression were expected to modulate bone quality, however, opposite to our assumption, these changes failed to increase bone quality of mice.

VI. CONCLUSION

Resistance training applied external load to muscle and bone to adapt onto the stress and from the result increase muscle and bone quality. However, although external load were applied to both of training group, only one group (sham treated group) was able to adapt with training. This might state that applying external load cannot overcome the adverse effect of FGFR signaling inhibition. In other word, FGFR signaling may have a potential role in strength, muscular and aerobic endurance, and bone quality during growth and resistance training. To clarify the close relationship between FGFR signaling and muscle and bone quality, further analysis are needed to elaborate muscle and bone crosstalk. As NVP-BGJ398 is not a specific inhibitor for certain FGFR, and presence of FGFR and FGF families are ubiquitous in whole organ in the body, specific knock out model might be required to test whether local inhibition changes whole body physiology.

VII. REFERENCE

- Amaya, E., Stein, P. A., Musci, T. J., & Kirschner, M. W. (1993). FGF signalling in the early specification of mesoderm in *Xenopus*. *Development*, 118(2), 477-487.
- Aspenberg, P., & Lohmander, L. S. (1989). Fibroblast growth factor stimulates bone formation bone induction studied in rats. *Acta orthopaedica Scandinavica*, 60(4), 473-476. doi: [http://dx.doi.org/10.1016/S0092-8674\(05\)80085-6](http://dx.doi.org/10.1016/S0092-8674(05)80085-6)
- Baker, J., Liu, J.-P., Robertson, E. J., & Efstratiadis, A. (1993). Role of insulin-like growth factors in embryonic and postnatal growth. *Cell*, 75(1), 73-82. doi:[http://dx.doi.org/10.1016/S0092-8674\(05\)80085-6](http://dx.doi.org/10.1016/S0092-8674(05)80085-6)
- Baylink, D. J., Finkelman, R. D., & Mohan, S. (1993). Growth factors to stimulate bone formation. *Journal of Bone and Mineral Research*, 8(S2), S565-S572. doi:10.1002/jbmr.5650081326
- Beavers, K. M., Brinkley, T. E., & Nicklas, B. J. (2010). Effect of exercise training on chronic inflammation. *Clinica Chimica Acta*, 411(11-12), 785-793. doi:<http://dx.doi.org/10.1016/j.cca.2010.02.069>
- Bikfalvi, A., Klein, S., Pintucci, G., & Rifkin, D. B. (1997). Biological Roles of Fibroblast Growth Factor-2 1. *Endocrine reviews*, 18(1), 26-45. doi: <http://dx.doi.org/10.1210/edrv.18.1.0292>
- Botezelli, J. D., Coope, A., Ghezzi, A. C., Cambri, L. T., Moura, L. P., Scariot, P. P., Pauli, J. R. (2016). Strength Training Prevents Hyperinsulinemia, Insulin Resistance, and Inflammation Independent of Weight Loss in Fructose-Fed Animals. *Scientific Reports*, 6. doi: 10.1038/srep31106.
- Canalis, E., Centrella, M., & McCarthy, T. (1988). Effects of basic fibroblast growth

- factor on bone formation in vitro. *Journal of Clinical Investigation*, 81(5), 1572. doi: 10.1172/JCI113490.
- Choi, S.-C., Kim, S.-J., Choi, J.-H., Park, C.-Y., Shim, W.-J., & Lim, D.-S. (2008). Fibroblast growth factor-2 and-4 promote the proliferation of bone marrow mesenchymal stem cells by the activation of the PI3K-Akt and ERK1/2 signaling pathways. *Stem cells and development*, 17(4), 725-736. doi:10.1089/scd.2007.0230.
- Christianson, M. S., & Shen, W. (2013). Osteoporosis prevention and management: nonpharmacologic and lifestyle options. *Clinical obstetrics and gynecology*, 56(4), 703-710.
- Clarke, M., Khakee, R., & McNeil, P. L. (1993). Loss of cytoplasmic basic fibroblast growth factor from physiologically wounded myofibers of normal and dystrophic muscle. *Journal of Cell Science*, 106(1), 121-133.
- Consensus, A. (1993). Consensus development conference: diagnosis, prophylaxis, and treatment of osteoporosis. *Am J Med*, 94(6), 646-650.
- Dailey, L., Ambrosetti, D., Mansukhani, A., & Basilico, C. (2005). Mechanisms underlying differential responses to FGF signaling. *Cytokine & Growth Factor Reviews*, 16(2), 233-247.
- Dono, R., Texido, G., Dussel, R., Ehmke, H., & Zeller, R. (1998). Impaired cerebral cortex development and blood pressure regulation in FGF-2-deficient mice. *The EMBO journal*, 17(15), 4213-4225.
- Eswarakumar, V. P., Lax, I., & Schlessinger, J. (2005). Cellular signaling by fibroblast growth factor receptors. *Cytokine & Growth Factor Reviews*, 16(2), 139-149. doi:10.1093/emboj/17.15.4213.
- Gómez-Cabello, A., Ara, I., González-Agüero, A., Casajús, J. A., & Vicente-

