Abstract

The Effects of Whole Body Vibration Exercises
on Bone Remodeling of Ovary-Excised Rats

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The current study applies regular whole body vibration exercise to rats, which are induced to suffer bone loss. OPG and RANKL expression levels of bone cell protein and bone density are examined to analyze how whole body vibration exercise affects bone remodeling. Therefore, the purpose of this research is to utilize the results to show that vibration exercises are effective in increasing bone health and preventing bone loss.

5-week-old female Sprague-Dawley(Daehan Biolink Co., Korea) rats were divided into a Sham group(n=35) and OVX group(n=35). After ovary excision operations, the two groups were once again divided into 4 groups: Sham-Exercise, OVX-Exercise, Sham-Nonexercise(Sham-Con), and OVX-Nonexercise(OVX-Con). The vibration exercise was applied at an intensity of 45 HZ 0.3g(g=9.8m/s2), 5 days a week. The intensity of vibration increased from the first 10 minutes up to the final 90 minute mark. 60 seconds of resting time was provided every 10 minutes. The exercise was applied for 12 weeks.

After 12 weeks of whole body vibration exercise, the bone marrow stromal cell's OPG expression showed statistical difference between group($p<.05$) and exercise($p<.001$). Also RANKL and RANKL/OPG ratio showed statistical difference based on exercise($p<.05$). There were statistical differences in BMD and BMC levels based on group($p<.01$) and exercise($p<.001$). Bone area showed statistical difference only in exercise($p<.01$).

As can be seen from above, whole body vibration exercises affect bone modeling stimulation and bone absorption prevention. In other words, it induces bone cell formation and improves bone mass and strength. In addition to previous studies that have shown that high intensity exercises induce bone formation, the current study shows that low intensity/high frequency exercises also help maintain bone health and support its growth.

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I. Introduction

1. Necessity of Research

With economic development and the advance in medical technology, the average life expectancy is growing, along with the number of retrogression diseases occurring(Morley & Thomas, 1998). A popular disease is osteoporosis, and it is becoming a social/economical problem as the number of senior citizens increase. However, not only old people experience this disease; even the young, who spend many hours sitting in chairs, suffer from this(Imanishi & Nishizawa, 2004). Also, women who carry out extreme diets and go under much pressure, suffer menopause and also from osteoporosis. According to the 2004 Korean National Fitness Survey(Ministry of Culture, 2004), although Korean children's physical characteristics have grown, their physical capabilities decreased, which was caused by less exercise. Teenagers who are not able to obtain maximum bone mass during their growth, are in danger of suffering from osteoporosis.

Osteoporosis is a disease in which bone formation slows and bone absorption increases to cause a decrease in bone mass, which results in bones breaking to even the slightest impact(Christodulou & Cooper, 2003). With the disease, disease and death rates are increasing as well as is the quality of life diminishing(Lips, 1997). Especially, the decrease in bone mass of women occurs dramatically after menopause. The biggest cause of the disease is that estrogen is not readily supplied after menopause(Charkoudian & Joyner, 2004). Especially, the speed of bone aging occurs most rapidly within 3 years of menopause(Lawrence etc., 2002). This is because the rate of bone substitution and loss increases with the lack of estrogen.

The first approach to bone loss, these days, is pharmacologic intervention. However, there are certain dangers associated with long-term intake(Laccy etc, 2002). On the other hand, although not specified in type, duration, frequency, and intensity, exercise seems to have relation with bone mass(Jang Jae Bong, 1997; Akiko etc., 2003; Fujimura etc., 1997; Yong etc., 1994). According to Lee(1995), exercise increases bone mass density(BMD), may prevent loss in BMD, and may reduce the dangers related to fracture. Especially regular exercise increases bone mass, bone intensity, and bone density(Kim Hyun Kuk etc., 2002; Notomi etc., 2000; Herard etc., 1997; Chilibeck etc., 1995; Snow-Harter, 1994; Suominen, 1993). Exercise is the best way that young people can maintain their maximum bone density(Holy & Zerath, 2000; Horcajadu etc.,

1997), and it also helps maintain adequate bone density with age to reduce dangers related to fracture(Vincent & Braith, 2002; Umemura etc., 1995). Regular exercise and physical activities also helps prevent bone density loss after menopause and may increase bone mass(Kim Young Pyo & Jung Il Kyu, 2004; Berard etc., 1997). Therefore, regular exercise is being recommended as a way to prevent and treat ostcoporosis. Many studies show that high intensity, resistance exercises are useful in increasing bone reformation and bone tissues(Kim Hyun Kuk etc., 2002; Boyde, 2003; Honda etc., 2003; Fuchs etc., 2001). However, recent studies argue that even low amounts of resistance can help in bone formation(Rubin etc., 2001), and draw questions against overload(Bauer & Snow, 2003). Therefore, studies on vibration exercises, as a new way to increase bone formation, are being conducted.

Vibration has been limited its use only in pain reduction(Yamamoto etc., 1995) and fracture treatment(Nakatsuchi etc., 1996). It has not been long since vibration has been thought of as a form of non-physiological mechanical stimulation(Shigeo etc., 2003; Eisman, 21; Rubin etc., 2001). Vibration improves muscular strength and power(Torvinnen etc., 2002; Bosco etc., 2000, 1999), and increases vertical jump capabilities(Bosco etc., 2000). Also, in a research where vibration exercises were employed on middle-aged women(Kim Jin Kuk, 2000), this form of exercise improved physical capabilities. Recently, results on the relationship between short and quick vibrations and bone metabolism were announced(Iwamoto etc., 2005; Stewart etc., 2004; Sugiyama & Kawai, 2004). Rubin(2001) argued the effectiveness of vibration stimulation on bone formation, and Tanaka(2001) argued that vibration exercises increased osteocalcin mRNA. Verschueren(2004) discovered an increase in bone density in the hips after long exposure to vibration exercises. There also studies that show that vibration exercises are effective in bone reformation for the elderly(Shigeo etc., 2003). However, Flieger(1998) argues that vibration exercises do not increase BMD in animal models that did not experience bone loss, and that response suppression could not be found in increased replacement rates. Torvinen(2003) conducted a study, in which healthy young adults were given vibration exercise, and the results showed that bone density did not increase. Furthermore, Hollins(2001) argues that if exposed to strong vibrations for long periods, a person may experience damage to other tissues.

The formation and reformation of bones is caused by the formation of osteoblast cells and resorption of osteoclast cells. Imbalances between osteoblasts and osteoclasts may cause various hormone changes and

inflammation. This might cause osteoporosis(Peacock etc., 2002). There are recent discoveries on osteoclasts as a bone absorbent, Receptor activator of nuclear factor(NF) - Bligand(RANKL), and osteoprotegerin(OPG) as the decoy receptor(Lacey etc., 1999).

OPG is a part of the TNF receptor superfamily secreted by bone marrow stromal cells, fibroblasts, and T-lymphocytes(Simonet etc., 1997). It is the soluble decoy receptor for the osteoclast differentiation factor(ODF)(Yasuda etc., 1998). OPG restrains the spread and survival of osteoclasts, prevents grown osteoclasts from activity, and entices osteoclasts to die(Grimaud etc., 2003; Kwon etc., 1998; Yasuda, 1998; Tsuda etc., 1997). Ueland(2003) argued that cortical bones and trabecular bones are both related with OPE decrease in a study conducted on menopause ostcoporosis patients. Also, Oh Ki Won(2004) found in his study that OPG of female adults had biological relations with bone metabolism. In animal experiments, reconbinant OPG increased bone mass increased. Especially, when recombinant OPG was injected into OVX animals, it perfectly prevented bone loss(Yasuda etc., 1998; Simonet etc., 1997). In the in vitro research in relation with OPG, OPG seems to induce osteoclast‘s apoptosis(Burgess etc., 1999; Hofbauer etc., 1999). There are also studies that prove that advanced osteoclast’s survival are restricted by limiting activity(Shalhoub etc., 1999).

On the other hand, RANKL induces osteoclast specialization(Malyankar etc., 2000), also activates them(Lacey etc., 1998), and becomes an essential stimulant for bone absorption(Hofbauer etc., 2000). Oliveira(1999) found that serious osteopetrosis was occurring in RANKL knock out mice. He argues that RANKL, which plays an esential role in osteoclast formation, might be related with anti-absorbtion of estrogen. Women who experienced early menopause due to estrogen deficiency, had an increase in RANKL levels and, this is how he explained the increase in absorption(Gutty etc., 2003).

