Effects of different intensities of jumping exercise on structures of femoral cortical bone and periosteum in rats

KANO, Tsuyoshi*
KIRA, Yuichiro*
OHSAKO, Masafumi**

Abstract

Structural changes of a periosteum at the central and distal portions of femur caused by jumping exercises under various conditions were investigated by relating to a change of the bone matrix in this study.

Seven-week-old male rats (wistar strain) were used as materials, and were divided into two groups (experimental group: EX, and control group: CO). Furthermore, EX divided into three subgroups that jumped at 30, 45 or 90% of maximal jumping height of each rat. Each EX accomplished jumping exercises in each heights, 100 times/day, 5 days/week, for 4, 7 or 14 days, and CO was fed ordinarily for same days as each EX. After each experimental period, samples from distal 2/5 (central) and 1/4 (distal) portions of femur were excised, and were observed histologically and measured bone morphometrically.

Compaction of the cortical bone was advanced accompanied with increase in exercise intensity at the central portion. Oppositely, active osteoid formation was recognized at the distal portion. Osteoid formation was activated at the periosteum side of central and distal portions. Compaction of the cortical bone was found only at the central portion, and expansion of Howship's groove was observed only at the distal portion. The periosteum showed acute increase in the thickness of the osteogenic layer and then returned to its original thickness.

* Master's Program in Graduate School of Welfare Society Design, Course of Human Centered Life Design, Concentration in Health Care and Sports, Second Year Students, Toyo University.

* Master's Program in Graduate School of Welfare Society Design, Course of Human Centered Life Design, Concentration in Health Care and Sports, First Year Students, Toyo University.

** Professor of the Faculty of Human Life Design, Toyo University.

From these results, it was understood that the jumping exercise at the various intensities caused characteristic changes to the bone matrix but little effects were given to the thickness of the periosteum.

Keywords: jumping exercise, mechanical loading, cortical bone, periosteum

Introduction

It is commonly known that mechanical loading promotes a bone formation. In previous studies, it was reported that a bone mineral density was correlated with extent of exercise and weight[1, 2], and a thickness of a cortical bone and a trabecular bone density of a cancellous bone increased by growth[3, 4]. It has been also recognized that a simple exercise like a muscle training is effective on an improving the bone mineral density in experiment using human subject[5]. However, it has been discussed how a mechanical stress causes bone modeling, remodeling and what type of exercise makes a promotion of bone formation[6-8]. It is showed that the mechanical loading to the bone affects to a bone mass and a bone strength affect[9]. It has been cleared that osteocytes existing in a bone matrix plays roles as a sensor of the mechanical loading and a control tower of a bone forming[10-17]. In the study of bone resorption suppression system by exercise, there was a report on the induction of osteoclast apoptosis through increased expression of transforming growth factor-β (TGF-β), osteoprotegerin (OPG) by tension stimulation[18]. It was reported that an apoptosis of a osteoclast induced by developments of TGF-β and OPG accompanied with tensile stimulation. It is thought that a microdamage appears in a fragile bone selectively, causes the bone remodeling by the osteoclast and results in a replacement to a new bone unit[19, 20]. As described above, osteocytes, osteoblasts and osteoclasts are respond flexibly to the mechanical loading and a mechanism of them has been gradually cleared[21].

High impact exercises such as gymnastics and/or ball games (basketball, volleyball and squash, etc.) improve the bone mineral density[22-24] and it was showed actually that distinct effects on the bone mineral density were found in calcaneus and tibia, not in radius[25]. To the contrary, little effects of the exercise on the bone mineral density were found by low impact exercises such as walking, running and swimming (diving)[22-24]. As an example of a high impact exercise, it was reported, in a clinical study about young women, that a maximum jumping motion of 10-times a day, 3 days a week increased the bone mineral density of the lumbar vertebra and femoral neck[26]. There were reports that 5-times-jumping was enough to increase the bone mass and the bone strength in young rats[27]. Therefore, it was speculated that the high impact exercise was effective to increase in the bone mass and the bone strength but more repetitions wasn't necessary.

The periosteum is an important tissue playing a central role in bone development and formation[28-30]. It has thought that the periosteum is divided into the outer and inner layer, the outer layer covered the bone surface and contributed to maintenance of bone strength[31], and pluripotential mesenchymal cells existing in the inner layer differentiate to osteoblasts[32]. Structural changes in the periosteum and the cortical bone by the jumping exercise hasn't been investigated in detail. In this study, the structural changes of the periosteum at the central and distal portions of femur caused by the jumping exercise under various conditions were investigated by relating to a change of the bone matrix.