- Rodríguez, G. (2012). Effects of Training on Bone Mass in Older Adults. *Sports Medicine*, 42(4), 301-325. doi:10.2165/11597670-000000000-00000
- Gunter, K. B., Almstedt, H. C., & Janz, K. F. (2012). Physical activity in childhood may be the key to optimizing lifespan skeletal health. *Exercise and Sport Sciences Reviews*, 40(1), 13. doi: 10.1097/JES.0b013e318236e5ee.
- Hamrick, M. W. (2011). A role for myokines in muscle-bone interactions. *Exercise and Sport Sciences Reviews*, 39(1), 43. doi: 10.1097/JES.0b013e318201f601
- Hamrick, M. W. (2012). The skeletal muscle secretome: an emerging player in muscle-bone crosstalk. *BoneKEY reports*, 1(4). doi:10.1038/bonekey.2012.60
- Hurley, M., Okada, Y., Xiao, L., Tanaka, Y., Ito, M., Okimoto, N., . . . Coffin, J. (2006). Impaired bone anabolic response to parathyroid hormone in *Fgf2*^{-/-} and *Fgf2*^{+/-} mice. *Biochemical and Biophysical Research Communications*, 341(4), 989-994. doi: <http://dx.doi.org/10.1016/j.bbrc.2006.01.044>
- Karsenty, G., & Olson, Eric N. (2016). Bone and Muscle Endocrine Functions: Unexpected Paradigms of Inter-organ Communication. *Cell*, 164(6), 1248-1256. doi:<http://dx.doi.org/10.1016/j.cell.2016.02.043>
- Kawao, N., & Kaji, H. (2015). Interactions Between Muscle Tissues and Bone Metabolism. *Journal of Cellular Biochemistry*, 116(5), 687-695. doi:10.1002/jcb.25040
- Kim, J.-S., Yoon, D. H., Kim, H.-j., Choi, M.-j., & Song, W. (2016). Resistance exercise reduced the expression of fibroblast growth factor-2 in skeletal muscle of aged mice. *Integrative Medicine Research*.
- Kohn, D. H., Sahar, N. D., Wallace, J. M., Golcuk, K., & Morris, M. D. (2008).

- Exercise alters mineral and matrix composition in the absence of adding new bone. *Cells Tissues Organs*, 189(1-4), 33-37. doi:10.1159/000151452
- Lee, S.-J., & McPherron, A. C. (2001). Regulation of myostatin activity and muscle growth. *Proceedings of the National Academy of Sciences*, 98(16), 9306-9311. doi: 10.1073/pnas.151270098
- Liang, H., Pun, S., & Wronski, T. J. (1999). Bone anabolic effects of basic fibroblast growth factor in ovariectomized rats. *Endocrinology*, 140(12), 5780-5788. doi:10.1210/endo.140.12.7195
- LIU, H. Z., Li, Q., YANG, X. Y., Liu, L., Liu, L., AN, X. R., & CHEN, Y. F. (2006). Expression of basic fibroblast growth factor results in the decrease of myostatin mRNA in murine C2C12 myoblasts. *Acta biochimica et biophysica Sinica*, 38(10), 697-703. DOI: 10.1111/j.1745-7270.2006.00215.
- Lupu, F., Terwilliger, J. D., Lee, K., Segre, G. V., & Efstratiadis, A. (2001). Roles of Growth Hormone and Insulin-like Growth Factor 1 in Mouse Postnatal Growth. *Developmental biology*, 229(1), 141-162. doi:http://dx.doi.org/10.1006/dbio.2000.9975
- Marie, P. (2003). Fibroblast growth factor signaling controlling osteoblast differentiation. *Gene*, 316, 23-32. doi: http://dx.doi.org/10.1016/S0378-1119(03)00748-0
- Marie, P., Coffin, J., & Hurley, M. (2005). FGF and FGFR signaling in chondrodysplasias and craniosynostosis. *Journal of Cellular Biochemistry*, 96(5), 888-896. doi: 10.1002/jcb.20582
- Meyer, G. E., Yu, E., Siegal, J. A., Petteway, J. C., Blumenstein, B. A., & Brawer, M. K. (1995). Serum basic fibroblast growth factor in men with and without