As these previous studies suggest, bone remodeling can be controled with RANKL/OPG balance(Thomas etc., 2001).

Although there have been studies that have monitored changes in bone density and intensity after various exercises, or that have looked in to hormone or biological traits in relation with bone metabolism(Honda etc., 2003; Barengolts etc., 1994), there are not enough research conducted on how cell formation and reformation are effected in the in vitro level. Also, since an in vitrol level research, based on the effects of physical stimulation, cannot be directly applied to the human body, it is realistically difficult to stimulate in vitro.

2. Research Objectives

During the current study, rats with bone loss went through regular whole body vibration exercises. Afterwards, by monitoring the OPG and RANKL levels and bone density of the rats, the study will show the effects of vibration exercises in bone formation and reformation. Therefore, based on the results, this research will show that physical vibration can make people healthy and prevent diseases.

3. Hypotheses

For the objectives of this research the following hypotheses have been established.

1. Vibration exercises will affect OPG manifestation(in both groups and whether exercise was provided).
2. Vibration exercises will affect RANKL manifestation(in both groups and whether exercise was provided).
3. Vibration exercises will affect BMD(in both groups and whether exercise was provided).
4. Vibration exercises will affect BMC(in both groups and whether exercise was provided).
5. Vibration exercises will affect bone area(in both groups and whether exercise was provided).

II. Research Methods

1. Experiment Animals

70 5-week-old female Sprague-Dawley(Daehan Biolink Co., Korea) rats were given 1 week to adjust to the new environment. These were half divided into a Sham group and OVX group. After the operations, they were given 1 week to recover. Afterwards, the two groups were once again divided into 4 groups: Sham-Exercise, OVX-Exercise, Sham-Nonexercise(Sham-Con), and OVX-Nonexercise(OVX-Con).

2. Breeding Methods

Animals were put into a special cage. The temperature(23°C±1°C) and humidity(50 - 60%) were regulated, and a 12/12 hour light-dark cycle was maintained. Harian Teklad Laboratory Animal Diets feeds were used, and the rats were allowed to drink water freely.

3. Ovary Resection Method

Ovary resection was conducted with Robertson's method(1984). Before the surgery, the animals were administered with 70mg/kg ketamine and 10mg/kg Xylazine for anesthesia. Hair was removed from the abdomen area and the operation area was cleansed with 10% betadine. 1.5 cm of skin and muscles were removed to expose the ovary, and silk thread was used to suture ovary blood vessels to remove the ovary. Afterwards, silk thread was used to suture skin and muscles. The Sham group went through a similar process but did not have their ovaries removed; only suture operations were conducted.

4. Whole Body Vibration Exercise Method

For the experiment, a vibration machine(Turbosonic, Korea) that vibrates up and down was used. The rats were put on the machine as exercise. The exercise was applied at an intensity of 45 HZ 0.3g(g=9.8m/s2), 5 days a week. The intensity of vibration increased from the first 10 minutes up to the final 90 minute mark. 60 seconds of resting time was provided every 10 minutes. The exercise was applied for 12 weeks.

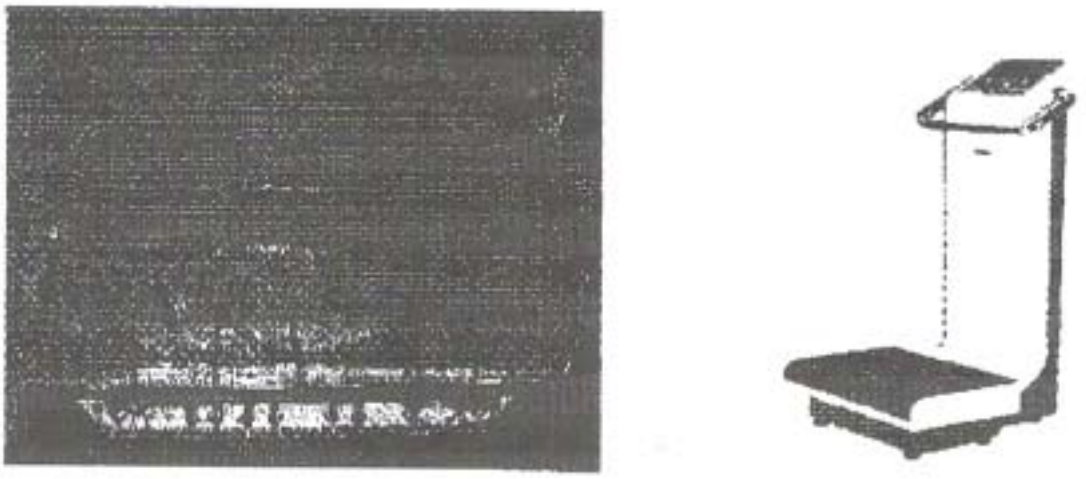
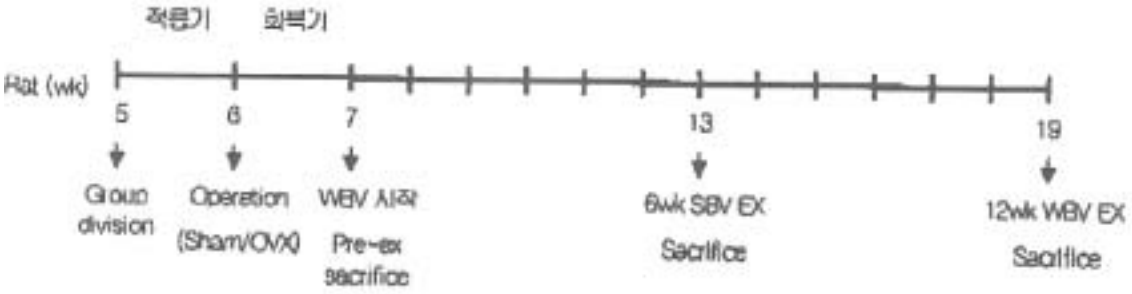


Figure 1. WBV Exercise machine

5. Experiment Method

Adjustment period/ recovery period



6. Analysis Method

1) Early Osteoblast Breeding

The enzymatic digestion method was used(Kim etc., 1998). A 1 day old rat was put into 70 EtOH and put to death. The head was opened and put into a-MEM to remove muscles and other material, and was cut up into pieces. Extracted bones were put into an enzyme mixture(0.1% collagenase; Wako, Japan, 0.05% trypsin, 0.5mM EDTA) for 20 minutes. After this calvaria cells were acquired through centrifugal separation. This was put into a-MEM(Gibco BRI, USA), which had 10% FBS and 100U/ml penicillin streptomycin, and was bred at conditions of 37°C, humidity 95%, and 5% CO2.

2) Coexisting Breeding of Osteoblasts and Marrow Cells

The cervical vertebrae and femur of rats were removed and tissues were removed from the bones. The thighs were washed in antibiotic(penicilin-streptomycin) PRS-CMF. Afterwards, using a 26.5-gauge needle a-MEM was injected into the marrow. Marrow cells were collected and were absolved using an 18-gauge needle. The separated marrow cells were treated with RBC lysis buffer(Sigma, USA) for 1 minute, and red cells were melted. Then, cells were collected using centrifugal methods(1,600rpm, 5 minutes), to be grown with early osteoblasts. Cells($1X10^6$/ml) in the coexisting breeding were spread on 100mm dishes of a-MEM(Gibco BRL, USA) containing 10% FBS, 1,25-(OH)2VitD3, and dexamethason. This was bred in conditions of 37°C, humidity 95%, and 5% CO2). The culture medium was changed every 2 days.

3) Western Blot Analysis

The following procedures were conducted to analyze levels of protein in RANKL and OPG.

Cells cultivated through coexisting methods were cleansed with PBS, and recollected with homogenization buffer 1ml(50mM HEPES(pH 8.0), 150mM NaCl, 1.5mM MgCl, 1% Triton, 10mM sodium pyrophosphate, 1mM PMSF). Collected cells were treated with sonication. Then cells were disassembled using centrifugal methods(14,000rpm, 4°C, 20 mins), and only the top layer were collected for use. For protein Bradford's method was used. The sample was put in using 12% gel, and protein was transferred using nitrocellulose membrane(Sigma, USA).

4) Femur DXA Scan

The DXA(Dual Energy X ray Absorptiometry; PIXImurs, LUNAR Co., USA) and small subject software program were used to measure the BMD, BMC, and bone area of the femur of the lab animals. All DXA scans were repeated and the average results were calculated.

