

Study in structures of tendon-attached portion and density of vessels in periosteum of femur in rat

NAKAI, Shingo^{*}

KIRA, Yuuichiro^{*}

OHSAKO, Masafumi^{**}

Summary

This study aimed to clarify the morphological characteristics of a periosteum at the portion that a muscle attached and to investigate a relationship between the structures and the vascularity of the periosteum. Ten male rats (wistar strain, 13-week-old) were used as materials. Femurs were excised from them, were fixed rapidly and were used for preparation of histological specimens.

Many muscles attached to the surface of the body of femur in rats. Most of tendon fibers at the anterior face of the femur were embedded in the bone in parallel direction against the bone surface, but most of them in the posterior face were embedded in nearly vertical direction against the bone surface.

Ends of the former tendons were embedded in a deep area of the bone matrix as Sharpy's fibers, but tendons of the latter connected loosely to fibers of periosteum. Thick blood vessels existing on the bone surface ran in mostly parallel direction to the bone surface and some of them ran so as to surround the tendon. The density of blood vessels in the posterior face was generally lower than the posterior face.

It was understood that the distribution and running direction of the blood vessels in the periosteum was related to the embedded direction of the tendon fibers.

Keywords : Tendon attaching portion, Bone structures, Distribution of vessels

^{*} Doctoral Program in Graduate School of Welfare Society Design, Course of Human Centered Life Design, Concentration in Health Care and Sports, Third Year Student, Toyo University.

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

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

1. Introduction

A muscle repeats hypertrophy and atrophy by changes of activity in daily living, and then, structural changes occur at the tendon-attached portion¹⁾. On the other hand, many blood vessels existed in the periosteum, but a site difference and a growth change are seen in that²⁾. A site that the muscle and the periosteum connect softly and other site that they connect tightly existed in a femur, but differences in structure of both sites hadn't been cleared. Furthermore, the differences in distribution of the blood vessel at those sites hadn't been reported.

This study, using rats' femurs, aimed to clear structural characteristics at the sites of different attaching patterns of the tendons and to investigate relationship between the distribution of the blood vessels and those characteristics.

2. Materials and methods

2.1 Materials

Ten male rats (wistar strain, 13-week-old) were used for materials.

2.2 Sampling and fixation

Pentobarbital (Somnopenil) solution was shot to rat's abdomen (40mg/kg) and they were fixed by a perfusing fluid contained 2.5% glutaraldehyde and 4% paraformaldehyde (4%PFA). Femurs were excised after that, and were immersed in 4%PFA or Karnovsky fixation fluid. Moreover, resin was put into an abdominal aorta for making the blood vessel-cast specimen. After that, those specimens were immersed in 15%KOH fluid for removing organic substances for two weeks and used for observation by a scanning electron microscope(SEM). An india ink diluted by physiological saline solution was also inserted to the blood vessel and those specimens were observed by a light microscope.

2.3 Preparation and observation of histological specimens

The specimens of femur fixed by Karnovsky fluid were cross-sectioned at the middle portion of the diaphysis and their surface were etched by 0.1 or 1mol HCl. After that, they were immersed in 4% osmium tetra-oxide, were dehydrated and freeze-dried, and were vacuum-evapolated with carbon and platinum. Anterior, posterior and medial faces of the femur were observed by a scanning electron microscope. The specimens fixed 4% PFA were dehydrated and cleared under the vacuum-condition, were embedded in Rigolac resin and used for the observation of running of the blood vessels.

3.1 Macroscopic observations

In the anterior face of the femur, tendon fibers attached from a vertical direction to the bone surface at the proximal portion. The tendon fibers of quadriceps femoris muscle attached from a parallel direction to the bone surface at the middle and distal portion, in the anterior face of the femur. Moreover, adipose tissues were recognized at the space between the quadriceps femoris muscle and the periosteum, and they existed remarkably from middle portion of diaphysis to metaphysis, especially. On the other hand, in the posterior face of the femur, some adductor muscles attached to the bone surface from vertical direction and thickness of the periosteum was thin in the anterior-medial face and was thick in the posterior-lateral face. (Fig.1)

3.2 Histological observations

A cross-sectional face of a bone matrix near the calcified cartilage matrix was smooth but the face of the bone matrix surrounding that bone was rough, when the periosteum and the bone matrix of the middle portion of the diaphysis of the specimens etched were observed by SEM (Fig 2). Connection between the bone and the periosteum was loose at the portion that the periosteum was thin, and thick collagen fibers existed between them at the portion that the periosteum was thick (Fig 3).

