Author's response to reviews

Title: Contributions of biarticular myogenic components to the limitation of the range of motion after immobilization of the rat knee joint

Authors:

Momoko Nagai (nagai.momoko.36v@st.kyoto-u.ac.jp)
Tomoki Aoyama (blue@hs.med.kyoto-u.ac.jp)
Akira Ito (ito.akira.27n@st.kyoto-u.ac.jp)
Hirotaka Iijima (iijima.hirotaka.75s@st.kyoto-u.ac.jp)
Shoki Yamaguchi (yamaguchi.shouki.26n@st.kyoto-u.ac.jp)
Junichi Tajino (tajino.junichi.57z@st.kyoto-u.ac.jp)
Xiangkai Zhang (zhang.xiangkai.48v@st.kyoto-u.ac.jp)
Haruhiko Akiyama (hakiyama@kuhp.kyoto-u.ac.jp)
Hiroshi Kuroki (kuroki.hiroshi.6s@kyoto-u.ac.jp)

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Author's response to reviews: see over
11 April, 2014

Dr Shigeyuki Muraki and Ms Ma. Luz de Gusman

Editor

*BMC musculoskeletal Disorders*

Dear Editor,

Enclosed please find the revised manuscript “*Contributions of biarticular myogenic components to the limitation of the range of motion after immobilization of the rat knee joint*” by M. Nagai, T. Aoyama, A. Ito, H. Iijima, S. Yamaguchi, J. Tajino, X. Zhang, H. Akiyama, H. Kuroki

Your kind consideration of our paper for publication in *BMC musculoskeletal Disorders* would be greatly appreciated.

Sincerely yours,

Hiroshi Kuroki, PhD.
Department of Physical Therapy
School of Health Sciences
Faculty of Medicine, Kyoto University
53 Kawahara-Cho, Shogoin, Sakyoku, Kyoto 606-8507 Japan
E-mail: kuro@hs.med.kyoto-u.ac.jp
TEL&FAX +81-75-751-3963
Response for the first Referee

Reviewer: Guy trudel

Title: Contributions of biarticular myogenic components to the limitation of the range of motion after immobilization of the rat knee joint

We are grateful to Editor and Reviewers for the critical comments and useful suggestions that have helped us to improve our manuscript considerably. As indicated in the responses that follow, we have taken all these comments and suggestions into account in the revised version of our manuscript.

All of the change points were shown with the under line.

Major compulsory revisions

#1:
Interesting question and proper study design. How do the authors explain that 80% of the contracture is myogenic within the first 4 weeks yet muscle CSA was maintained. What is the relationship between myogenic contractures and muscle CSA? That is the second objective of the study and not completely addressed. Maybe just admit that the two appear unrelated.

Reply 1:
Authors appreciate and agree with the Reviewer’s suggestion. In the present study, we observed the CSAs at 1 week and 16 weeks post operatively. The CSA at 2, 4, and 8 weeks postoperatively were not observed. Therefore, we could not mention about the changes in the first 4 weeks. Eight-week-old rats with immature skeletons were treated in this study. We thought that the skeletal immaturity was one of the factors that led to changes in the muscle CSA. The myofiber CSA increased dramatically during the early growth of the animals (Reference no.14). Owing to aging, the compensatory hypertrophy of some fibers brought about by the normal increase in body weight led to increased load on the muscle (reference
We thought, therefore, the myofiber CSA increased in the experimental period because it coincided with the growth process. We have included these points in the discussion paragraph (Page 17, Line 5-13).

With regard to the relationship between myogenic contracture and muscle CSA, to the best of our knowledge, no study has looked into the relationship between ROM restrictions and changes in CSAs. Therefore, we were interested in this theme. Our research project confirmed the hypothesis that the changes in muscle CASs lead to limitations in the joint extensibility. We could not discuss the relationship between ROM restrictions and changes in CSAs because only morphological data and ROM data were presented in our manuscript.

# 2:

**Gastrocnemius does not “consist almost entirely of fast-twitch fibers.”**

**Reply 2:**

We checked the relevant references. This sentence was replaced with the following: “In the rat hind limb, the hamstrings and gastrocnemius muscles have similar fiber type: high proportion of Type II myosin heavy chain isoform [25].” (Page 6, Line 10-12). The type of fiber differs in humans and rats, so we mentioned about the type of fiber in rats only.

# 3:

**Tension on the strings does not provide torque; was direction of pull changed as ROM progressed?**

**Reply 3:**

Authors agree with the Reviewer’s comment. We measured the ROM for the knee that was extended passively for measurement (Page 9, line 8-10). To measure identical direction and tension (0.49 N), force gauge was used. The numerical data about the loss of angle and gradual increase in the arthrogenic components after 4, 8, and 16 weeks in our study were similar to those of previous studies (Reference no.11, and Lee S, et al., 2010). We consider that our method was reasonable and accurate. However, we are also aware that a better method could be used to provide torque. We rewrote “0.49Nm” to “0.49N” (Page 9, Line 12) and “ROM analysis” paragraph (Page 8, Line 15 - Page 10, Line 2). We have added a new “limitations”
paragraph (Page 19, Line 17, to Page 20, Line 2) to discuss this specific issue.