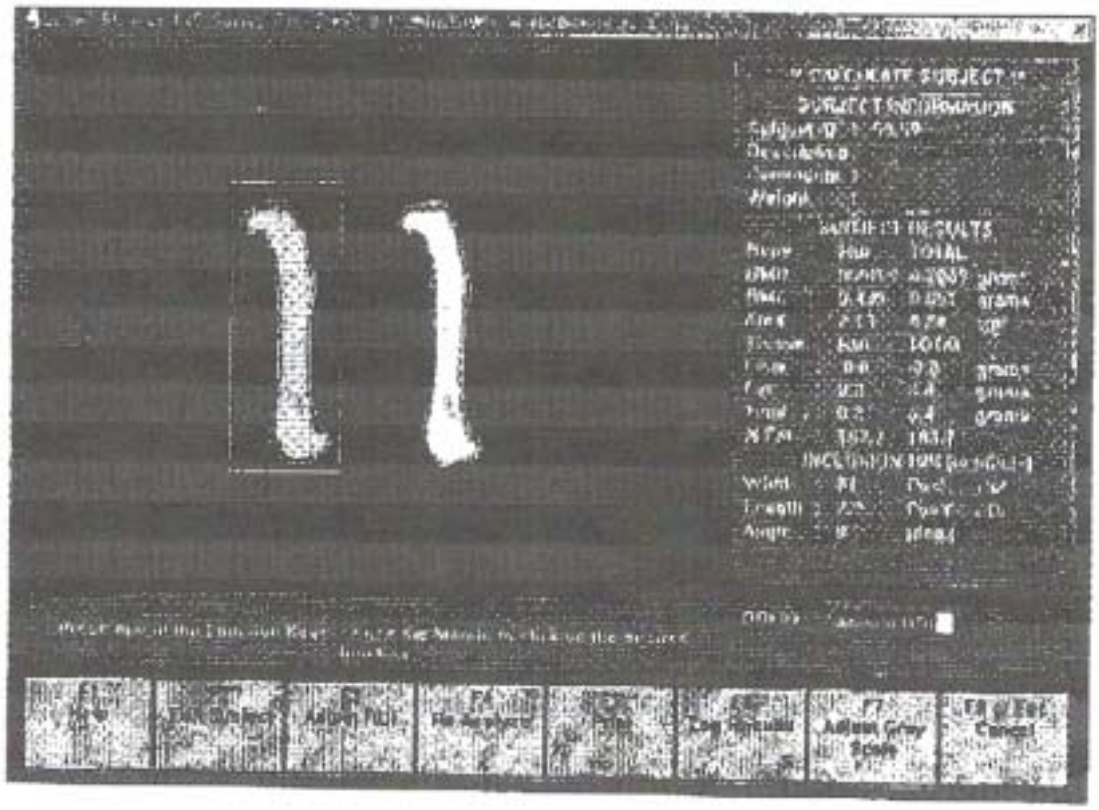


Figure 2. Animal Femur DXA Measurement

7. Data Management

The sample size of animals in experiments was set by G*program software, and data acquired were processed through SPSS(ver. 11.0) for

averages and standard deviations(mean±SE). A two-way ANOVA was used to differentiate groups(Sham/OVX) and exercise(Ex/non-Ex). Statistical level of significance was set at p<.05.

III. Results

1. OPG Expression Difference Based on Group and Exercise

The OVX group(851.583±331.905) showed less OPG expression than the Sham group(1020.872±330.043)(p<.05). The exercised group(11223.719±338.037) showed more OPG expression than the non-exercised group(811.233±280.453)(p<.001).

Chart 1. OPG Expression average and standard deviation based on group and exercise.

data unit : Intensity

집 단	운동여부	Mean	SE
Sham	운 동 군 (n=14)	1158.055	374.782
	비운동군 (n=21)	929.416	268.183
	전 체 (n=35)	1020.872	330.043
OVX	운 동 군 (n=14)	1089.383	307.127
	비운동군 (n=21)	693.050	244.923
	전 체 (n=35)	851.583	331.905
전 체	운 동 군 (n=28)	1123.719	338.037
	비운동군 (n=42)	811.233	280.453
	전 체 (n=70)	996.228	339.449

집단: Group
운동여부:Exercise
운동군: exercise
비운동군:non-exercise
전체:total

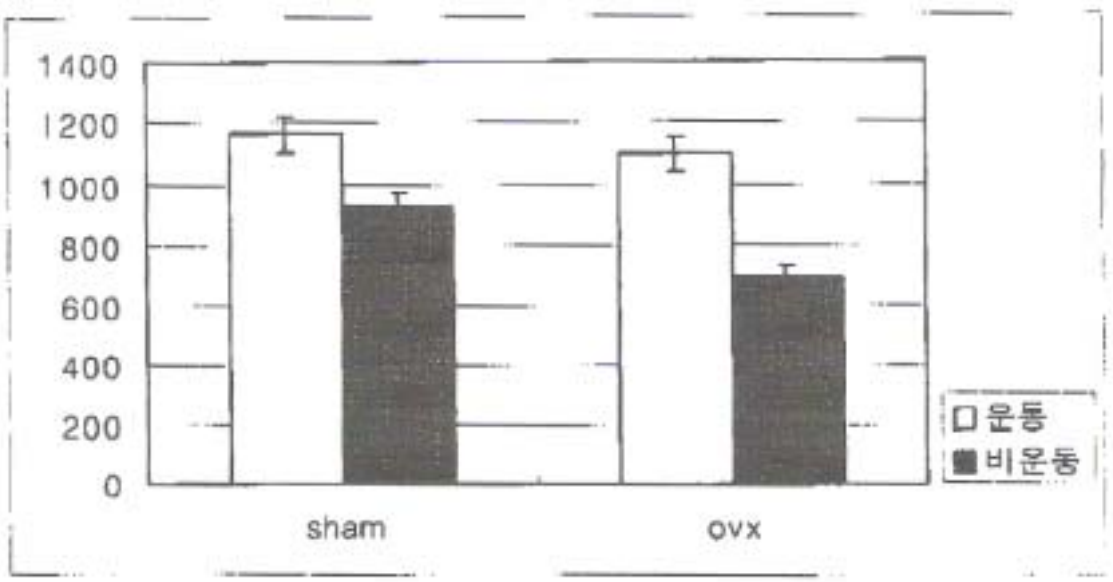


Figure 3. OPG Expression based on group and exercise

In the main effects of group and exercise, the OPG expression difference based on group(Sham/OVX) was F=4.5333, and the level of significance 5 % showed difference(p<.05). The difference in OPG expression for exercise(Ex/no-EX) was F=19.027, and the level of significance 5% showed difference(p<.001). However, the 2 factors(group & exercise) of OPG change did not statistically show any difference in interaction. In other words, interaction between group and exercise did not affect changes in OPG.

Chart 2. Analysis of OPG based on group and exercise

	제 III 유형 제곱합	자유도	평균제곱	F
집 단	390801.935	1	390801.935	4.533*
운동여부	1640480.547	1	1640480.547	19.027***
집단 * 운동여부	118109.177	1	118109.177	1.370
오 차	5680448.289	66	86218.913	
전 체	69307094.774	70		

