Regional Differences in Elements in the Peroneus Longus Tendon in Humans

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Abstract

Many studies have been performed on the structure, molecular composition, and biochemical properties of tendons. However, comparatively little research has been conducted on the content of various trace elements within tendons. Six elements were analyzed in four regions of the peroneus longus tendon: the tensional part of the tendon immediately proximal to the lateral malleolus (region A), the compressive region of the tendon in contact with the lateral malleolus (region B), the compressive region of the tendon in contact with the deep surface of the cuboid (region C), and the tensional part of the tendon between the cuboid and first metatarsal to which the tendon is attached (region D). Regions B and C are wrap-around regions. The calcium content was higher in region C than in both A and D, which is likely related to regional differences in cartilage degeneration. The phosphorus content was also higher in region C, possibly because of low alkaline phosphatase activity in this region. The sulfur content was higher in the wrap-around regions; sulfur content is thought to be influenced by tendon-bone compression. Finally, the magnesium content in the wrap-around regions was also higher, which is probably related to a higher level of fibrocartilage. No significant relationships were found with regard to zinc or iron. Overall, the findings of the present study indicate that element contents are related to function and anatomical differences in tendons and that they may even vary within the same tendon.

Keywords: Peroneus longus tendon · Wrap-around tendon · Proteoglycans · Calcium · Sulfur

Introduction

The peroneus longus muscle originates in the lateral compartment of the leg. The musculotendinous junction of the peroneus longus tendon is located proximal to the superior peroneal retinaculum. The peroneus longus tendon courses distally in the leg posterior to the lateral malleolus toward its insertion in the foot. After passing posterior to the lateral malleolus, the peroneal tendon passes inferior to the cuboid in a canal formed by the cuboid groove superiorly and inserts onto the medial cuneiform and base of the first metatarsal [1, 2]. Macroscopically, the peroneus longus tendon has two flat regions that are capable of withstanding compression in regions where they press against the lateral malleolus and cuboid bones. These are considered to be a natural adaptation to compressive forces acting at the site of bony pulleys. A striking feature of many tendons is their fibrocartilaginous character. There are intriguing differences among different tendons. Despite the fact that numerous reviews have detailed the structure, molecular composition, and biomechanical properties of tendons, far less attention has been paid to their content of trace elements [2-4]. Therefore, the authors investigated regional variations of elements in the peroneus longus tendon and the relationships among element contents by direct chemical analysis.

Materials and Methods

Sampling

The cadaveric subjects comprised 8 men and 5 women ranging in age from 77 to 98 years (average age, 87.5±7.8 years). They were treated by injection of a mixture of 36 % ethanol, 13 % glycerin, 6% phenol and 6 % formalin through the femoral artery. The insertion tendon of the peroneus longus muscle (peroneus longus tendon) was resected from the 13 subjects. All unilateral peroneus longus tendons were harvested. Specimens with macroscopically obvious calcification were excluded from the present study.

Four regions of the peroneus longus tendon were analyzed in terms of their elements contents: the tensional part of the tendon immediately proximal to the lateral malleolus (region A), the compressive region of the tendon in contact with the lateral malleolus (region B), the compressive region of the tendon in contact with the deep surface of the cuboid (region C), and the tensional part of the tendon between the cuboid and first metatarsal to which the tendon is attached (region D) (Fig. 1).

Determination of Elements

The tendon samples were thoroughly washed with distilled water and dried at 80°C for 16 h. After 1 ml of concentrated nitric acid was added to the samples, the mixtures were heated at 100°C for 2 h. After the addition of 0.5 ml of concentrated perchloric acid, they were heated at 100°C for an additional 2 h. The

samples were adjusted to a volume of 10 ml by adding ultrapure water and were filtered through filter paper (No. 7; Toyo Roshi, Osaka, Japan). The resulting filtrates were analyzed with an inductively coupled plasma-atomic emission spectrometer (ICPS-7000; Shimadzu, Kyoto, Japan). The conditions were as follows: power of 1.2 kW from a radio-frequency generator, plasma argon flow rate of 1.2 l/min, cooling gas flow of 14 l/min, carrier gas flow of 1.0 l/min, entrance slit of 20 μm, exit slit of 30 μm, observation height of 15 mm, and integration time lapse of 5 s. The amount of element was expressed on a dry-weight basis.

Statistical Analysis

Statistical analyses were performed using Statcel version 3 (OMS, Tokyo, Japan). A two-tailed unpaired Student's t-test was used to compare differences between regions. A p-value of <0.05 was considered to be significant. Data were expressed as the mean±standard deviation.

Results

Table 1 lists the average contents of calcium, phosphorus, sulfur, magnesium, zinc and iron of the human peroneus longus tendon that were resected from four regions used in the ordinary dissection.

Calcium Content

Figure 2 shows the calcium contents of the four regions investigated. The average calcium contents were 1.25±0.51 mg/g in region A, 1.54±0.47 mg/g in region B, 2.10±0.93 mg/g in region C, and 1.43±0.41 mg/g in region D. The wrap-around regions (regions B and C) showed a higher content than regions A and D, which are subject to tensional force. With regard to the wrap-around regions (regions B and C), region C showed a higher content than region B. In addition, region C showed a significantly higher content than regions A ($p<0.01$) and D ($p<0.05$).