Materials and methods

Animals and tissue collection site

Seven-week-old male rats (wistar strain) were used as materials, and were divided into two groups (experimental group: EX, and control group: CO). Furthermore, EX divided into three subgroups that jumped at 30, 45 or 90% of maximal jumping height after jumping heights of each rats were measured at their maximal effort. Each EX accomplished jumping exercises in each heights, 100 times/day, 5 days/week, for 4, 7 or 14 days, and CO was fed ordinarily for same days as each EX. After each experimental period, samples from distal 2/5 (central) and 1/4 (distal) portions of femur were excised, and were cut in horizontal, frontal or sagittal directions. Those specimens were embedded in paraffin wax or resin by ordinary methods, were cut and stained by various methods and were observed histologically and measured bone morphometrically (Fig. 1).

Mechanical loading experiments

Each EX jumped in according to methods of Umemura et al.[27] The maximal jumping height in each rats were measured at the start of the experiments, were re-measured at 1 and 2 weeks after that, and mechanical loads in each rats were corrected weekly.

Collection of the specimen and fixation

Rats were anesthetized deeply by aspiration of carbon dioxide gas, and euthanized before sampling. Soft tissues containing skin and muscles were removed from hind

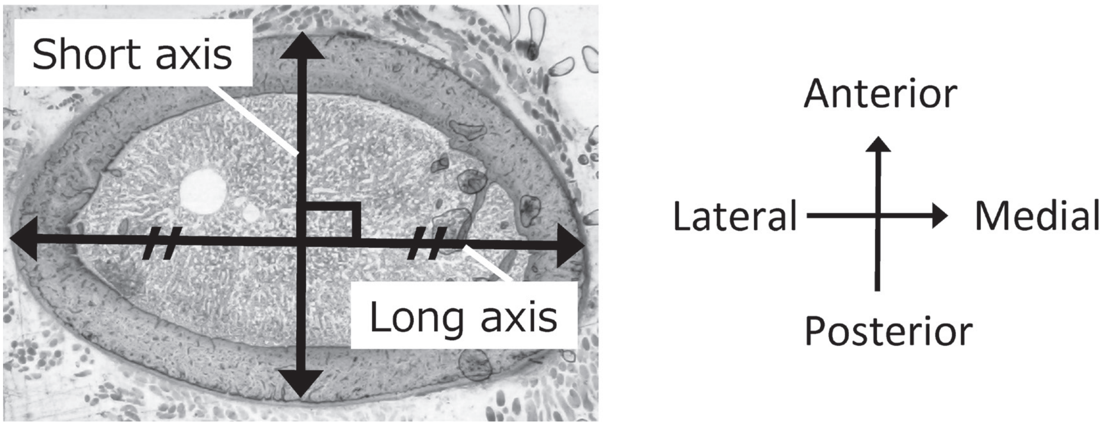

Fig. 1　Description of the diameter and face

limbs, without damage to a periosteum, after concerning cardiac arrests. Samples that thicknesses were 2-3mm were excised from distal and central portions of right femurs after their morphologies were measured by caliper exactly. They were immersed in Karnovsky's solution containing 5% glutaraldehyde and 4% paraformaldehyde at 4 ℃ in overnight, and immersed and kept in 0.1M cacodylate buffer containing 0.2M saccharide until proceeding next step of sample preparation. Using left hindlimbs, specimens that length were about 7mm were excised, cut in direction of frontal and sagittal, and were rapidly fixed by 4% paraformaldehyde at 4℃.

Preparation of undecalcificated resin-embedded specimens

The samples excised were dehydrated by alcohol series consisting of 70, 95, and 100% ethanol, and were similarly immersed to acetone, at the vacuum condition (20 minutes × 2 times). Thereafter, they were immersed to mixed solution of acetone and Rigolac resin (1:1, 1:3, 1:7), were embedded in Rigolac resin (one day, twice), were polymerized at 37, 45, 55 and 60℃ for one day per step. Polymerized specimens were trimmed by a Bandsaw and they were carefully ground until 100 μm thickness by the abrasive film. Those specimens were etched by 1N hydrochloric acid, were stained by toluidine blue and were observed with a light microscope.

Preparation of decalcified semi-ultrathin sections

Fixed samples were decalcified by 8% EDTA solution for 21-35 days (pH 7.4, 4 ℃). Samples pre-fixed by Karnovsky's fluid were, after decalcification, post-fixed by 1% osmium tetroxide in 0.1M cacodylate buffer (pH 7.4) for 4 hours at 4℃. Those samples were dehydrated by method described above, were cleared by the acetone and were embedded in epoxy resin. Semi-thin sections were cut using those resin blocks, were stained by toluidine blue, and were observed with the light microscope.

Preparation of decalcified paraffin-wax embedded specimens

Samples fixed by 4% paraformaldehyde solution were decalcified and embedded in paraffin-wax, in accordance with ordinal methods.

Statistical analysis

Data were analyzed by two-way layout ANOVA for exercise intensity and exercise period, and their interaction. Post multiple comparisons were performed using the Bonferroni test. SPSS Statistics software (Version 24, SPSS Inc., Chicago, IL, USA) was used for the analyses and $P < 0.05$ was considered to be statistically significant.