*p<.05, ***p<.001

제III형 제곱합:Sum of squares of Type III

자유도:Degree of freedom

평균제곱:Average square

집단*운동여부:group*exercise

오차:difference

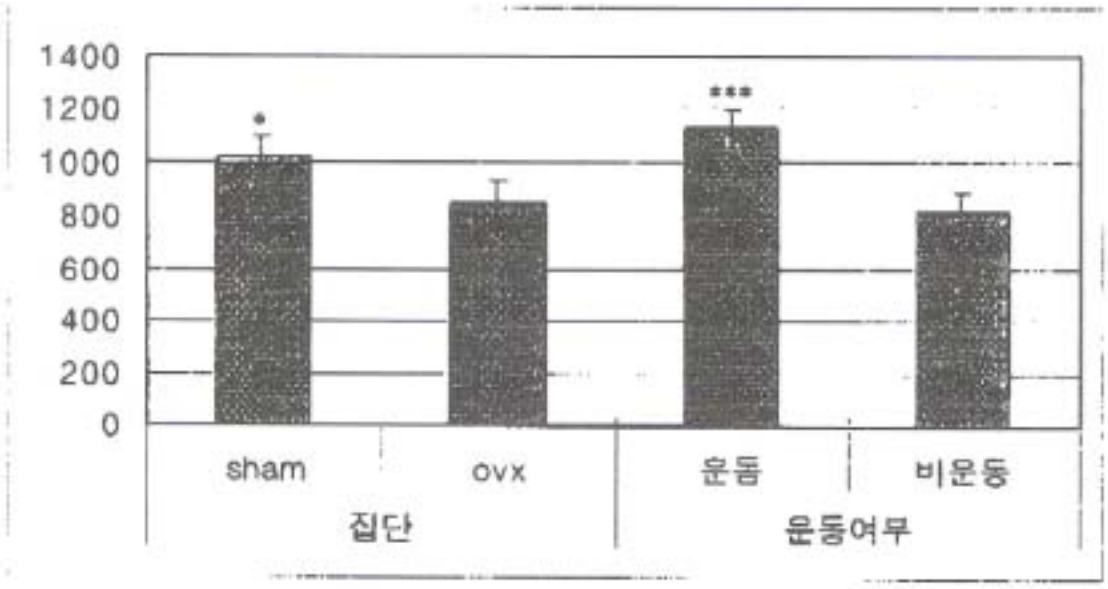


Figure 4. OPG expression difference based on group and exercise

2. RANKL Expression Difference Based on Group and Exercise

The difference in RANKL expression did not show between OVX group(628.244±171.850) and Sham group(683±203.054). However, the exercised group(711.057±183.379) did show higher results than the non-exercised group(618.670±185.254)($p<.05$).

Chart 3. RANKL expression averages and standard deviation based on group and exercise

data unit : Intensity

집 단	운동여부	Mean	SE
Sham	운 동 군 (n=14)	724.154	206.880
	비운동군 (n=21)	655.573	200.742
	전 체 (n=35)	683.005	203.054
OVX	운 동 군 (n=14)	697.959	163.320
	비운동군 (n=21)	581.767	164.914
	전 체 (n=35)	628.244	171.850
전 체	운 동 군 (n=28)	711.057	183.379
	비운동군 (n=42)	618.670	185.254
	전 체 (n=70)	665.624	188.757

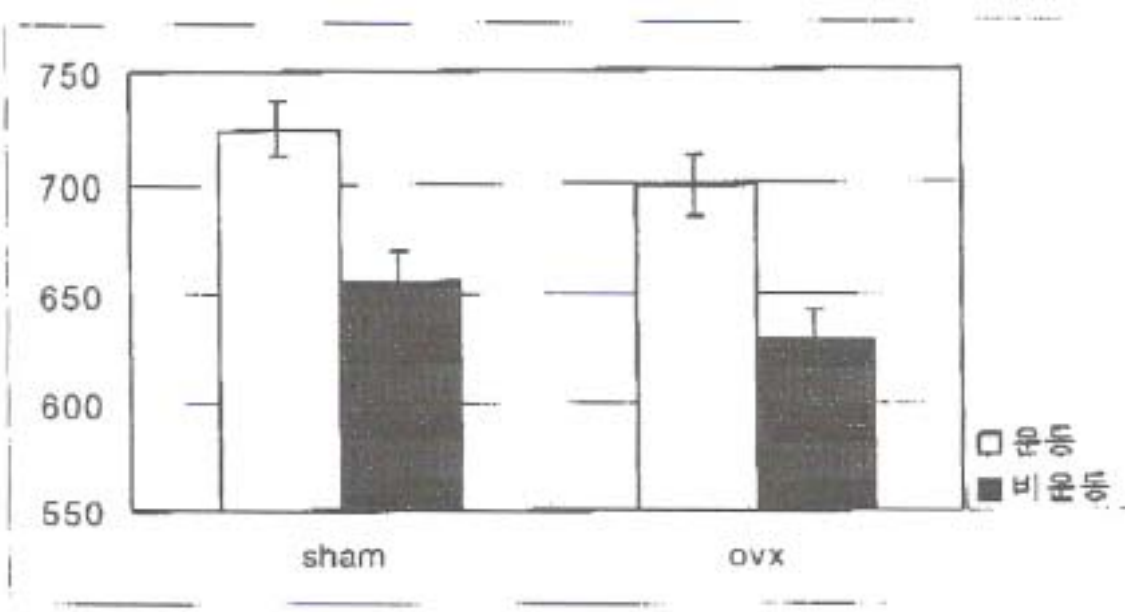


Figure 5. RANKL expression based on group and exercise

In the main effects of group and exercise, the RANKL expression difference based on group(Sham/OVX) was F=1.230, and the level of significance 5% did not show difference(p<.05). However, the difference in RANKL expression for exercise(Ex/no-EX) was F=4.201, and the level of significance 5% showed difference(p<.001). The 2 factors(group & exercise) of RANKL change did not statistically show any difference in interaction. In other words, interaction between group and exercise did not affect changes in RANKL.

Chart 4. Analysis of RANKL based on group and exercise

	제 III 유형 제곱합	자유도	평균제곱	F
집 단	42000.523	1	42000.523	1.230
운동여부	143394.013	1	143394.013	4.201*
집단 * 운동여부	9520.698	1	9530.698	.279
오 차	2253027.597	66	34136.782	
전 체	32547466.528	70		

*p<.05

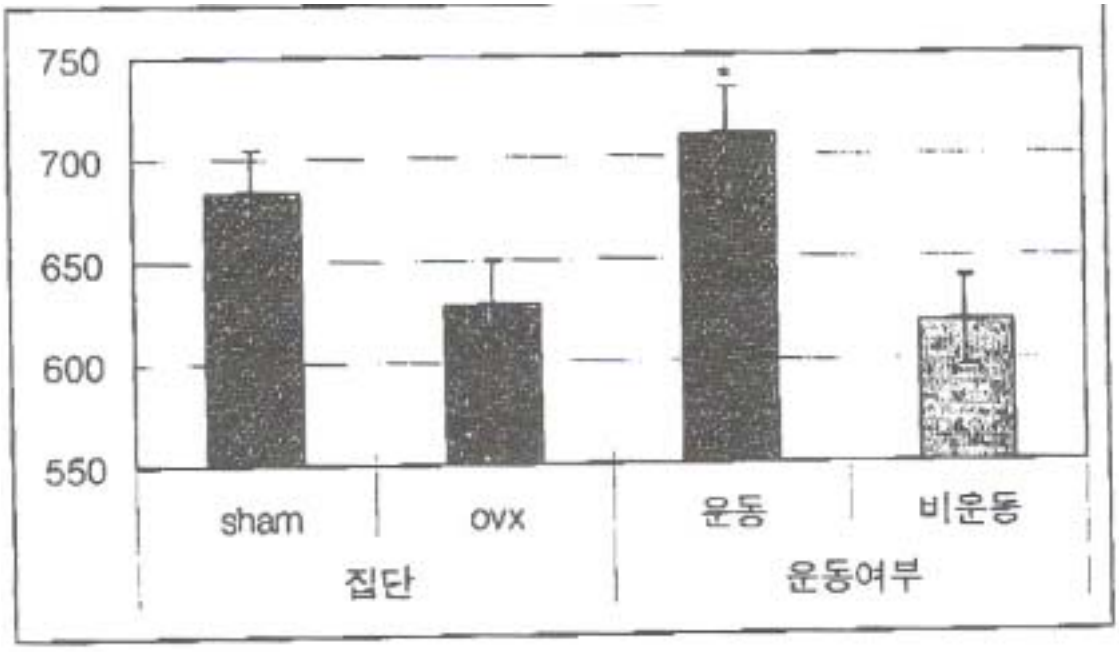


Figure 6. RANKL expression difference based on group and exercise

3. RANKL/OPG ration difference based on group and exercise

The difference in RANKL/OPG ratio did not show between OVX group(.762±.273) and Sham group(.668±.143). However the non-exercised group(.764±.260) did show higher results than the exercised group(.642±.115)($p<.05$).