Phosphorus Content

Figure 3 shows the phosphorus contents of the four regions investigated. The average phosphorus contents were 0.23±0.17 mg/g in region A, 0.26±0.13 mg/g in region B, 0.47±0.38 mg/g in region C, and 0.27±0.08 mg/g in region D. Region C showed a significantly higher content than region A ($p<0.05$).

Sulfur Content

Figure 4 shows the sulfur contents of the four regions investigated. The average sulfur contents were 0.83±0.11 mg/g in region A, 0.98±0.09 mg/g in region B, 1.24±0.19 mg/g in region C, and 0.83±0.1 mg/g in region D. There was no difference between regions A and D. Region B showed a higher content than regions A and D, which were not wrap-around regions ($p<0.01$). Region C also showed a higher content than regions A and D ($p<0.01$). With regard to the wrap-around regions (regions B and C), region C showed a higher content than region B.

Magnesium Content

Figure 5 shows the magnesium contents of the four regions investigated. The average magnesium contents were 121±83.3 μg/g in region A, 177±98.8 μg/g in region B, 216±107 μg/g in region C, and 152±90.3 μg/g in region D. Region C showed a significantly higher content than region A ($p<0.05$).

Zinc Content

Figure 6 shows the zinc contents of the four regions investigated. The average zinc contents were 27.7±17.4 μg/g in region A, 22.6±13.6 μg/g in region B, 22.9±13 μg/g in region C, and 23.1±12.3 μg/g in region D. A relationship among the four regions in terms of zinc content was not found.

Iron Content

Figure 7 shows the iron contents of the four regions investigated. The average iron contents were 33.7±16.5 μg/g in region A, 28.4±14.9 μg/g in region B, 30.1±19 μg/g in region C, and 27.3±14.6 μg/g in region D. A relationship among the four regions in terms of iron content was not found.

Discussion

Although numerous reviews have detailed structure, molecular composition, and biomechanical properties of tendon and ligament, far less attention has been paid to their content of trace elements. In a previous study, we collected peroneus longus tendon specimens from four structurally different sites in each of seven cadavers and investigated the sulfur content of each site. This study provided information on site-related changes in the element contents of tendons, which can be useful for interpreting and evaluating morphological and biochemical changes in tissues and alterations in their mechanical properties [5]. In the present study, we investigated the various element contents of these four regions of the peroneus longus tendon in further detail.

One of the main functions of tendinous tissue is to transmit tension through the muscular tissue.

When tension is produced, there are compression regions between the tendon and bony tissue where the run of the tendon changes. Benjamin et al. found that these regions were flatter and glossier in appearance than the tensional parts. Thus, these tendinous tissues were referred to as wrap-around tendons in these reports [6-8]. These reports also pointed out that wrap-around tendons could be found in various locations, such as the tendons of the musculus flexor carpi radialis, musculus extensor digitorum, and biceps brachii muscles of the upper limb and the tendons of the peroneus longus, peroneus brevis, posterior tibial, and flexor hallucis longus muscles of the lower limb. In addition, there is abundant fibrous cartilage tissue in wrap-around regions.

It was also reported that the tissue structure of tendons might vary depending on the strength of tendon tension and compression [3]. The peroneus longus tendon has two characteristic wrap-around regions, and it plays a more important role in supporting weight compared with the tendinous tissue of the upper limb. Thus, the tendinous and muscular tissues connected to it are thicker than those of the upper limb, and the compression between the wrap-around tendons and the bony tissue is stronger. Based on this, we designed a study on the differences in compositional changes and functions of such tissues, which revealed to us that anatomical differences might also be present within the same tendon.

The sulfur content was higher in regions B and C (wrap-around regions) compared with the other regions ($p<0.01$). According to previous histological studies [2, 4], metaplasia may occur in fibrocartilage tissue containing cartilaginous matrix produced due to tendon-bone compression in wrap-around regions. We can conclude that there is an increase in the amount of glycosaminoglycans accompanied by rich sulfate regions such as chondroitin sulfate, dermatan sulfate, and keratin sulfate, which comprise the cartilaginous matrix. Furthermore, we found that the sulfur content even varied between the wrap-around regions. The sulfur content was significantly higher in the wrap-around region in contact with the deep surface of the cuboid (region C) compared with that behind the lateral malleolus (region B). We consider that the sulfur content is influenced by tendon-bone compression, which might be due to weight bearing in the area of region C. Moreover, we thought that the wrap-around region behind the lateral malleolus performed better in terms of the tendon's sliding ability, so the tendon-bone compression was dispersed here. However, because the tendon-bone compression was larger in the area of contact with the deep surface of the cuboid and in areas with low sliding ability, the sulfur content obviously reached a higher level in region C.