Results

Body weight

No significant differences were found in body weight of each group at 7 days, but a significant difference was recognized at 4 and 14 days. The body weight of CO was lesser significantly than EX30 and EX45 at for 4-day-groups and higher significantly than the other EX at 14 days. (Fig. 2)

Bone morphometric analyses

Comparison of the Long and a short axis

When comparing there were compared for the length of long and short axes of transversal sections at the central and distal portions of the femur in each group,

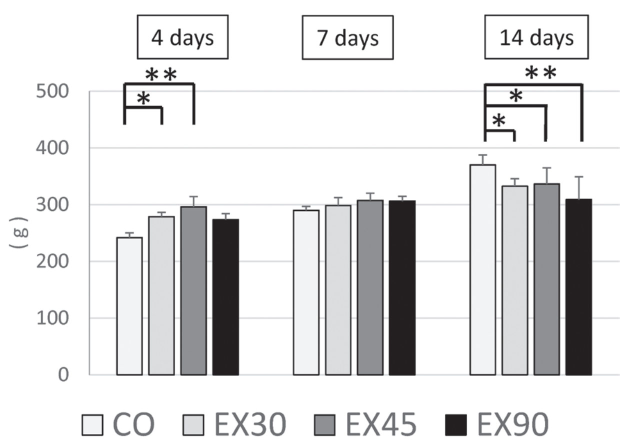

Fig. 2 The Body weight in each group (*:p<0.05, **:p<0.01)

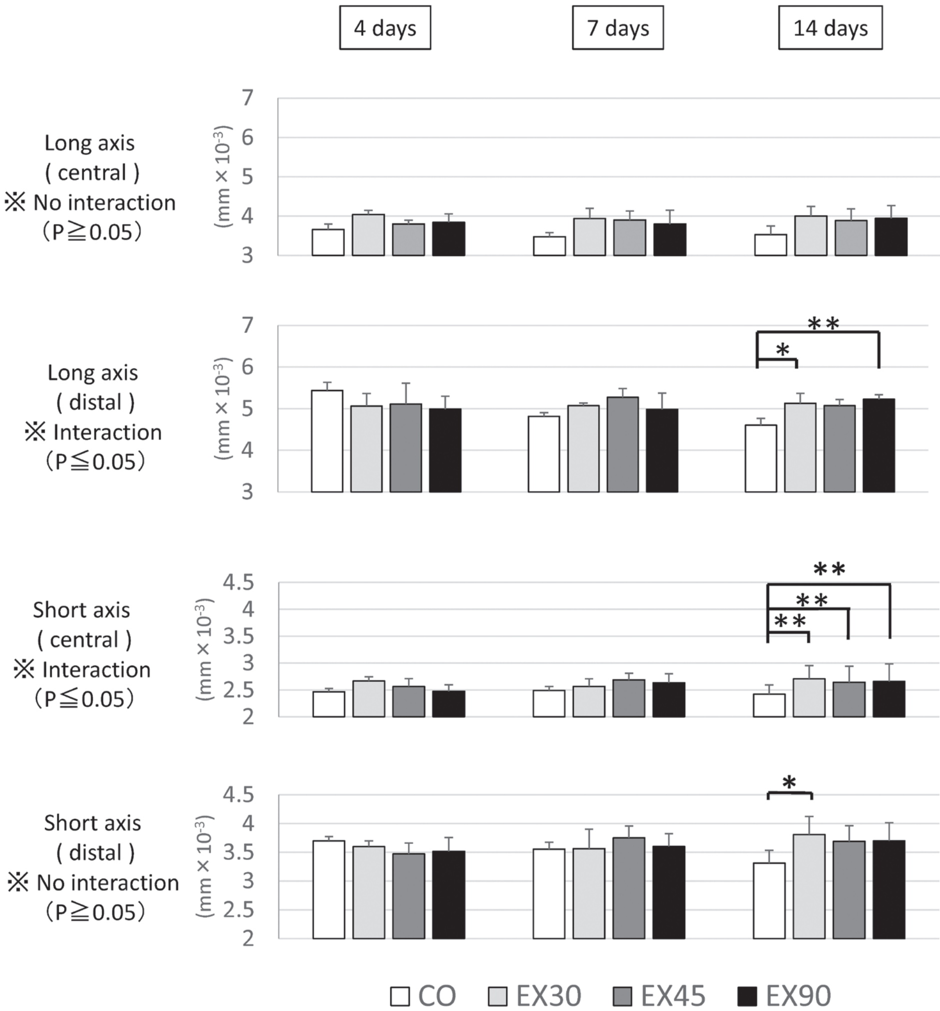

Fig. 3 The Bone morphometric analysis (＊:p<0.05, ＊＊:p<0.01)

no significant differences were observed in the length of the long axis at the central portion between any groups in 14-day-groups. However, those of EX30 and EX90 were significantly longer than CO in the distal portion (Fig. 3). As for the length of short axes of that, significant differences were observed at the central and distal portions, and all of EX showed higher values than CO in only 14-day-groups.