**# 4:**
Always detaching the UL muscles first introduces a systematic bias to be mentioned in the limitations. Randomly detaching LL and UL would be a better study design.

Reply 4:
Authors agree with your suggestion. This was added in the limitation paragraph (Page 20, Line 3-5).

**# 5:**
P11 Line 11. Why is there a myogenic restriction in sham-operated? This is inconsistent with the authors own definitions P8 lines 3-4.

Reply 5:
There are two differences between the previous study (Reference no.11) and this study. First, the fixator creation method was differed. In our method, the wires were screwed into the bones and inserted into the muscles, which enclosed the bone, so the skin and muscles were affected by the surgical invasion. We considered that adhesion of the soft tissues in the skin and muscles during the healing process after the surgical procedure caused the myogenic restriction. Second, the sham-operation was performed on the opposite knee of the immobilized knee, so it is possible that the changes in the load or walking style caused by the immobilization lead to the muscular changes in the opposed side.

**# 6:**
This claim in unfounded: “This means that the volumes and CSAs of the UL (including the hamstrings) were larger than those of the LL (including the gastrocnemius), suggesting that heavier muscles with larger myofiber CSAs are more susceptible to increases the arrangement of collagen fibers in their connective tissues.”

Reply 6:
We rewrote this part of the Discussion. Please see Page 17, Line 18, to Page 18, Line 10.
We have confirmed the weights of several samples. The timing and numbers of sampling were diverse in this experiment, so we could not show the numerical data pertaining to the muscle mass; the hamstrings were heavier than the gastrocnemius. We had mentioned about muscle mass based on the basis formula (Reference no.32). We considered that the muscle mass were calculated by using the CSA data which we gained and the muscle length data which obtained based on the cited reference (Reference no.25).

# 7:
This claim is speculative: “The different contributions of the UL and LL may also stem from their different lever arms, defined as the distance between the point at which a force is applied and the axis.”

Reply 7:
We agree with your suggestion. In this study, we did not assess the lever arm. However, some previous studies showed a relationship between the moment arm and muscle length (Page 18, Line 14-17). We added a reference to the relationship between the moment arm and sarcomere length (Reference no.6). Furthermore, stretch and isometric tension are suggested to stimulate protein synthesis and increase extensibility (Reference no. 9, 22, 34). Therefore, we considered that the muscle extensibility restriction caused by immobility lead to the muscle atrophy more on the PT than the PL.

# 8:
This claim is speculative: “This suggests that we must consider the influences of changes not only in the muscle fascicle, myofibril CSAs, and collagen fiber content but also in the muscle-tendon complex on joint extensibility after contracture.”

Reply 8:
We agree with your suggestion. The relevant text in the Conclusions and Discussion were deleted.

# 9:
The result in the abstract that: “The myofiber CSAs was significantly lower
16 weeks post-surgery than 1 week post-surgery only in the hamstrings.” Is incorrect. Table 3 shows the CSA to increase from 2645 to 1921.

Reply 9:
Your valuable suggestion was considered. Please see Page 4, Line 6-7. We rewrote “the myofiber CSAs” to “the ratio of the myofiber CSAs relative to those of the sham side.” We remade Figure 6, which showed the change in the CSAs for each group (control, sham side, immobilized side) and the ratio of the myofiber CSAs of the control and immobilized to those of sham side. The data of Table 3 were shown in the Figure 6 and Table 3 was deleted. Which was suggested by reviewer 2. We used the ratio of the myofiber CSAs of the immobilized knees to the sham side not control side. Because muscles changes could have been induced by the surgical invasion, e.g., damage or inflammation in the early phase after the surgery, we chose the sham for comparison with the subject to exclude the influence of surgical invasion.

Minor Essential Revisions

# 10:
UL and LL is confusing; clinically refers to upper limb and lower limb and UL is incorrect because leg is from knee to ankle. Suggests post thigh and post leg.

Reply 10:
We appreciate for the Reviewer’s suggestion. We rewrote “upper limb” and “lower limb” to “post thigh” and “post leg,” respectively.

# 11: Sample size calculation missing.

Reply 11:
We confirmed that the sample size of this study was small. The small sample size was mentioned in the limitation paragraph (Page 20, Lines 3-5).

# 12: K-wire and cerclage does not prevent motion in flexion.

Reply 12:
This method used was based on a previously reported method (N. Tatsuta, et al., 2008, Japanese). The resin we used underwent hardening and this did not allow the knee to flex or extend. We checked the restriction of the knee motion after the surgical procedure. The numerical data on the loss of angle and gradual increase in the arthrogenic contracture after 4, 8, and 16 weeks in our study were similar to those reported in previous studies (Reference no.11 and S. Lee, et al., 2010). We considered that our method using K-wire and resin is suitable and the obtained data are acceptable.