A higher calcium content was clearly shown in region C compared with both A and D ($p<0.05$). Although region C showed a higher content than region B, this difference did not reach statistical significance among wrap-around regions. It is considered that this is related to cartilage degeneration. It has been reported that calcium strongly increases on the degeneration of menisci composed of fibrocartilage [9]. In the present study, we excluded tendons with calcification. Interestingly, region C is an area that may contain the sesamoid bone (os peroneum) [10]. We believe that the calcium content in region C was higher than that in region B in areas of the same wrap-around part because region C is an

area in which the tendon has a lower sliding ability and the tendon-bone compression is stronger in the wrap-around part. Thus, the calcium content reached a higher level in region C.

The phosphorus content was significantly higher in region C than in region A. Region C also had a high calcium content. In a study by Archer et al. (1992), the light microscopic histochemistry of specimens with calcifying tendinitis demonstrated a low level of alkaline phosphatase activity. They described that alkaline phosphatase activity would be an extra factor to promote calcification. This is considered to explain why region C had a high phosphorus content.

We also observed that the magnesium contents in regions B and C (wrap-around regions) were higher than those in regions A and D. Region C showed a significantly higher content than region A ($p<0.05$). Tohno et al. found very high correlations between calcium and magnesium contents and between phosphorus and magnesium contents [11]. It has long been reported that a low magnesium intake affects growth plates and cartilage. Growth plates of magnesium-restricted animals showed reduced chondrocyte column formation. The extracellular matrix of both articular cartilage and growth plates in experimental animals contained reduced amounts of proteoglycans [12]. Therefore, we considered that region C had a high magnesium content because it is rich in fibrocartilage. We believe that the magnesium content, like the calcium, phosphorus, and sulfur contents, reflects the amount of fibrocartilage contained in wrap-around regions.

We did not observe any apparent statistically significant differences in zinc and iron contents in different regions.

In conclusion, the elementary composition of tendons might change with different functions in different regions defined in terms of anatomy, even within the same tendinous tissue.

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Figure Legends

Table 1 Average Element Contents in the four regions of the peroneus longus tendon

Fig. 1 The four regions of the peroneus longus tendon investigated in terms of element content
Region A: The tensional part of the tendon immediately proximal to the lateral malleolus. Region B: The compressive region of the tendon in contact with the lateral malleolus. Region C: The compressive region of the tendon in contact with the deep surface of the cuboid. Region D: The tensional part of the tendon between the cuboid and the first metatarsal (1st Meta) to which the tendon is attached.

Fig. 2 Calcium contents in the four regions of the peroneus longus tendon

Fig. 3 Phosphorus contents in the four regions of the peroneus longus tendon

Fig. 4 Sulfur contents in the four regions of the peroneus longus tendon

Fig. 5 Magnesium contents in the four regions of the peroneus longus tendon

Fig. 6 Zinc contents in the four regions of the peroneus longus tendon

Fig. 7 Iron contents in the four regions of the peroneus longus tendon

Figure
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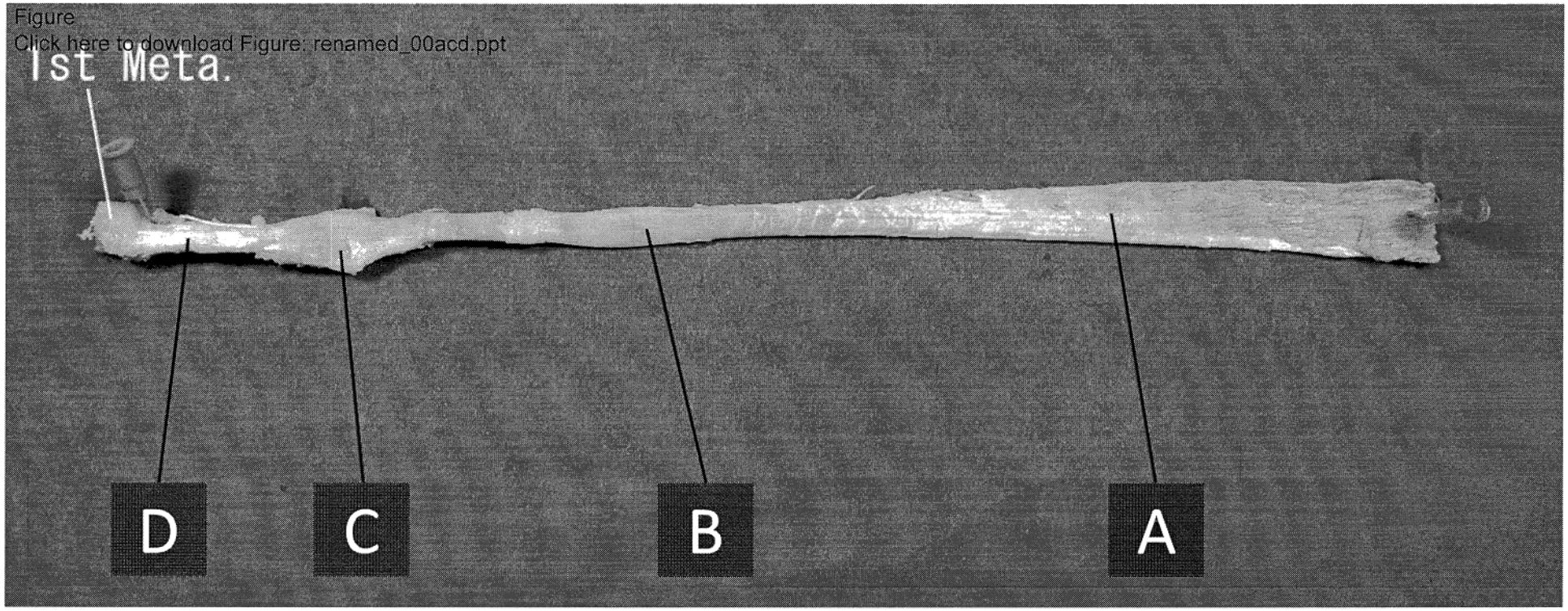