Comparison of the thickness of the periosteum

Significant differences in thickness of periosteum were found at the anterior face of femur only at 7-day-group and weren't in other groups. No significant differences in the periosteum were recognized fundamentally at the posterior face of that, except for 14-day-groups (Fig.4). Concerned to medial and lateral faces of that, significant differences in thickness of the periosteum were found at the lateral face in 4 and 14-day-groups but other groups showed non-significant difference at both medial and lateral faces (Fig. 5).

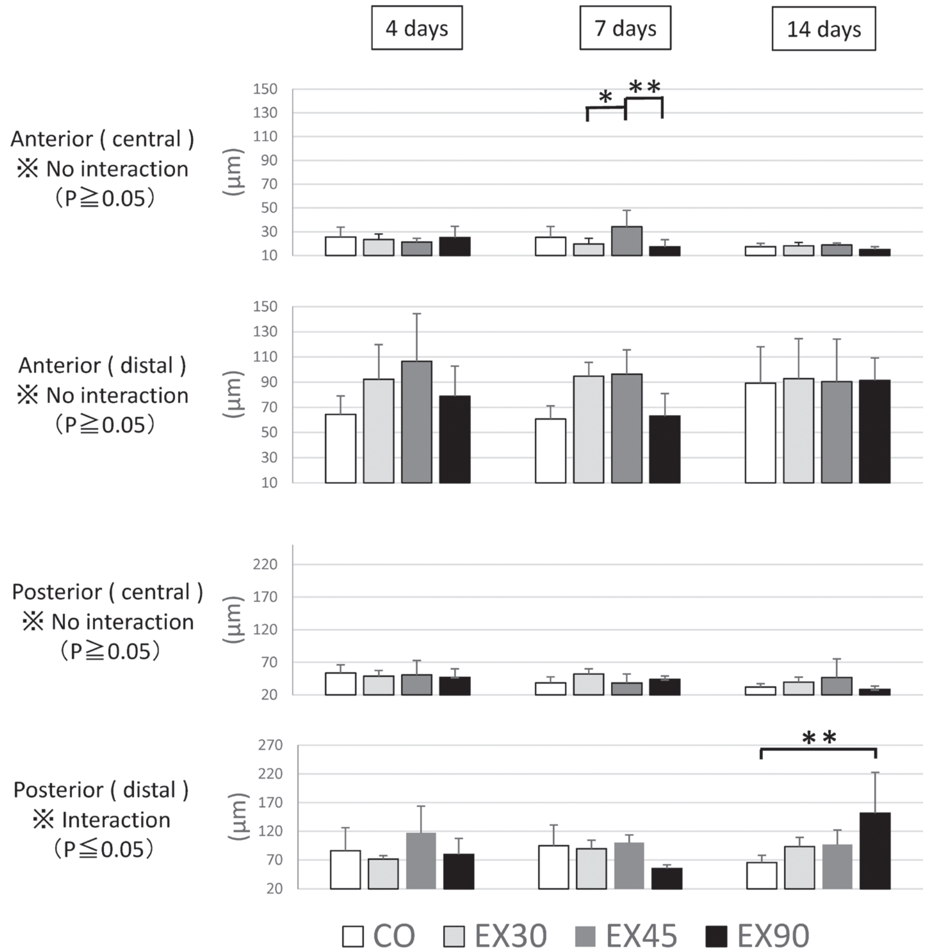

Fig. 4 Thickness of the periosteum morphometric analysis, anterior and posterior face (＊:p<0.05, ＊＊:p<0.01)

Histological observations

We observed all sections of central and distal portions of femurs in every groups. As a result, obvious differences in histological structures were recognized at posterior and medial face in 14-day-groups, compared to 4 and 7-day-groups. Therefore, data of them were used for morphological analyses in this study. Thick osteoid were formed below the periosteum in both the posterior and medial site at the central portion, accompanied with the increase in the exercise intensity. Blood vessel lumens were narrowed in the cortical bone, and the bone matrix was indicated compaction (Fig. 6). At the distal portion, unlike the central portion, along with the exercise intensity increases, significant bone resorption from periosteal side was confirmed (Fig. 7, yellow arrowhead), and vascular cavities had many occurrences in the periosteum side of the compact bone. Many bone resorption images were observed at below the periosteum and the blood vessel cavities were formed and expanded at the distal portion accompanied with that (Fig. 7). To