On the other hand, the blood vessels ran in parallel direction of the bone surface at the portion that the tendon fibers embedded in parallel direction to the bone surface (parallel-embedded portion: PEP), and a density of blood vessels at this portion was lower than the former portion (Fig. 4). The blood vessels of the vertical-embedded portion surrounded the muscle fibers. Those vessels of the parallel-embedded portion run in parallel direction to the muscles fibers, and some of the blood vessels anastomosed in each other and invaded in the bone (Fig 4,5).

4. discussion

In this study, many tendon fibers that arranged in parallel direction to the bone surface were recognized in anterior face at the distal portion of femur. Adipose tissues existed in the space between the quadriceps femoris muscle and the periosteum, and these tissues were found remarkably at distal portion of femur, especially. It was known that many adipose tissues existed, at the portion where the friction occurred by the muscle contraction. And then, it was supposed that strong frictions occurred at the diaphysis of femur that the vastus intermedius muscle attached. On the other hand, the muscles attached from vertical

direction existed in posterior face. Then, it is thought that it is necessary to be able to resist to the traction force from not only direction of a long axis of the femur but also a horizontal direction

A hip and a knee joints of rat was always flexed. Then, it is necessary to greatly extend with a hip joint in comparison with *Homo sapiens* of the vertical bipedalism at the time of a walk and the jump. Therefore, in the case of a rat, it was thought that a contracting force was brought in hip joint extension position by a muscle attaching to the bone surface from the vertical direction. It was thought that this result meant a characteristic of quadrupedal animals, and gave an important suggestion in examining basic bone structure of the *Homo sapiens*. The periosteum was thin in an anterior-medial face and was thick in posterior-lateral face, at the middle portion of diaphysis. It was speculated that a tension from the tendon affected to these results. It was supposed that loose connection between the periosteum and the bone surface reduced the friction because a priority was given when the muscle run on the periosteum at PEP in anterior-medial face. On the other hand, the periosteum was thick and the connection between the periosteum and the bone surface was tight at VEP in posterior face. It was thought that it was necessary for the periosteum to resist to the pulling force from the muscle by increase in the thickness of the periosteum.

The periosteum was composed of outer fibrous and inner cellular layers. It was known that the former layer has strong mechanical characteristic and the latter layer has rich vessel-network²⁾. Smooth areas existed around the calcified cartilage at the deep portion of the cortical bone when observing a ground face of that bone etched by HCl. Areas that the bone surface was rough (rough area) were found near the smooth area. Organic quality such as the matrix fibers was exposed at rough area. Lamellar structures were recognized at periosteum and endosteum sides of the cortical bone.

Ohsako³⁾ had classified the bone matrix in three types (A, B and C) from the point of view of density and arrangement of the matrix fibers, mainly. According to this report³⁾, it was showed that Type A existed around the calcified cartilage, Type B was found near Type A, and Type C has lamellar structure. From existing portion of each type bone, it was assumed that the bones of smooth area and rough area corresponded to Type A and B, respectively. Furthermore, the lamellar bone in periosteum and endosteum sides of the cortical bone corresponded to Type C, similarly.

Type A existed together with the calcified cartilage at the deep area of the cortical bone. Type B was distributed in wide extent of posterior-lateral face of the femur. Type C was formed thickly in anterior-medial face of that. The diaphysis of rat's femur showed the

curve that was a convex forward. And then, it was thought that this portion bended to anterior direction by a mechanical stress from the long-axis direction of this bone. It was supposed that Type C existing in anterior-medial face of the femur functioned to resist the extension of that portion⁴⁾. Furthermore, the connection between the periosteum and the bone surface was loose at the site that the periosteum was thin. To the contrary, at the site that it was thick, the periosteum didn't peel from the bone surface because many thick fibers existed between the periosteum and the bone surface and connected them each other. Thus, the periosteum-rich-site corresponded to Type B-forming site. At this portion, it was thought that thick fibers penetrating the periosteum gave the structure that was able to resist to the mechanical stress. On the other hand, Type C was formed in anterior-medial face of the femur. It was understood that Type C could resist to extension force by the mechanical stress was given, but connected loosely to the bone surface because the fibers penetrating the periosteum were thin. The tendon of the muscle in anterior face of the femur arranged in parallel direction to the bone surface, and the blood vessels also ran in parallel direction to those tendon fibers. It was thought that such an arrangement of the muscle and the vessel were effective for the pumping action by the muscle. The tendon fibers were arranged in vertical direction to the bone surface in posterior face. At there, the blood vessels showed small ring-like shape and surrounded the tendon fibers that were embedded in vertical direction to the bone surface. It was speculated that the blood vessels ran not to disturb an arrangement of the tendon fibers.