Fig. 1

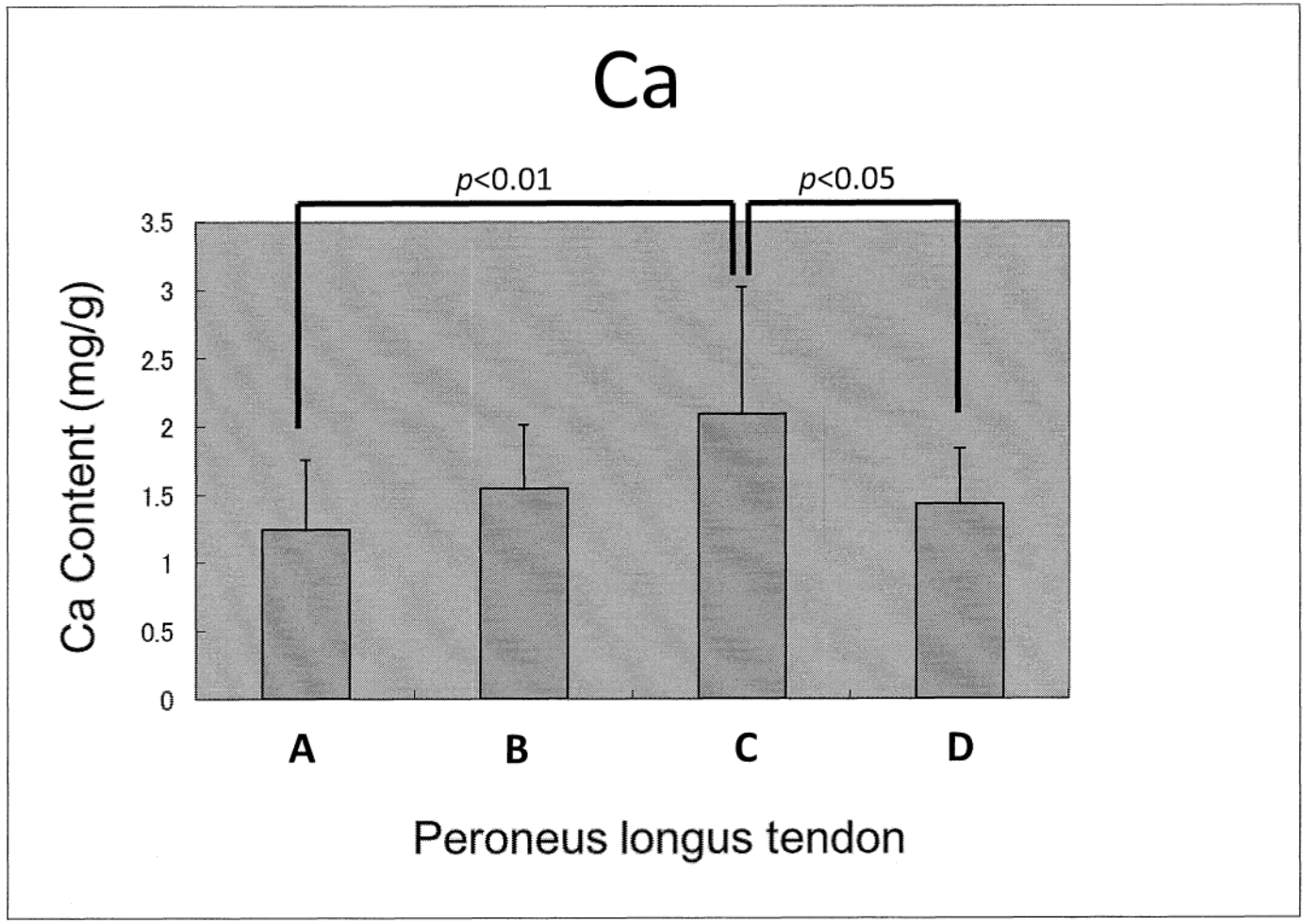
Ca
p<0.01
p<0.05
Ca Content (mg/g)
3.5
3
2.5
2
1.5
1
0.5
0
A
B
C
D
Peroneus longus tendon