Chart 5. RANKL/OPG ration averages and standard deviation based on group and exercise

data unit : ratio

집 단	운동여부	Mean	SE
Sham	운 동 군 (n=14)	.630	.110
	비운동군 (n=21)	.691	.159
	전 체 (n=35)	.668	.143
OVX	운 동 군 (n=14)	.654	.123
	비운동군 (n=21)	.834	.322
	전 체 (n=35)	.762	.273
전 체	운 동 군 (n=28)	.642	.115
	비운동군 (n=42)	.764	.260
	전 체 (n=70)	.715	.235

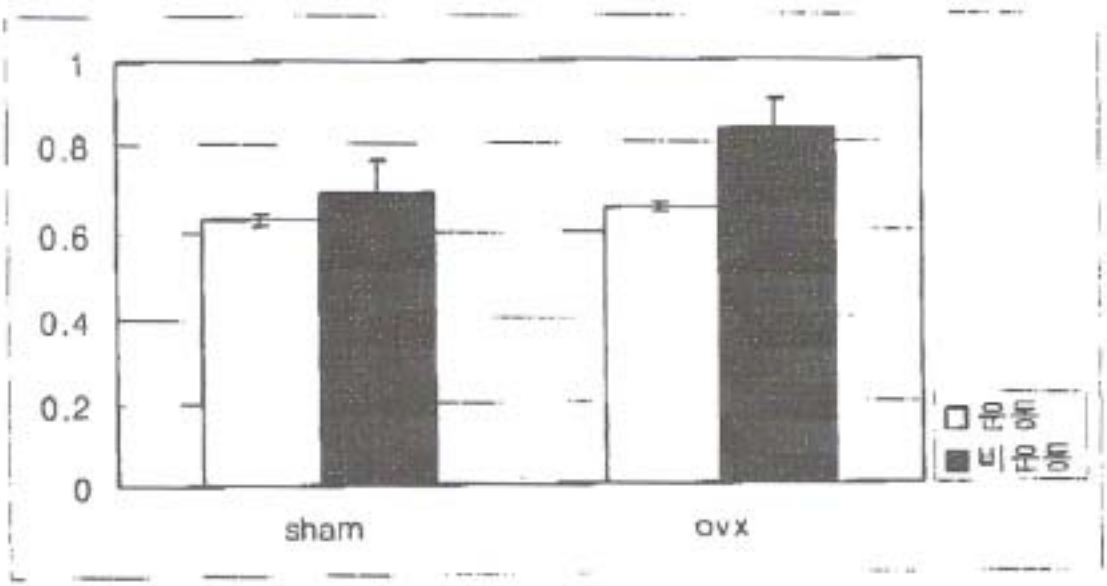


Figure 7. RANKL/OPG ratio based on group and exercise

In the main effects of group and exercise, the RANKL/OPG ratio difference based on group(Sham/OVX) was F=2.564, and the level of significance 5% did not show difference($p<.05$). However, the difference in RANKL/OPG ratio for exercise(Ex/no-EX) was F=5.617, and the level of significance 5% showed difference($p<.001$). The 2 factors(group & exercise) of RANKL/OPG ratio did not statistically show any difference in interaction. In other words, interaction between group and exercise did not affect changes in RANKL/OPG ratio.

Chart 6. Analysis of RANKL/OPG ratio based on group and exercise

	제 III 유형 제곱합	자유도	평균제곱	F
집 단	.114	1	.114	2.564
운동여부	.249	1	.249	5.617*
집단 * 운동여부	.056	1	25.644E-02	1.273
오 차	2.927	66	1.435E-02	
전 체	39.195	70		

*$p<.05$

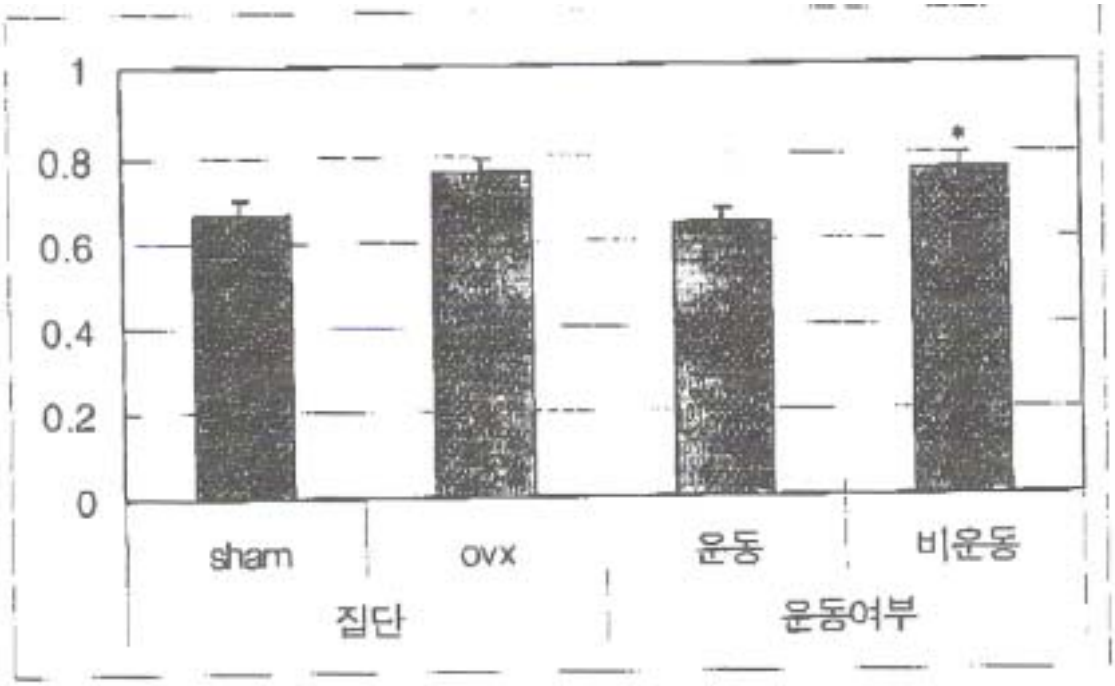


Figure 8. RANKL/OPG ratio difference based on group and exercise

4. BMD difference based on group and exercise

The difference in BMD showed between OVX group(.189±.012) and Sham group(.196±.014). The exercised group(.202±.012) did show higher results than the non-exercised group(.190±.010)(p<.001).

Chart 7. BMD means and standard deviations based on group and exercise

data unit : g/cm²

집 단	운동여부	Mean	SE
Sham	운 동 군 (n=14)	.207	.009
	비운동군 (n=21)	.188	.011
	전 체 (n=35)	.196	.014
OVX	운 동 군 (n=14)	.198	.012
	비운동군 (n=21)	.182	.010
	전 체 (n=35)	.189	.012
전 체	운 동 군 (n=28)	.202	.012
	비운동군 (n=42)	.190	.010
	전 체 (n=70)	.192	.013

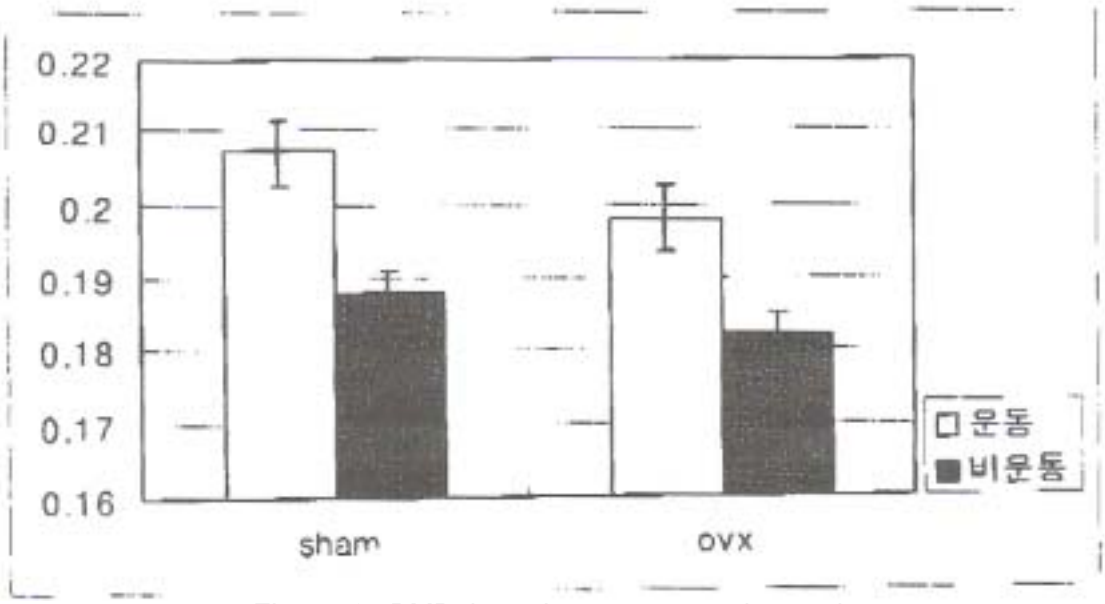


Figure 9. BMD based on group and exercise

In the main effects of group and exercise, the BMD difference based on group(Sham/OVX) was F=10.414, and the level of significance 5% did show difference(p<.01). The difference in BMD for exercise(Ex/no-EX) was F=49.641, and the level of significance 5% showed difference(p<.001). The 2 factors(group & exercise) of BMD change did not statistically show any difference in interaction. In other words, interaction between group and exercise did not affect changes in BMD.