From the above, it was thought that the thickness of the periosteum and the distribution of the blood vessels closely related to the arrangement of the muscle.

5. Conclusion

It was understood that the running direction of the blood vessels in the periosteum was related to the embedded direction of the tendon fibers.

6. 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.

7. Acknowledgements

This study was accomplished by the help of the Inoue research grant. We would like to thank our Lab members for their kind help and support.

8. References

- 1) LE Lanyon : Control of bone architecture by functional load bearing. Journal of Bone and Mineral Research 7 : 369-375, 1992.
- 2) Turner CH, et al. : Basic biomechanical measurements of bone : a tutorial. Bone 14 : 595-608,1993.
- 3) M. Ohsako : Histological Studies on Growth and Function of Condylar Process in Rat Mandible. The Journal of the Stomatological Society 60 : 47-96, 1995.
- 4) T. Tateishi, et al. : Viscoelasticity and strength of the cortical bone. The Japan Society of Mechanical Engineers 46 : 97-106, 1980.

9. Explanation of the figure

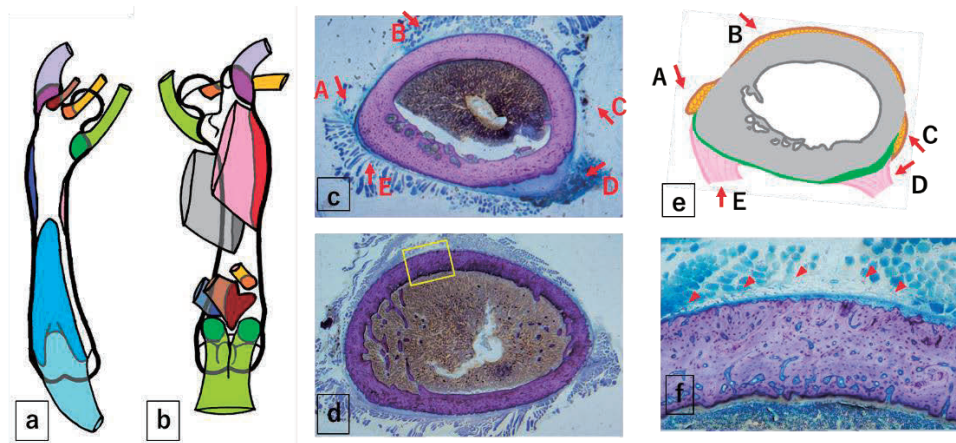


Fig.1. Relationship between muscle attaching portion and periosteum

a, b: scheme of muscle attaching portion of femur

(a: anterior face, b:posterior face)

c, d: low-magnified images of cross-sectioned femur (c: middle portion of diaphysis, d: metaphysis)

e, f: muscle attaching portions, f: magnified image of square of Fig d

A : Lateral vastus m. B : Intermedius m. C : Medial vastus m. D : Long adductor m. E : adductor magnus m.

Red arrow heads: adipose tissue

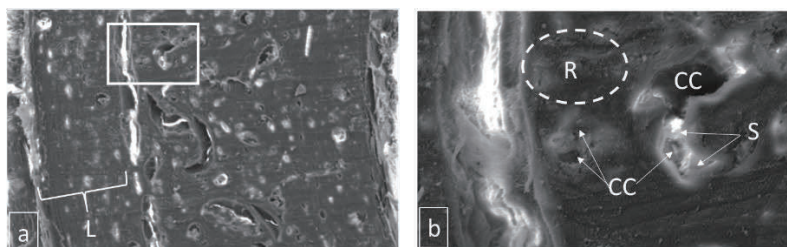


Fig.2 Classification of bone matrix

a: structure of cortical bone, b: magnified image of square of Fig.a

L: lamellar bone,

R: rough area, S: smooth area

(R and S was classified by structure of cross-sectioned bone surfaces that were etched by 0.1N HCL.)