Fig. 2

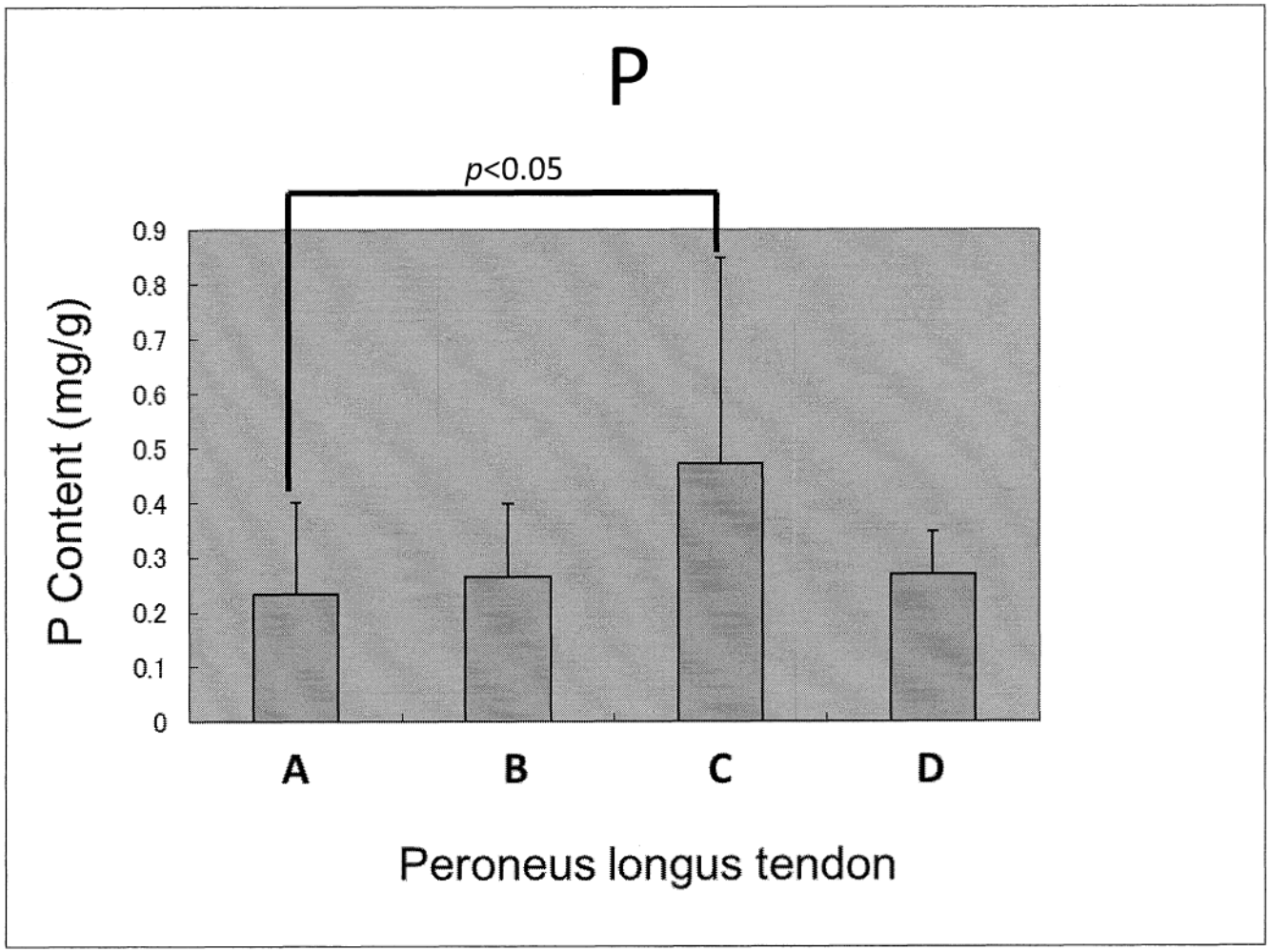
P
p<0.05
P Content (mg/g)
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
A
B
C
D
Peroneus longus tendon