- prostate carcinoma. *Cancer*, 76(11), 2304-2311. doi: 10.1002/1097-0142(19951201)76:11<2304::AID-CNCR2820761119>3.0.CO;2-N
- Nagai, H., Tsukuda, R., & Mayahara, H. (1995). Effects of basic fibroblast growth factor (bFGF) on bone formation in growing rats. *Bone*, 16(3), 367-373. doi: [http://dx.doi.org/10.1016/8756-3282\(94\)00049-2](http://dx.doi.org/10.1016/8756-3282(94)00049-2)
- Nakamura, Y., Tensho, K., Nakaya, H., Nawata, M., Okabe, T., & Wakitani, S. (2005). Low dose fibroblast growth factor-2 (FGF-2) enhances bone morphogenetic protein-2 (BMP-2)-induced ectopic bone formation in mice. *Bone*, 36(3), 399-407. <http://dx.doi.org/10.1016/j.bone.2004.11.010>
- Okada-Ban, M., Thiery, J. P., & Jouanneau, J. (2000). Fibroblast growth factor-2. *The International Journal of Biochemistry & Cell Biology*, 32(3), 263-267. doi:[http://dx.doi.org/10.1016/S1357-2725\(99\)00133-8](http://dx.doi.org/10.1016/S1357-2725(99)00133-8)
- Ornitz, D. M., & Marie, P. J. (2002). FGF signaling pathways in endochondral and intramembranous bone development and human genetic disease. *Genes & development*, 16(12), 1446-1465. doi: 10.1101/gad.990702
- Pedersen, B. K. (2011). Muscles and their myokines. *Journal of Experimental Biology*, 214(2), 337-346. doi:10.1242/jeb.048074
- Shackelford, L., LeBlanc, A., Driscoll, T., Evans, H., Rianon, N., Smith, S., Lai, D. (2004). Resistance exercise as a countermeasure to disuse-induced bone loss. *Journal of Applied Physiology*, 97(1), 119-129. doi: 10.1152/japplphysiol.00741.2003.
- Wallace, J. M., Rajachar, R. M., Allen, M. R., Bloomfield, S. A., Robey, P. G., Young, M. F., & Kohn, D. H. (2007). Exercise-induced changes in the cortical bone of growing mice are bone-and gender-specific. *Bone*, 40(4), 1120-1127. doi: <http://dx.doi.org/10.1016/j.bone.2006.12.002>.

- Warburton, D. E., Nicol, C. W., & Bredin, S. S. (2006). Health benefits of physical activity: the evidence. *Canadian medical association journal*, 174(6), 801-809. doi: 10.1503/cmaj.051351.
- White, K. E., Cabral, J. M., Davis, S. I., Fishburn, T., Evans, W. E., Ichikawa, S., McLellan, N. J. (2005). Mutations that cause osteoglophonic dysplasia define novel roles for FGFR1 in bone elongation. *The American Journal of Human Genetics*, 76(2), 361-367. doi: <http://dx.doi.org/10.1086/427956>
- Wilkie, A. O. (2005). Bad bones, absent smell, selfish testes: the pleiotropic consequences of human FGF receptor mutations. *Cytokine & Growth Factor Reviews*, 16(2), 187-203. doi: <http://dx.doi.org/10.1016/j.cytogfr.2005.03.001>.
- Wolff, I., Van Croonenborg, J., Kemper, H., Kostense, P., & Twisk, J. (1999). The effect of exercise training programs on bone mass: a meta-analysis of published controlled trials in pre-and postmenopausal women. *Osteoporosis international*, 9(1), 1-12. doi: 10.1007/s001980050109.
- Yu, K., Xu, J., Liu, Z., Sasic, D., Shao, J., Olson, E. N., . . . Ornitz, D. M. (2003). Conditional inactivation of FGF receptor 2 reveals an essential role for FGF signaling in the regulation of osteoblast function and bone growth. *Development*, 130(13), 3063-3074. doi:10.1242/dev.00491