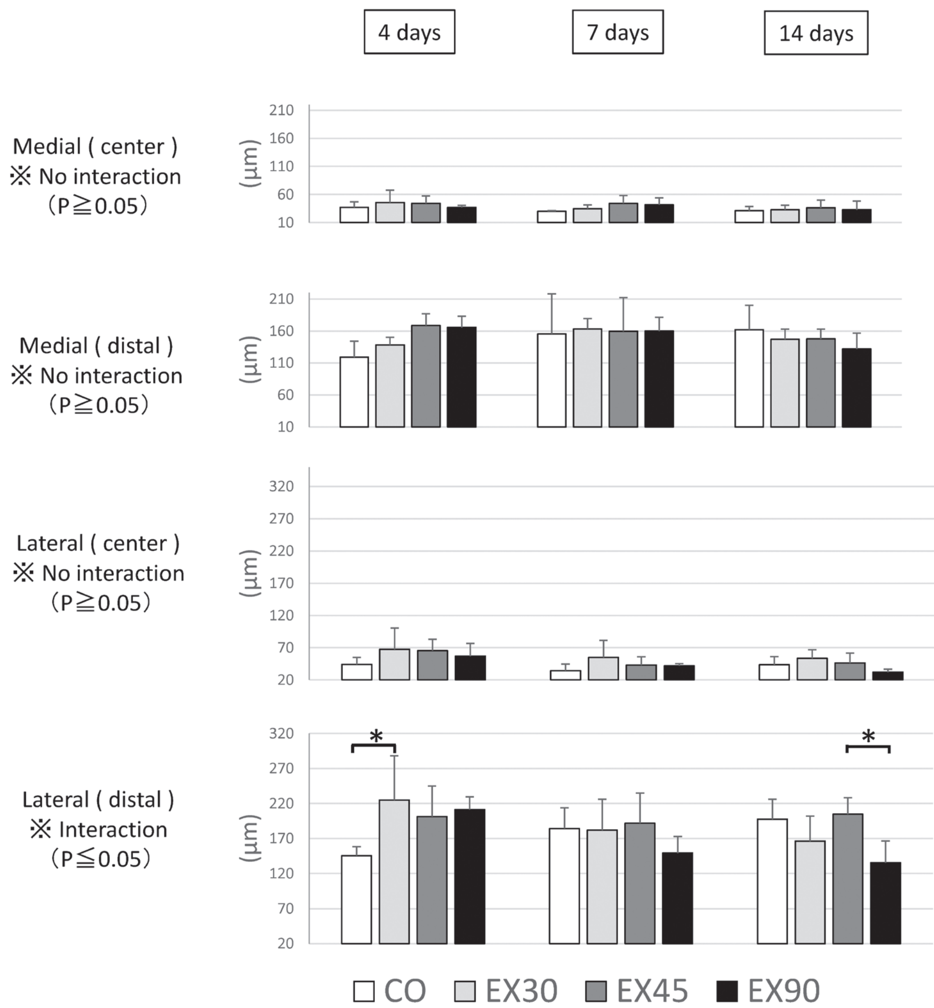

Fig. 5 Thickness of the periosteum morphometric analysis, medial and lateral face (＊:p<0.05, ＊＊:p<0.01)

confirm the change in the details of the periosteal tissue, with respect to the tissue of the central portion of the femur, by creating a decalcified semi-ultrathin sections were observed under a light microscope (Fig. 8). Thickness of the periosteum of the medial face increased at the central portion in EX30 of 4-day-groups and decreased until a same level of CO in 14-day-groups. Furthermore, at that face, a bone forming layer of the periosteum thickened especially and an extent of cell aggregation was same as CO.

Discussion

Structural changes of a periosteum at the central and distal portions of femur caused by jumping exercises under various conditions were investigated by relating to a change of the bone matrix in this study.

Body weight of CO increased gradually with growth and was higher level than any

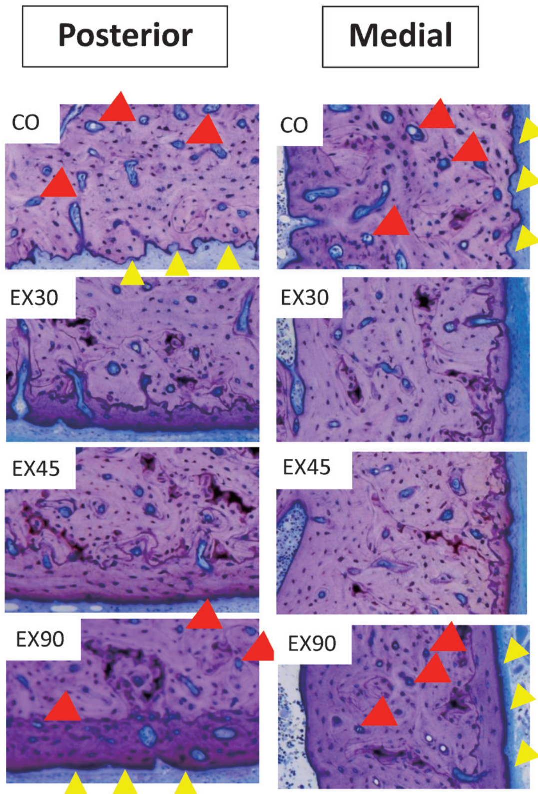

Fig. 6 Structural changes in the cortical bone of the central portion for 14 days (Undecalcificated resin-embedded specimens, toluidine blue stain, Red arrowhead: blood vessel lumens, Yellow arrowhead: osteoid)

groups of EX in 14-day-groups (Fig. 2) . This result agreed with observations of Rabey et al.[33] that made mice running or climbing. It was thought that there were little effects of the body weight on the bone formation, because the body weight of EX didn't increase necessarily.