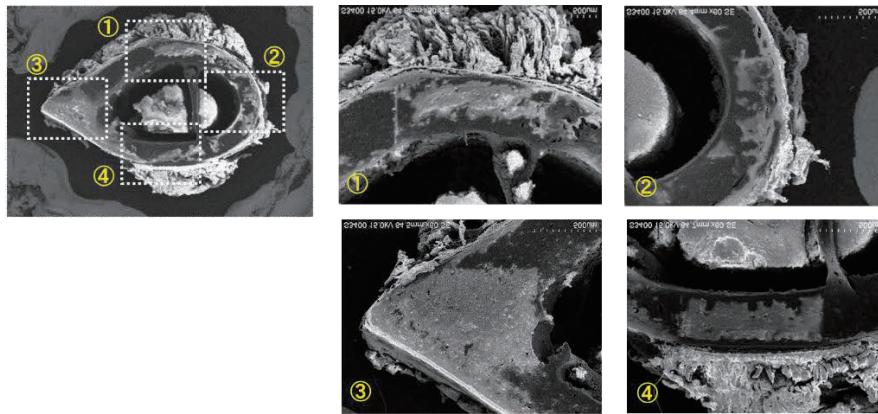


Fig.3 Differences in thickness of periosteum in each face of femur

①: anterior face, ②: medial face, ③: lateral face, ④: posterior face
Arrows of Fig. ① shows thin periosteum.

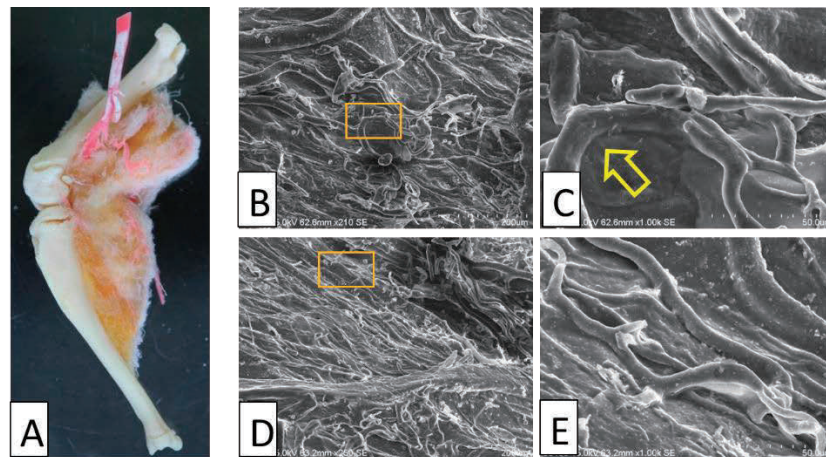


Fig.4 Distribution of vessels in the periosteum (vessel-casting specimen)

A: macroscopic image, B-E: SEM images

B, C: running of vessels at the portion that tendon fibers attached in vertical direction to the bone surface. C: magnified image of square of Fig B

D, E: running of vessels at the portion that tendon fibers attached in parallel direction to the bone surface. E: magnified image of square of Fig D

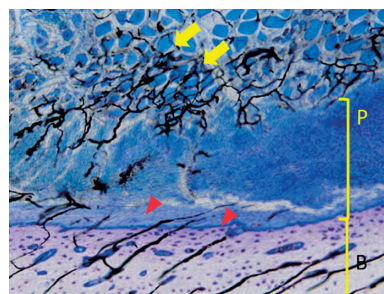


Fig.5 Running of vessels at the portion that tendon fibers attached in vertical direction to the bone surface.

P: periosteum, B: bone

Yellow arrows: vessels surrounding the muscle fibers cross-sectioned

Red arrow heads: vessels invading into the bone

ラット大腿骨における腱付着部の構造と 骨膜の血管分布に関する研究

福祉社会デザイン研究科ヒューマンデザイン専攻博士後期課程3年

中井 真悟

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修了

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

【目的】

本研究は、ラット大腿骨を用いて、異なる筋の付着様式の部位における骨膜の構造上の特徴を明らかにするとともに、それらと血管分布との関連性について検討することを目的とした。

【方法】

材料として13週齢のウィスター系雄性ラット10匹を用いた。それらから大腿骨を摘出し、速やかに固定して非脱灰の組織観察用標本作製した。さらに、還流固定したラットから血管鋳型標本作製し、KOHにて有機質を溶解して走査電子顕微鏡により観察した。

【結果および考察】

ラット大腿骨体の表面には多くの筋が付着しているが、その後面では主として腱が骨表面にほぼ垂直な方向に埋入され、逆に前面ではその長軸にほぼ平行な方向に埋入されるものが多かった。前者の腱の末端はシャープな線維となって骨基質内に深く埋入されていたが、後者の腱は骨膜の線維にゆるく連結していた。骨表面の血管に関しては、太いものが基本的に骨の長軸とほぼ平行に走行するが、それと同時に腱付着部位を取り巻くように走行し、全体的に大腿骨前面より疎であった。

【結論】

骨膜内の血管の分布および走行方向には、腱線維の骨内への埋入方向が関わっていることが理解された。

キーワード：腱付着部、骨構造、血管分布