Fig. 3

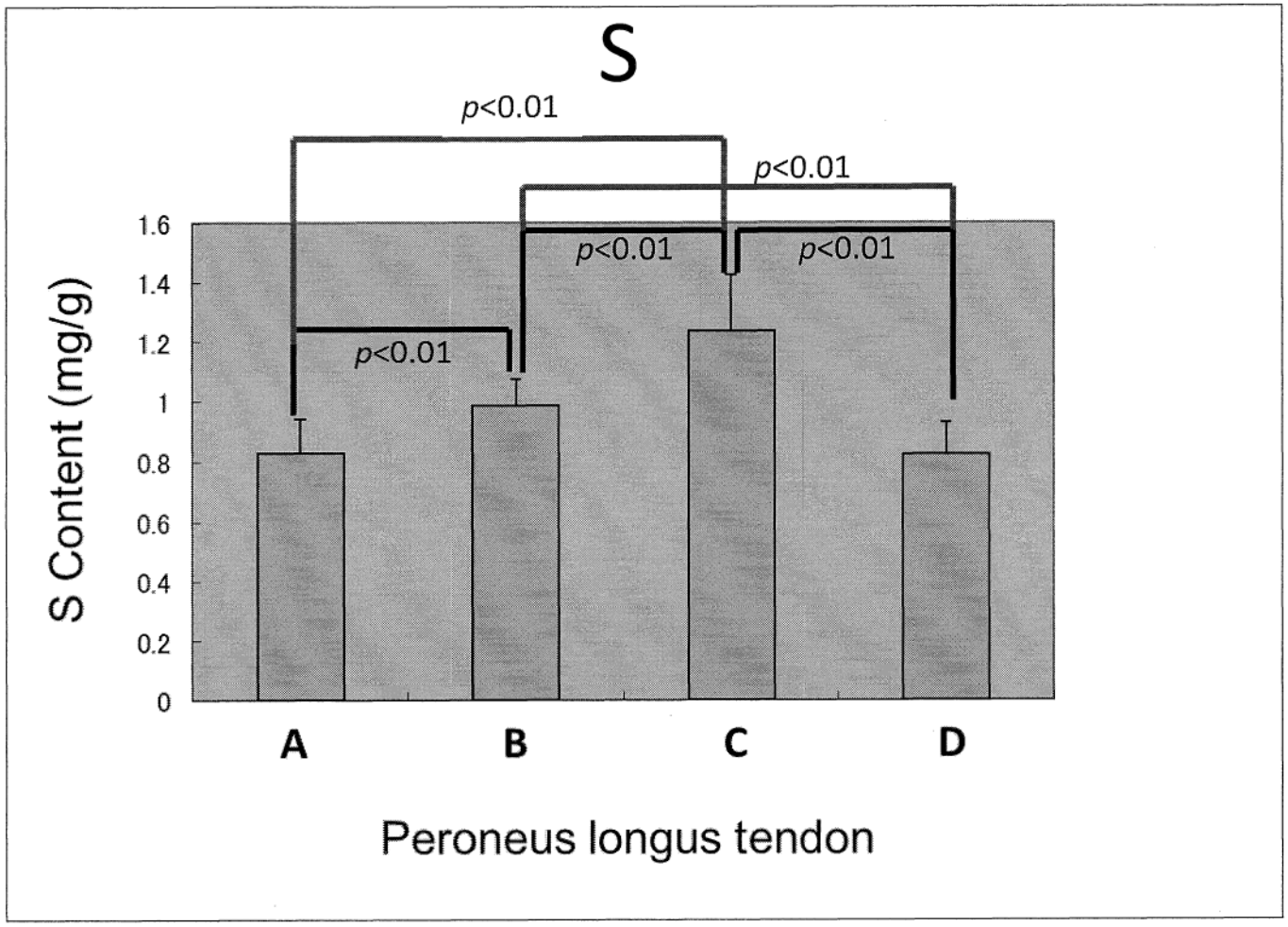
S
p<0.01
p<0.01
p<0.01
p<0.01
p<0.01
S Content (mg/g)
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
A
B
C
D
Peroneus longus tendon

Fig. 4

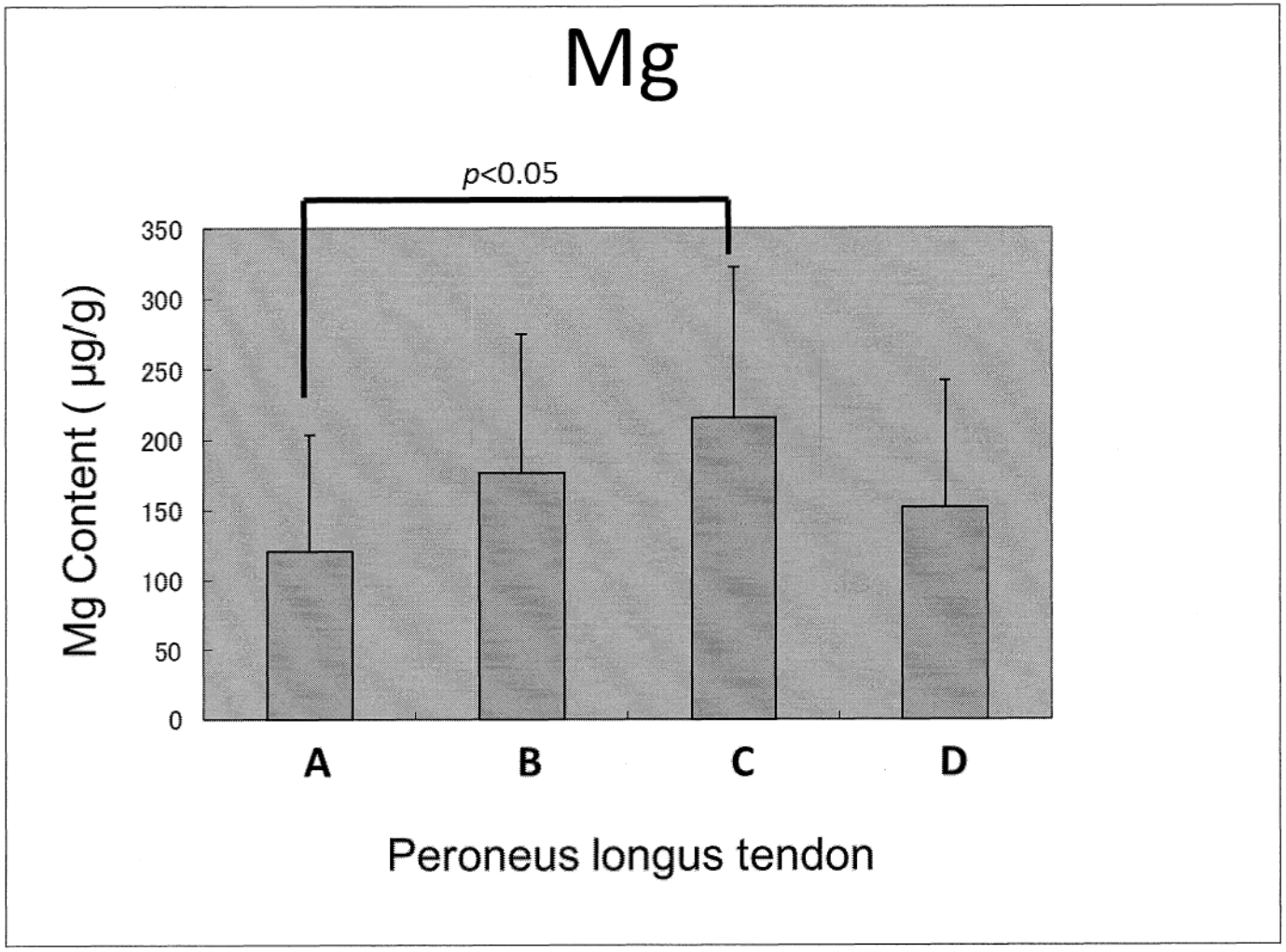
Mg
p<0.05
Mg Content (μg/g)
350
300
250
200
150
100
50
0
A
B
C
D
Peroneus longus tendon