Chart 8. Analysis of BMD based on group and exercise

	제 III 유형 제곱합	자유도	평균제곱	F
집 단	1.015E-03	1	1.015E-03	10.414**
운동여부	4.840E-03	1	4.840E-03	49.641***
집단 * 운동여부	2.037E-05	1	2.037E-05	.209
오 차	6.434E-03	66	9.749E-05	
전 체	2.588	70		

**p<.01; ***p<.001

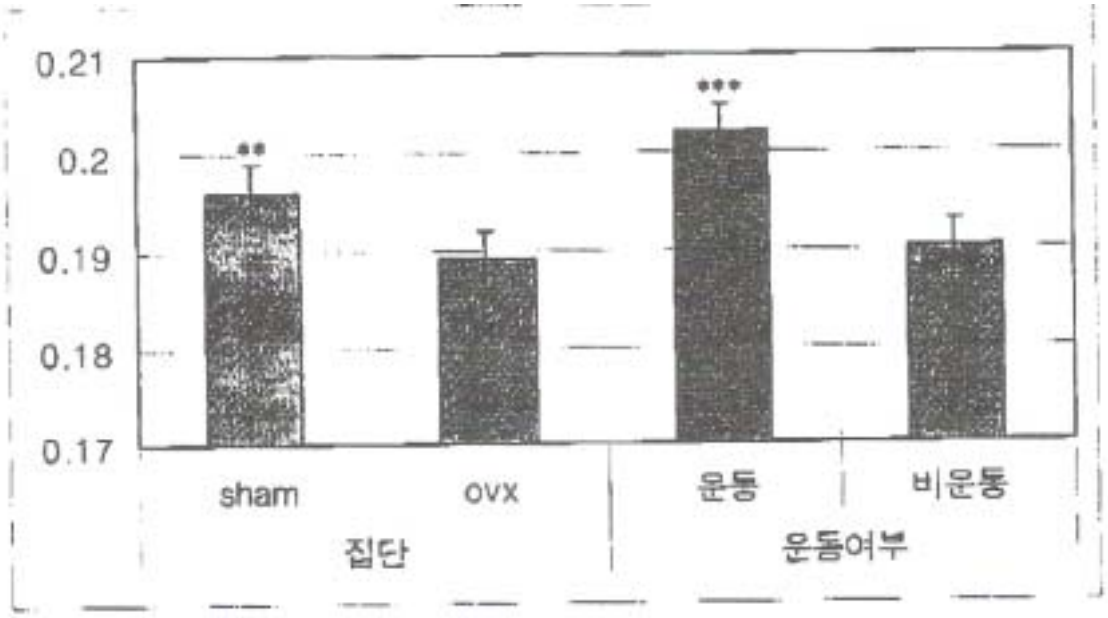


Figure 10. BMD difference based on group and exercise

5. BMC difference based on group and exercise

The difference in BMC showed between OVX group(.350±.052) and Sham group(.386±.048)($p<.01$). The exercised group(.408±.036) did show higher results than the non-exercised group(.341±.045)($p<.001$).

Chart 9. BMC means and standard deviation based on group and exercise

data unit : g

집 단	운동여부	Mean	SE
Sham	운 동 군 (n=14)	.416	.034
	비운동군 (n=21)	.366	.046
	전 체 (n=35)	.386	.048
OVX	운 동 군 (n=14)	.399	.037
	비운동군 (n=21)	.317	.031
	전 체 (n=35)	.350	.052
전 체	운 동 군 (n=28)	.408	.036
	비운동군 (n=42)	.341	.046
	전 체 (n=70)	.368	.053

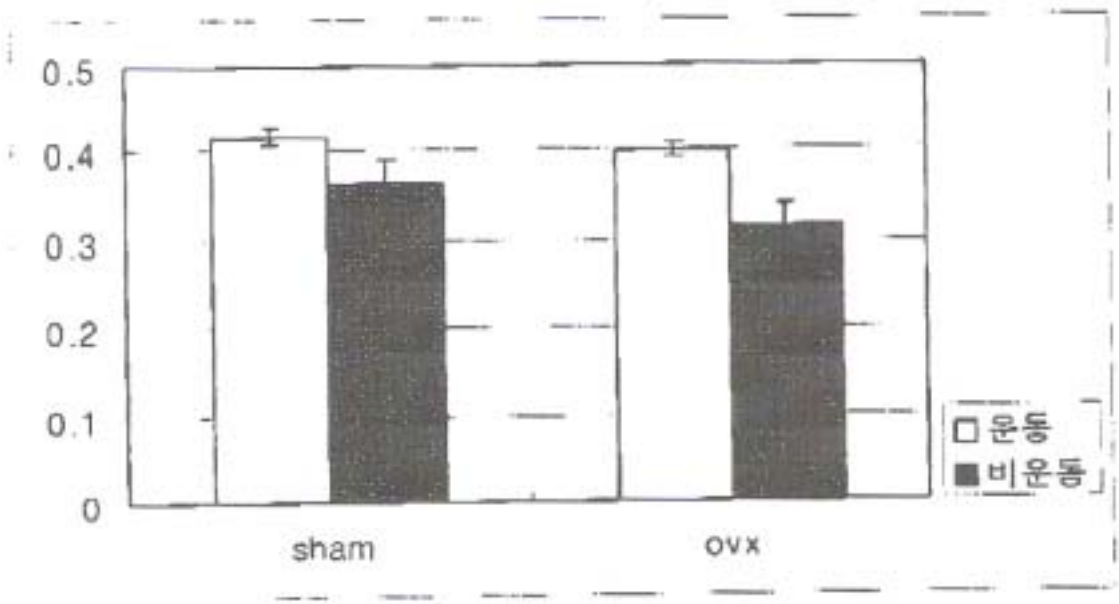


Figure 11. BMC based on group and exercise

In the main effects of group and exercise, the BMC difference based on group(Sham/OVX) was F=13.164, and the level of significance 5% did show difference($p<.01$). The difference in BMC for exercise(Ex/no-EX) was F=51.821, and the level of significance 5% showed difference($p<.001$). The 2 factors(group & exercise) of BMC change did not statistically show any difference in interaction. In other words, interaction between group and exercise did not affect changes in BMC.

Chart 10. Analysis of BMC based on group and exercise

	제 III 유형 제곱합	자유도	평균제곱	F
집 단	1.865E-02	1	1.865E-02	13.164**
운동여부	7.340E-02	1	7.340E-02	51.821***
집단 * 운동여부	4.298E-03	1	4.298E-03	3.034
오 차	9.349E-02	66	1.416E-03	
전 체	9.664	70		