국 문 초 록

저항성 운동이 C57BL/6N 쥐의 근육과 뼈의 질에 미치는 영향: Fibroblast Growth Factor Signaling에 관하여

조 수 한

서울대학교 대학원

체육교육과

서론:

근육의 질과 뼈의 질을 좋은 상태로 유지하는 것은 노년의 삶의 질 향상과 밀접한 연관성을 지닌다. 규칙적인 운동은 근육뿐만 아니라 뼈의 질 향상에 중요한 역할을 지니는 것으로 알려져 있다. 하지만 운동으로 인해 유도되는 상호작용의 기전에 대해서는 잘 알려져 있지 않다.

근육과 뼈는 서로 이웃한 기관으로써 기능적으로 밀접한 관계를 지닌다. 여러 선행 논문들에 따르면 근육과 뼈의 상호 커뮤니케이션에 있어서 마이오카인(myokine)이 잠재적인 중재자 역할을 할 것이라고 얘기하고 있다. 이 중 FGF-2는 저항성 운동을 통해 분비될 것으로 기대되며, FGF 수용체에 결합하여 뼈의 질에 긍정적인 영향을 미칠 신호전달을 유도할 것이라고 사료된다.

따라서 이 연구에서는 FGF-2와 FGFR 신호전달 체계가 저항성 운동으로 인해 유도되는 근육과 뼈의 질 향상에 중요한 역할을 하는지 알아보려고 하였다. 이를 확인하기 위해 FGFR 억제 물질을 투여하여 정확한 사실관계를 확인하고자 하였다.

방법: 성장기의 8주령 C57BL/6N 쥐를 다음과 같이 임의로 분배하였다; 억제 물질 투여 통제군 (Inh-C), vehicle 물질 투여 통제군 (Sham-C), 저항성 운동과 억제물질 투여군 (RT-Inh), 저항성 운동과 vehicle 물질 투여군 (Sham-C). FGFR 억제 물질로는 NVP-BGJ398 (50mg/kg body weight; Novartis, Switzerland)가 사용되었으며, vehicle로는 PEG-300을 Glucose 5% 와 2:1 비율로 혼합하여 사용하였다. 저항성운동은 주 3회의 빈도로 8주간 실시 되었으며, 매주 몸무게, 악력을 측정하였고, 마지막 운동 프로그램 종료 시점으로부터 48시간 후 운동관련 지표를 측정하였다. 운동관련 지표 측정 후 48시간의 회복기간을 가진 다음 근육과 뼈, 그리고 장기를 적출하여 생물학적 지표를 분석하였다.

결과:

FGFR 억제 물질 투여군은 악력, 근지구력, 심폐지구력 관련 지표를 저하시키는 것으로 나타났다. 신체 조성과 근육 무게는 크게 영향을 미치지 않는 것으로 나타났다. 이 효과는 운동군에서도 동일하게 나타났다으며, 운동으로 인해 나타나는 근력 증가와 근 지구력 향상, 뼈의 질 증가는 FGFR 억제 물질 투여로 인해 억제되는 것으로 나타났다.

억제물질 투여는 뼈의 질 또한 유의미하게 낮추는 것으로 나타났으며, FGF2 유전자와 myostatin 유전자 발현 또한 근육과 뼈에서 낮추는 것으로 나타났다.

결론:

FGFR 억제 물질은 근육의 질과 뼈의 질을 낮추며, 이 효과는 8주간의 저항성 운동으로 인해 회복되지 않는 것으로 나타났다. Myostatin과 FGF2의 유전자 발현은 근육과 뼈에서 상반되는 발현양상을 보였으며, 이는 이 연구의 가설과는 다른 결과를 도출하였다. 운동과 FGFR 억제 물질, 그리고 유전자 발현의 관계를 정확히 규명하기 위해서는 추가적인 실험이 필요할 것으로 사료된다.

학번: 2014-20986