Fig. 5

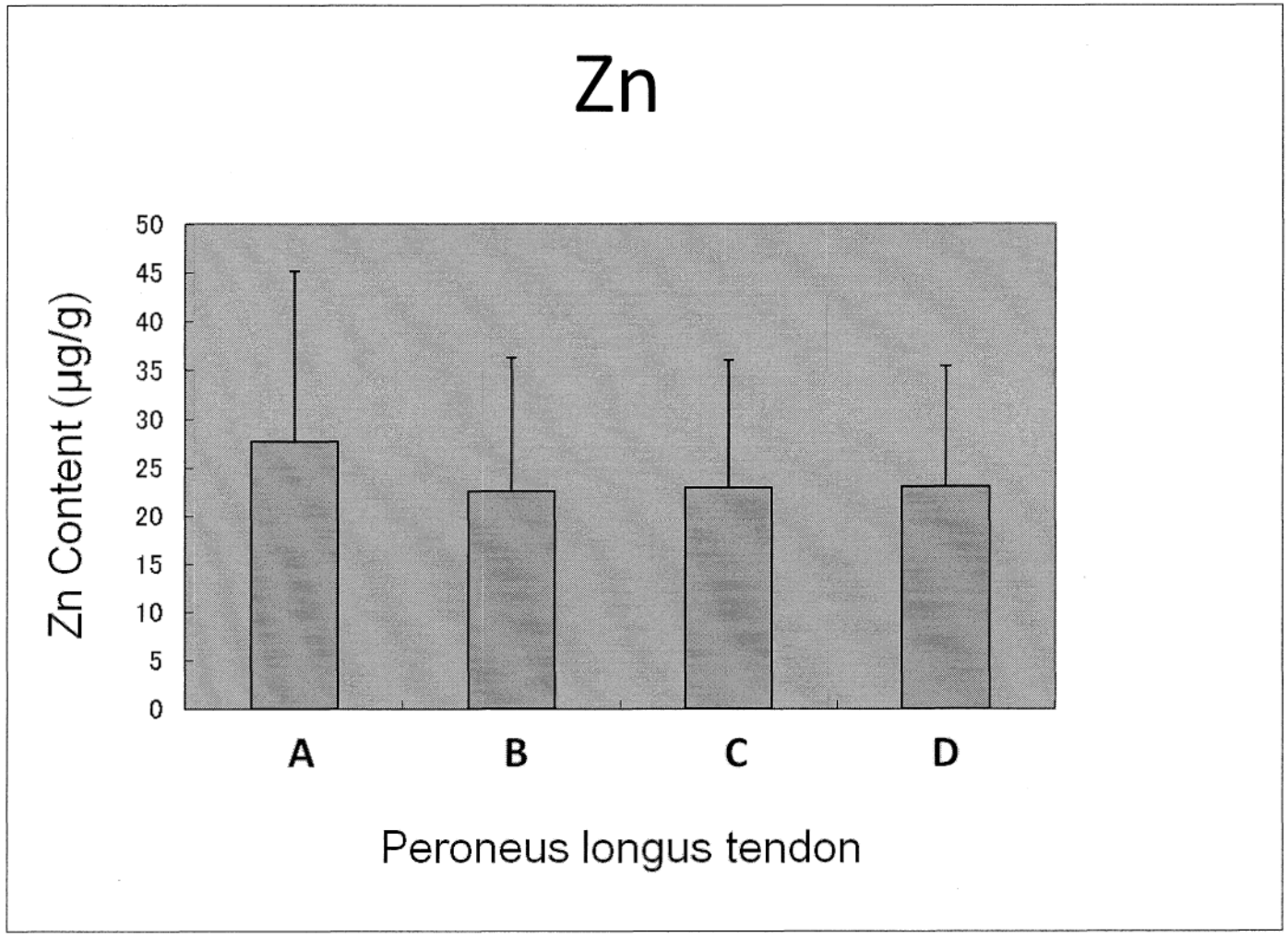
Zn
Zn Content (μg/g)
50
45
40
35
30
25
20
15
10
5
0
A
B
C
D
Peroneus longus tendon