**$p<.01$; ***$p<.001$

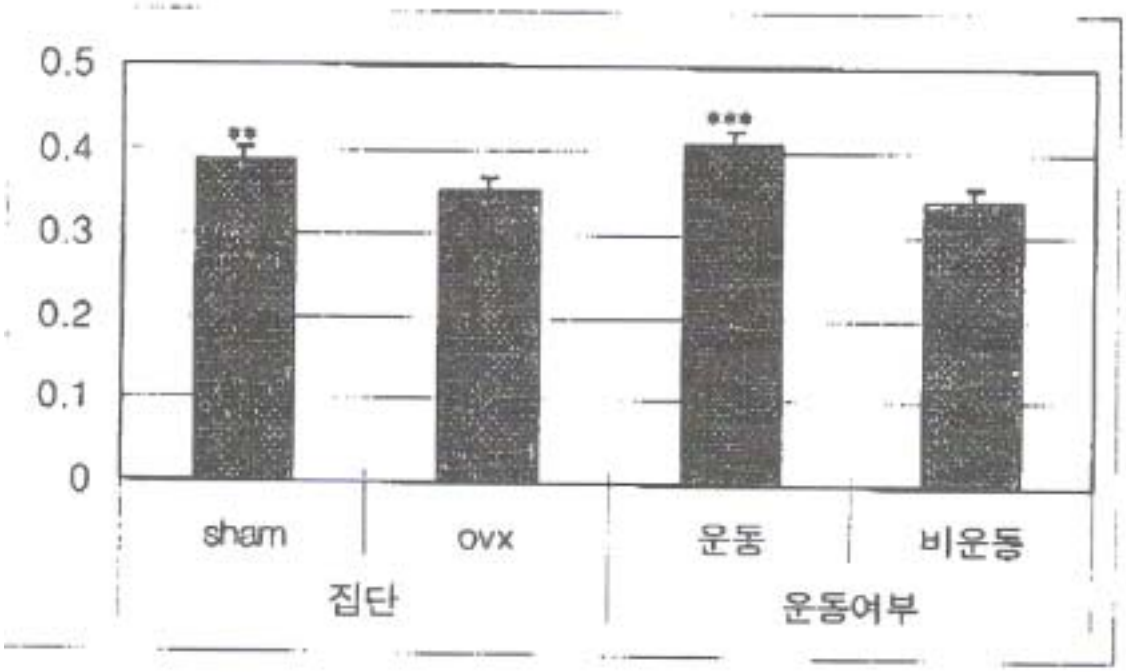


Figure 12. BMC difference based on group and exercise

6. Bone area difference based on group and exercise

The difference in bone area did not show between OVX group(1.923±.167) and Sham group(1.972±.152). The exercised group(2.009±.082) did show higher results than the non-exercised group(1.906±.187)($p<.01$).

Chart 11. Bone area means and standard deviation based on group and exercise

data unit : cm^2

집 단	운동여부	Mean	SE
Sham	운 동 군 (n=14)	2.015	.079
	비운동군 (n=21)	1.944	.183
	전 체 (n=35)	1.972	.152
OVX	운 동 군 (n=14)	2.004	.087
	비운동군 (n=21)	1.809	.187
	전 체 (n=35)	1.923	.167
전 체	운 동 군 (n=28)	2.009	.082
	비운동군 (n=42)	1.906	.187
	전 체 (n=70)	1.948	.161

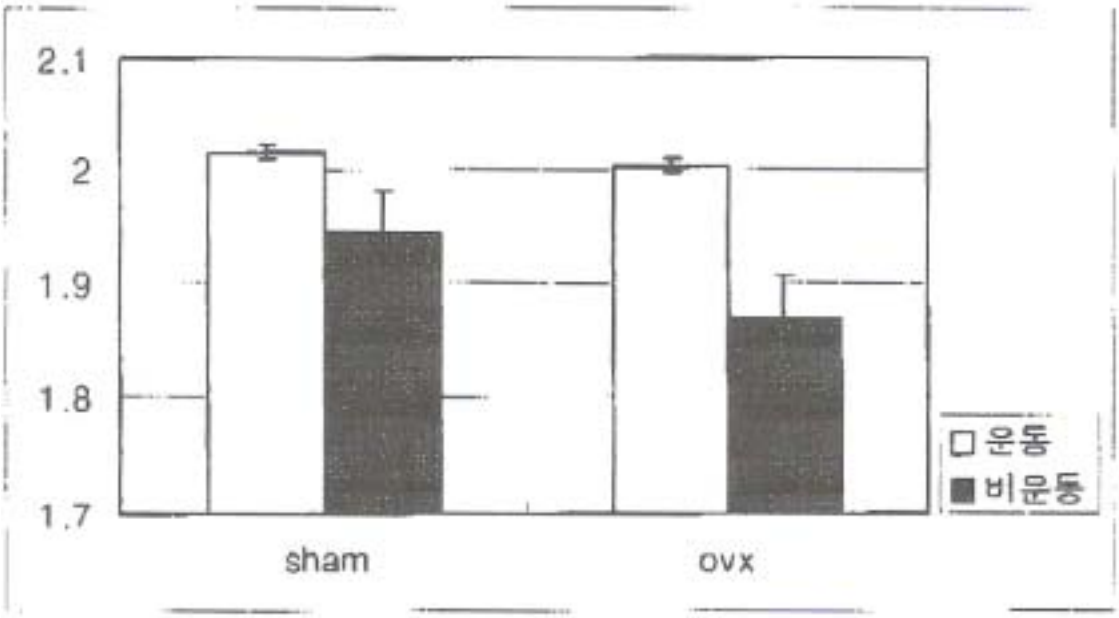


Figure 13. Bone area based on group and exercise

In the main effects of group and exercise, the bone are difference based on group(Sham/OVX) was F=1.330, and the level of significance 5% did not show any difference. However, the difference in bone area for exercise(Ex/no-EX) was F=7.638, and the level of significance 5% showed difference($p<.01$). The 2 factors(group & exercise) of bone area change did not statistically show any difference in interaction. In other words, interaction between group and exercise did not affect changes in bone area.

Chart 12. Analysis of bone area based on group and exercise

	제 III 유형 제곱합	자유도	평균제곱	F
집 단	3.116E-02	1	.0	1.330
운동여부	.179	1	.179	7.638**
집단 * 운동여부	1.701E-02	1	.012	.726
오 차	1.546	66	.02	
전 체	267.265	70		

**p<.01; ***p<.001

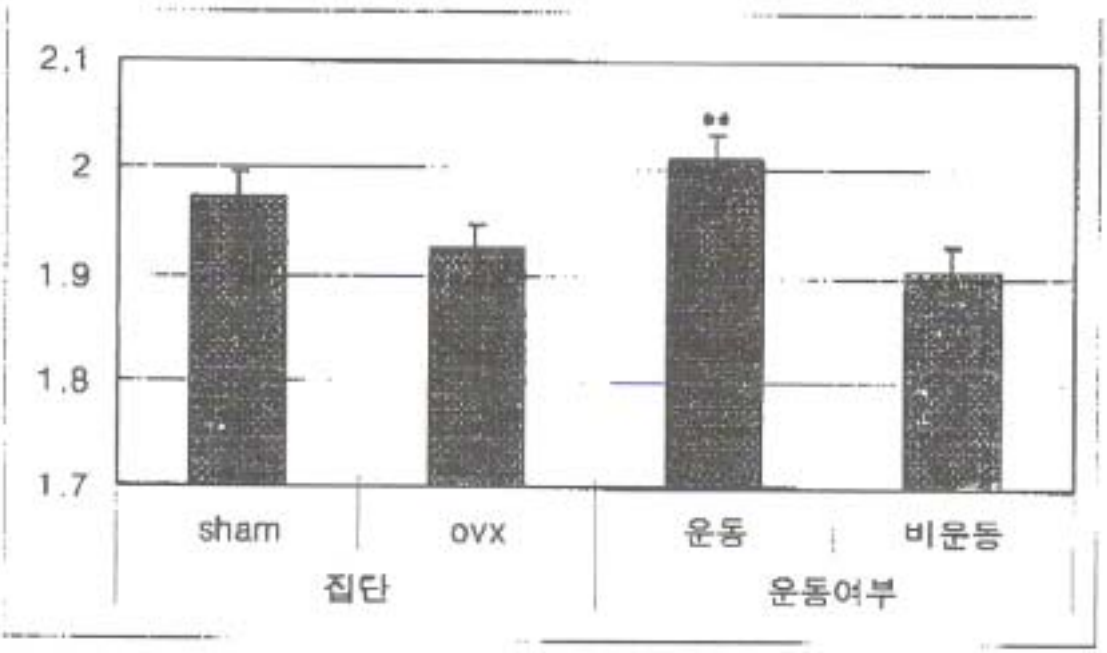


Figure 12. Bone area difference based on group and exercise

IV. Discussion

In relation with the prevention of osteoporosis, when the physical force has been applied, the level of bone formation increases with larger amounts of force being applied to the bone. Therefore, physical stimulation from exercise plays an important role in bone formation. Various exercise methods, such as jogging(Peng etc., 1997), walking(Chien etc., 2000), and jumping(Umemura etc., 2002), are being recommended to help bone formation. In particular, exercises

with high impact and resistance show greater osteogenic response than other kinds(Akiko etc, 2003; Cheng etc., 2002; Fuchs & Snow, 2002; Bassey etc., 1998). Although many studies recommend exercise as a way to prevent and cure osteoporosis, none have given a clear answer to the intensity and results of exercise.