Significant differences in diameters of the femurs were found between CO and EX only in 14-day-groups (Fig. 3) . No significant differences were recognized in data concerned to morphology of muscle-attaching potion at the experiment of a treadmill running or climbing using mice[33] .

The periosteum of the femur was thin at the center portion, to the contrary, was thick at the distal portion, and these results were agreed with results of Fan et al.[33, 34] . Furthermore, it was reported that the thickness of the periosteum of rat's femur decreased with growth[33] . Same results were also obtained in this study, it was supposed that increase in thickness of the periosteum with growth was physiological change simply (Fig. 4 and 5) .

Compaction of the cortical bone were found accompanied with increase in exercise

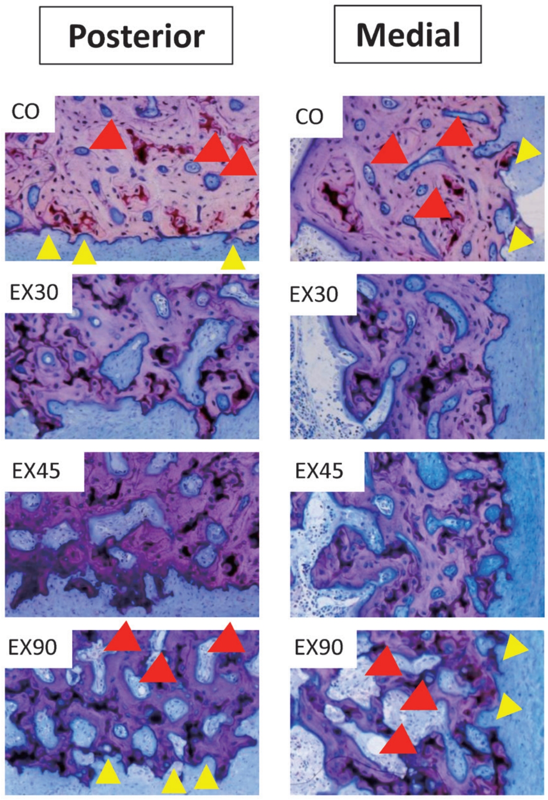

Fig. 7　Structural changes in the cortical bone of the distal portion for 14 days (Undecalcificated resin-embedded specimens, toluidine blue stain, Red arrowhead: blood vessel lumens, Yellow arrowhead: bone resorption)

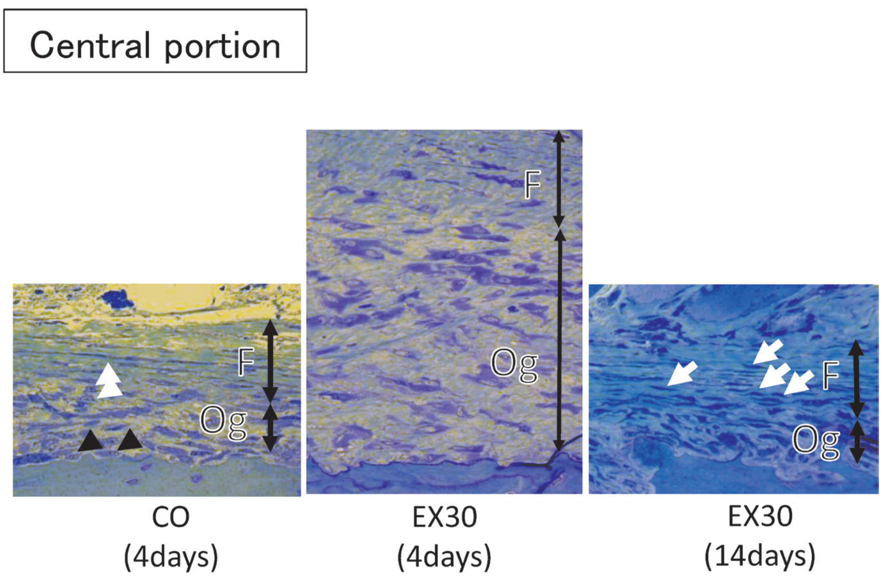

Fig. 8　Differences in the periosteum of the medial face (Decalcificated semi-ultrathin sections, Toluidine blue stain, White arrowhead: undifferentiated cell, Black arrowhead: osteoblast cell, White arrowed line: fibrobrast cell)

intensity at the central portion (Fig. 6). Oppositely, active osteoid formation were recognized at the distal portion (Fig. 7). Osteoid formation was activated at the periosteum side of central and distal portions. Compaction of the cortical bone was found only at the central portion and expansion of Howship's groove was observed only at the distal portion. The cortical bone showed porous structures but was going to be a compact bone[3]. It was thought that this change was due to mechanical reaction of the bone accompanied with increase in body weight and activity[36, 37]. Obvious compaction of the cortical bone was recognized at the central portion by increase in the exercise intensity in this study. This result suggested that mechanical loading affected the cortical bone formation. It was suggested that process of healing was different between diaphysis and metaphysis, from the report investigating the healing process by drug after bone injury[38-40]. This was assumed to be resulted from difference in cell reaction to mechanical loading between central and distal portions.