Fig. 6

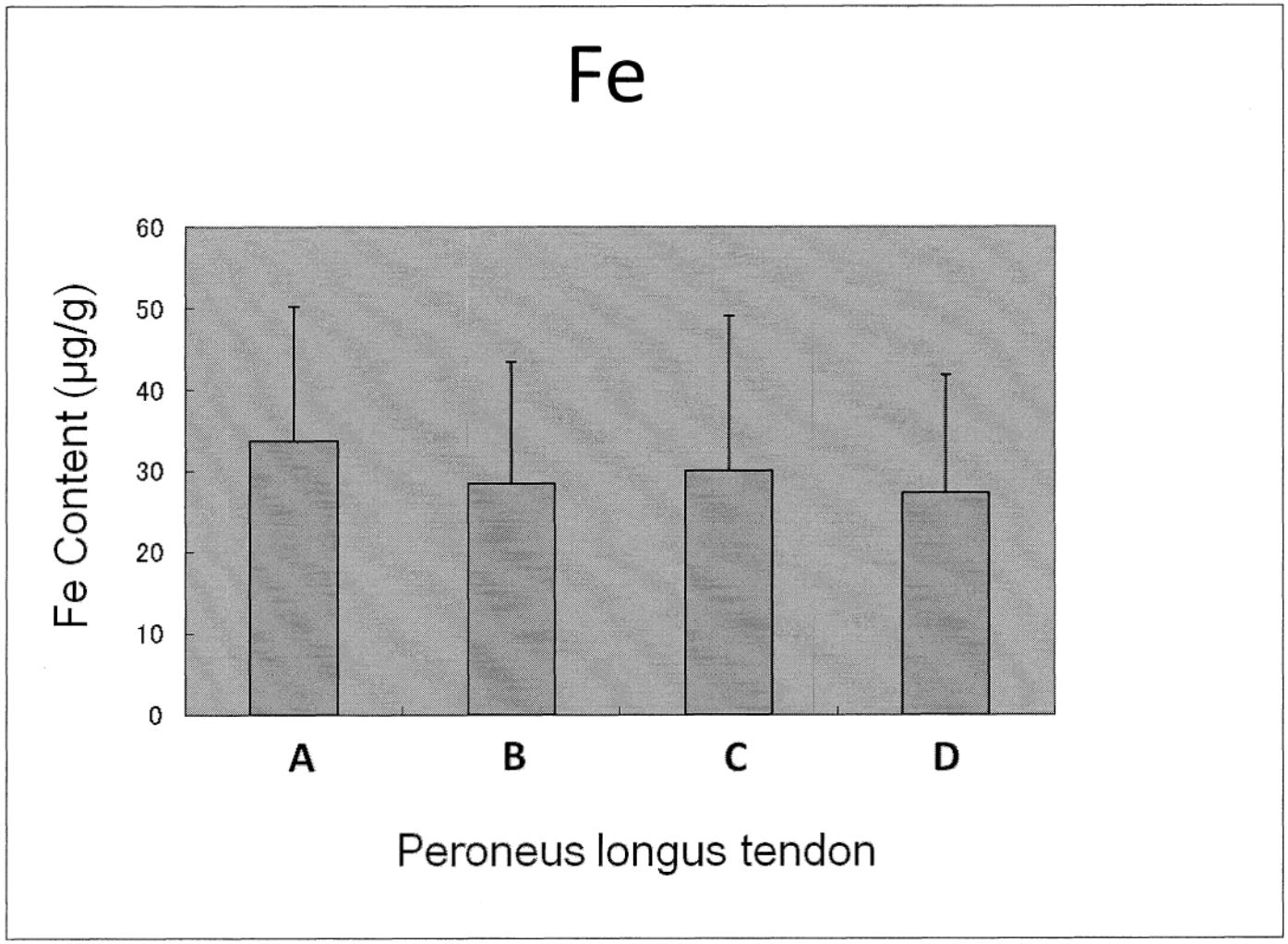
Fe
Fe Content (μg/g)
60
50
40
30
20
10
0
A
B
C
D
Peroneus longus tendon

Fig. 7

Table
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Table 1

	Ca (mg/g)	P (mg/g)	S (mg/g)	Mg (μg/g)	Zn (μg/g)	Fe (μg/g)
region A	1.25±0.51	0.23±0.17	0.83±0.11	121±83.3	27.7±17.4	33.7±16.5
region B	1.54±0.47	0.26±0.13	0.98±0.09	177±98.8	22.6±13.6	28.4±14.9
region C	2.10±0.93	0.47±0.38	1.24±0.19	216±107	22.9±13	30.1±19
region D	1.43±0.41	0.27±0.08	0.83±0.1	152±90.3	23.1±12.3	27.3±14.6