Contrary to past beliefs that 'the stronger the intensity, the greater the results', recent studies have shown that high-frequency, low magnitude physical signals are more effective in bone formation(Verschueren etc., 2004; Rubin etc., 2001). However, only recently have researchers looked into the effects of vibrational physical stimulation. Ward(2004) provided exercises of 90Hz, 0.3 intensity in 10 minute intervals to handicapped children, and found that vibration exercises helped in bone formation in these children. Also, Rubin(2004) conducted experiments using vibration exercise on menopause women, and as a result BMD increased to stop bone loss. However, Fliger(1998) argued that vibration exercises were only effective in bone loss groups, caused by ovary surgery, and that there were no changes in BMD in the regular group. Also, Torvinen(2003) argued that vibration exercises were not effective in helping bone formation in young healthy women.

Normal bone metabolism is maintained with a balance in osteoblast and osteoclast. This bone remodeling is controled by the balance of RNKL/OPG, which relates to the differentiation of osteoblasts and osteoclasts(Thomas etc., 2001). OPG controls the differentiation and survival of osteoclasts(Okada & Tsuda, 2002; Tsuda etc,. 1997), stops active growth of osteoclasts(Grimaud etc., 2003), and induces the death of osteoclasts(Kwon etc., 1998).

As results of applying whole body vibration exercise to rats in the current study, the expression of OPG in bone marrow stromal cells was lower in the OVX group than the Sham group($p<.05$). The reason behind this results from bone loss caused by less estrogen after ovary surgery. Therefore, it is shown that OPG is related to osteoblast formation. This coincides with Oh Ki Won's(2004) research, which shows that women before menopause have higher levels of OPG in their blood. Also, this relates to the previous study(Yasuda etc., 1998; Simonet etc., 1997) that proved recombinant OPG prevented bone loss.

However, differences did not show between the groups in bone marrow stromal cell RANKL expression. Previous research show that RANKL adhere to osteoclasts to stimulate osteoclast growth and differentiation, which increases bone absorption(Eghbali-Fatourechi etc., 203; Duong etc., 2001). This role of RANKL was shown in researches, such as osteoporosis occurring in RANKI

knock-out rats and osteoblasts not helping the formation of osteoclasts(Malyankar etc., 2000; Lacey etc., 1998; Fuller etc., 1998), and the growth of teeth being suppressed(kong etc., 1999). In particular, Eghbali-Fatourenchi(2003) showned that estrogen deficiency caused higher levels of RANKL. However, the current study differs from preceding one in that RANKL expression did not differ between the groups. This result can probably be explained with RANKL's relationship with aging(Cao etc., 2005; Frost, 2002). Previous experiments were conducted on middle-aged women, who were past their menopause, or animals with RANKL knocked out. Because osteoclasts become more active than osteoblasts with age, the RANKL levels of subjects of previous experiments were high. However, rats used in the current research can be viewed as young adults. Therefore, these did not have any relation with aging effects; and as a result, statistical differences were not found in RANKL and RANKL/OPG ratio between the groups.

The above results of difference in OPG and RANKL between groups can be explained with DXA. Even BMD($p<.01$) and BMC($p<.01$) differed between groups. The Sham group, which had normal estrogen levels, had higher levels of BMD and BMC than the OVX group. In relation to this, Paul(2001) reported that OVX rats showed increase in bone mass and BMD after being admitted with OPG. Also, Rogers(2002) argues that there is a positive interrelation between serum OPG levels and BMD. However, Browner(2001) reported that BMD reduction did not necessarily result in OPG reduction. This kind of discrepancy results from analysis method(Oh Ki Won etc., 2004; Thomas etc., 2001), wide range of subject(Ueland etc., 2003; Oliveria etc., 1999), and various ages(Cao etc., 2005).

It is widely believed that bone density increases as bone area increases, owing to more bone mass within an area. However, in the current study, although there were differences in BMD and BMC, there weren't any statistical differences in bone area. This agrees with Richman's(2001) results, where although there were not any differences in external bone size between C3H and B6 mice, C3H showed higher BMC. Therefore, bone area is a factor in bone growth, but is not an absolute standard.

Mechanical stimulation plays an important role in bone remodeling, growth, and fracture remedy. After 12 weeks of applying whole body vibration exercise to rats, the exercise group showed higher bone marrow stromal cell OPG expression than the non-exercise group($p<.001$). This result agrees with Tang's(2006) report, which shows that stimulation to osteoblastic cells increases OPG mRNA expression and reduces RANKL nRNA expression. Also, the results

coincide with Kwon Ki Ok's(2004) report that showed jump training affects the bone marrow stromal cell OPG expression in rats. Therefore, increased OPG, caused by vibration exercise, stimulates osteoblasts and reduces osteoclasts to prevent further bone loss.

Bone marrow stromal cell RANKL expression was higher in the exercised group than the non-exercised group($p<.05$). This is contrary to previous studies: Rubbin (200) reported that mechanical stimulation to stromal cells prevented osteoclast differentiation, Tang(2006) argued that physical stimulation reduced osteoblast RANKL mRNA levels, and Saunders(2006) reported that when MG-63 cells were physically stimulated, OPG increased but RANKL was not effected. On the other hand Mehrotra(2006) showed that after mechanical loading, RANKL mRNA increase caused bone remodeling to start, which supports the current study. The reason for RANKL expression being high in the exercise group is that bone formation was increased in the exercised group to maintain balance between formation and absorption. However, since there are not many previous studies on exercise and OPG/RANKL, more research should follow in the future.

Whole body vibration exercise for 12 weeks also affected DXA. The exercised group showed higher femur BMD than the non-exercised group did($p<.001$). This agrees with Fliger's(2003) research, which shows that OVX rat bone density increased with vibration exercise. Also Rubin(2001) reported that vibration exercises improved bone density by 32% and space within the bone trabecular became more tight. It seems that OPG secreted by osteoblast cells increased to cause an increase in the amount of bone minerals, and also in increase in bone density.

During the 12 weeks of vibration exercise, the exercised group showed higher increases in femur BMC quantity($p<.001$). BMC, along with BMD, are usually used as a standard for bone health. Especially, BMC is important in comparing bone size. In other words, greater the amount of BMC, greater the bone size. Therefore BMC and bone area can be used as measurements of growth(Mozess etc., 1990). The current study shows that, with vibration exercise, BMC and bone area increased in relation with the comparison group<.01). This is contrary to the results where there were no differences in bone area between the groups. This result implies that whole body vibration exercise could affect the overall bone development in rats.

Therefore, whole body vibration exercises can be used by not only healthy people but also by osteoporosis patients to stimulate bone formation. Generally, exercise helps improving bone mass and bone strength in children and

teenagers. The effectiveness of exercise dramatically drops after that period(Khan etc., 2000; Turner etc., 1995). Therefore, there should be future research conducted on age, vibration exercise intensity and duration.

The current study shows that vibration exercises stimulate bone formation and prevent bone loss, which could potentially become an alternative to current treatment. Considering bone remodeling and modeling are controled by reformation of microdamage(Burr etc., 1997), vibration is transformed into shear stress in the bone. This stimulation is passed on deep inside the bone, and bone cells are stimulated and interstitial fluid flow is increased to bring an increase in BMD and BMC. However, it has yet to be shown how this mechanical signal can affect the bone tissue. Therefore, future research should be conducted on this area of vibration exercise.

V. Conclusion

This research analyzes the potential of vibration exercise in increasing bone formation and preventing bone loss. Rats, which went through ovary removal surgeries, were used to study the levels of OPG and RANKL expression after regular whole body vibration exercise. The results concerning vibration exercise and bone modeling/remodeling are as follows.

1. Statistical differences were found in bone cell OPG expression based on group($p<.05$) and exercise($p<.001$).

2. Statistical differences were found in bone cell RANKL expression based on exercise($p<.05$). However, there were not any differences between groups.

3. Statistical differences were found in RANKL/OPG based on exercise($p<.05$). However, there were not any differences between groups.

4. Statistical differences were found in BMD based on group($p<.01$) and exercise($p<.001$).

5. Statistical differences were found in BMC based on group($p<.01$) and exercise($p<.001$).

6. Statistical differences were found in bone area based on exercise($p<.001$).

However, there were not any differences between groups.

As can be seen from above, whole body vibration exercises affect the analyzed factors. Therefore, whole body vibration exercises stimulate bone formation, prevent bone loss, and is an effective treatment method. However, since research on vibration exercise has only been initiated recently, research on the absolute intensity that can reach the peak-bone mass in bone formation should be conducted. Moreover, further research on the mechanism associated with vibration exercise being passed on to the bone should follow.