It was supposed that little changes in the thickness of the periosteum were observed because it didn't resist to mechanical loading directly. It might be that changes in blood circulation and releasing of growth factor from muscle tissue by exercise stimulation gave an acute influence on the periosteum (Fig. 8) because rich vessels and many sensory nerves existed in the periosteum[28-30].

From these results, it was understood that the jumping exercise at various intensities caused characteristic changes in the bone matrix structures but little effects gave to the thickness of the periosteum. Furthermore, these findings could be contribute to understanding of mechanism of pathological condition, that is, shin splints, tibial stress fracture and medial tibial stress syndrome (MTSS).

Conclusion

It was understood that the jumping exercise at the various intensities caused characteristic changes to the bone matrix but little effects was given to the thickness of the periosteum.

Committee of Animal Experiment and Ethics

This study was approved by committee of Animal Experiment and Ethics for the research, Graduate School of Welfare Society design, TOYO University.

Acknowledgments

This work thanks to the support of graduate and undergraduate laboratory member and the cooperation with you for guidance and encouragement.

This work was supported by the INOUE ENRYO Memorial Grant, TOYO University.

Reference

1) Pocock N., et al. : Muscle strength, physical fitness, and weight but not age predict femoral neck bone mass. J. Bone Miner. Res. 4 : 441-448, 1989.

2) Bevier WC., et al. : Relationship of body composition, muscle strength, and aerobic capacity to bone mineral density in older men and women. J. Bone Miner. Res. 4 : 421-432, 1989.

3) Ohsako M., et al. : Characteristics of bone structural changes by growth and mechanical stress in growing rats. J. Phys. Fitness Sports Med. 3 : 327-333, 2014.

4) Takahashi M., et al. : Effects of various mechanical loading on osteogenetic processes of tibial cancellous bone in rats. Bull. Grad. Sch. Toyo Univ. 52 : 241-253, 2015.

5) Pruitt LA., et al. : Effects of a one-year high-intensity versus low-intensity resistance training program on bone mineral density in older women. J. Bone Miner. Res. 10 : 1788-1795, 1995.

6) Ehrlich PJ., et al. : Mechanical strain and bone cell function: a review. Osteoporos Int. 13 : 688-700, 2002.

7) Pavalko FM., et al. : A model for mechanotransduction in bone cells: the load-bearing mechanosomes. J. Cell Biochem. 88 : 104-112, 2003.

8) Shipp KM. : Exercise for people with osteoporosis: translating the science into clinical practice. Curr. Osteoporos Rep. 4 : 129-133, 2006.

9) Iwamoto J., et al. : Differential effect of treadmill exercise on three cancellous bone sites in the young growing rat. Bone. 24 : 163-169, 1999.

10) Han Y., et al. : Mechanotransduction and strain amplification in osteocyte cell processes. Proc. Natl. Acad. Sci. USA. 101 : 16689-16694, 2004.

11) Vatsa A., et al. : Bio imaging of intracellular NO production in single bone cells after mechanical stimulation. J. Bone Miner. Res. 21 : 1722-1728, 2006.

12) Wang Y., et al. : A model for the role of integrins in flow induced mechanotransduction in osteocytes. Proc. Natl. Acad. Sci. USA. 104 : 15941-15946, 2007.

13) Tatsumi S., et al. : Targeted ablation of osteocytes induces osteoporosis with defective mechanotransduction. Cell Metab. 5 : 464-475, 2007.

14) Fritton SP., et al. : Fluid and Solute Transport in Bone: Flow-Induced Mechanotransduction. Annu. Rev. Fluid Mech. 41 : 347-374, 2009.

15) Bonewald LF. : The amazing osteocyte. J. Bone Miner. Res. 26 : 229-238, 2011.

16) Dallas SL., et al. : The osteocyte: an endocrine cell ... and more. Endocr. Rev. 34 : 658-690, 2013.

17) Klein-Nulend J., et al. : Mechanosensation and transduction in osteocytes. Bone. 54 : 182-190, 2013.

18) Kobayashi Y., et al. : Force-induced osteoclast apoptosis in vivo is accompanied by elevation in transforming growth factor beta and osteoprotegerin expression. J. Bone Miner. Res. 15 : 1924-1934, 2000.

19) Burr DB. : Targeted and nontargeted remodeling. Bone. 30 : 2-4, 2002.

20) Chapurlat RD., et al. : Bone microdamage: a clinical perspective. Osteoporos Int. 20 : 1299-1308, 2009.

21) Nakashima T. : Bone Homeostasis and Mechano biology. Clinical Calcium 26 : 1685-1695, 2016.

22) Fehling PC., et al. : A comparison of bone mineral densities among female athletes in impact loading and active loading sports. Bone. 17 : 205-210, 1995.

23) Taaffe DR., et al. : Differential effects of swimming versus weight-bearing activity on bone mineral status of eumenorrheic athletes. J. Bone Miner. Res. 10 : 586-593, 1995.

24) Mudd LM., et al. : Bone mineral density in collegiate female athletes: comparisons among sports. J. Athl. Train. 42 : 403-408, 2007.

25) Heinonen A., et al. : Bone mineral density in female athletes representing sports with different loading characteristics of the skeleton. Bone. 17 : 197-203, 1995.

26) Kato T., et al : Effect of low-repetition jump training on bone mineral density in young women. J. Apple. Physiol. 100 : 839-843, 2006.

27) Umemura Y., et al. : Five jumps per day increase bone mass and breaking force in rats. J. Bone. Miner. Res. 12 : 1480-1485, 1997.

28) Rhodin JAG. : 9 BONE. Histology A TEXT AND ATLAS by Oxford University Press Inc. USA, pp196, 1974.

29) Ham AW., et al. : 15 Bone and Bones. HISTOLOGY EIGHTH EDITION by J. B. Lippincott Company. USA, pp389, 1979.

30) Mescher AL. : 8. Bone. JUNQUERIRA'S Basic Histology Twelfth Edition by The McGraw-Hill Companies Inc. USA, pp122-127, 2010.

31) Chang H., et al. : Concise review: the periosteum: tapping into a reservoir of clinically useful progenitor cells. Stem Cells Transl. 1 : 480-491, 2012.

32) Malizos K.N., et al. : The healing potential of the periosteum molecular aspects. Injury. 36 :

13-19, 2005.

33）Rabey KN. at el. : Locomotor activity influences muscle architecture and bone growth but not muscle attachment site morphology. Journal of Human Evolution. 78 : 91-102, 2015.

34）Fan W., et al. : Structural and cellular differences between metaphyseal and diaphyseal periosteum in different aged rats. Bone. 42 : 81-89, 2008.

35）Fan W., et al. : Structural and cellular features in metaphyseal and diaphyseal periosteum of osteoporotic rats. J. Mol. Histol. 41 : 51-60, 2010.

36）Dalzell N., et al. : Bone micro-architecture and determinants of strength in the radius and tibia: age-related changes in a population-based study of normal adults measured with high-resolution pQCT. Osteoporos Int. 20 : 1683-1694, 2009.

37）Adachi A. : Modeling and simulation of trabecular pattern formation in a cancellous bone defect under mechanical forces. Clinical Calcium 26 : 1779-1787, 2016.

38）Sandberg O., et al. : Different effects of indomethacin on healing of shaft and metaphyseal fractures. Acta. Orthop. : 86, 243-247, 2015.

39）Sandberg O., et al. : Glucocorticoids inhibit shaft fracture healing but not metaphyseal bone regeneration under stable mechanical conditions. Bone Joint Res. 4 : 170-175, 2015.

40）Sandberg O., et al. : Earlier effect of alendronate in mouse metaphyseal versus diaphyseal bone healing. J. Orthop. Res., doi : 10. 1002/jor. 23316. 2016.

異なる強度の跳躍運動がラット大腿骨の皮質骨と骨膜に及ぼす影響

福祉社会デザイン研究科ヒューマンデザイン専攻博士前期課程２年
神尾　強司
福祉社会デザイン研究科ヒューマンデザイン専攻博士前期課程１年
吉良　裕一郎
ライフデザイン学部健康スポーツ学科教授
大迫　正文

要　旨

本研究では、異なる条件の跳躍運動によってもたらされる大腿骨中央および遠位部の骨膜の構造変化に骨基質の変化を関連づけて検討することを目的とした。

材料として 7 週齢のウィスター系雄性ラット 48 匹を用い、それらを跳躍運動群（EX）および対照群（CO）に分類した。EXは、さらに最大跳躍高の 30、45 または 90％の高さで跳躍運動する 3 群に分けた。各EXは、それぞれの高さの跳躍運動を、100 回/日、5 日/週、最大 14 日間行わせた。またCOも同様の期間を通常飼育した。実験期間終了後、大腿骨の遠位 2/5 部（中央部）ならびに 1/4 部（遠位部）の各種標本を作製し、組織学および形態学的に観察した。

運動強度の増加に伴って中央部では緻密化が確認され、遠位部では活性化した類骨が形成されていた。類骨形成は中央および遠位部ともに骨膜側で活性化していた。皮質骨の緻密化は中央部のみに見られ、ハウシップ窩は遠位部のみに観察された。骨膜は一過性に骨形成層の厚さを増し、その後は元の厚さに戻った。

これらの結果から、異なる強度の跳躍運動は、骨基質に特徴的な構造変化をもたらすのに対し、骨膜の厚さにはあまり影響をもたらさないことが理解された。

キーワード：　跳躍運動、加重負荷、皮質骨、骨膜