# 13: Details of ROM testing not available in this manuscript.

Reply13:
We rewrote the part on “ROM analysis.” Please see Page 8, Line 18, to page 10, Line 2.

“ROM analysis
At the end of the immobilization period, the animals were sacrificed under anesthesia with Nembutal and exsanguination. The wire and resin were removed from the joint and ROM analysis was performed. The macroscopic images were photographed with a digital camera (EX-V7, Casio, Japan) from the upper side. Thereafter the ROM was calculated using the Image J software package (National Institutes of Health, USA). To measure identical direction and tension, force gauges (DS2 series, Imada, Japan) was used, as previously described [27] with modifications. The ROM was defined as the angle (0° to 180°) between a straight line connecting the greater trochanter and the caput fibulae to a line connecting the caput fibulae and lateral malleolus with the hip joint at 90° of flexion. The maximum knee extension was defined as an extension of 180°. As the knee was extended passively for measurement, the trunk and pelvis were held manually to prevent the animal’s body from sliding forward. As previously described [28], the probe of the force gauges used to measure the ROM was fitted to the distal part of the ankle and then the knee joint was extended by pulling the strings with a tension of 0.49 N. The maximum knee extension was measured three times: (1) on the intact limb, (2) again after removal of the skin and PT from the hind limb, and (3) after removal of the PL from the lower leg. The muscles were removed beginning with their distal attachments. Distal incisions of PT that attached broadly to the front of the tibia were made from the tibia to their origins.
(proximal attachments) at the ischial tuberosity. Distal incisions of the PL were made from the distal Achilles tendon, which had adhered to the calcaneus, to their origins (proximal attachments) on the femur. The incisions were made with caution to avoid damage to any additional muscles.”

**# 14: Multiple typos p11 line 2: title**
Reply14: We rewrote word “PL” to “PT” and “LL” to “PL” (Page 13, Line 8).

**# 15: Table 1: Arthrogenic**
Reply15: We rewrote “arthrogenic” to “arthrogenic” (Page 31, Line 1).

**# 16: Table 3: date**
Reply16: We deleted Table 3 and revised Figure 6 using the same data (Figure 6).

**# 17: Widespread grammatical and syntactic errors.**
Reply17:
The revised manuscript has been checked by native English of Editage Co., Ltd.
The proofread copy is attached.

**# 18:**
The meaning of some sentences is unclear: “Especially the hamstrings and gastrocnemius, which are often used as an object of approach to knee joint contracture [2,22-24].
Reply18:
The following explanatory sentence was added: “In the clinical situation, the hamstrings and gastrocnemius are often manipulated to prevent the progressive contracture or muscle atrophy when joint ROM is restricted [2,22-24].” (Page 6, Lines 8–10).

**Revision 19:** Others are right out incorrect: “They consist almost entirely of fast-twitch fibers [25,26].
Reply19: Please check “Major revision 2” reply.

**# 20:**
Figure 6 is incomprehensible: error bars missing, twice % sign in Y1 axis, knee CSA does not exist, only CSA of muscles exist.

Reply20:
Figure 6 shows the ratio of the myofiber CSAs of the relative to the sham. According to the deleted Table 3 data, the ratio of the gastrocnemius CSAs at 16 weeks was $69.80 \pm 11.36$. The ratio of the hamstring CSAs in 16 weeks was $58.67 \pm 6.91$. So the lower error bar of gastrocnemius in 16 weeks (=58.44) and the average data of hamstrings in 16 weeks (=58.67) is overlapping. We deleted the minus error bars in Figure 6-d.
Response for the second Referee

Reviewer: Hiromu Ito

Title: Contributions of biarticular myogenic components to the limitation of the range of motion after immobilization of the rat knee joint

We are grateful to Editor and Reviewers for the critical comments and useful suggestions that have helped us to improve our manuscript considerably. As indicated in the responses that follow, we have taken all these comments and suggestions into account in the revised version of our manuscript.

All of the change points were shown with the under line.

Major compulsory revisions

# 1
As the reviewer pointed out above, the overall construction of the manuscript should be reconsidered. Other researchers would have very hard time to understand the results and discussions of the present manuscript. In addition, the manuscript must be checked by an experienced native English editor.

Reply1
The revised manuscript has been checked by native English of Editage Co., Ltd. The proofread copy is attached.

# 2
Table 3 shows interesting data. However, this table can be confusing because actual CSA is INCREASED despite of immobilization. To make this table straightforward, the reviewer would advise that this table be shown by the ratio relative to the control. Or the authors can show the change of each group in Fig. 6. In addition, the authors should be cautious in describing the results of CSA. For example, the authors described ‘the myofiber CSAs was
significantly lower 16 weeks,’ in the results section of the abstract. This
description is not true. This should be ‘the ratio of the myofiber CSAs relative
to the control was significantly lower.’

Reply2

We appreciate with reviewer’s suggestion. Please see Figure 6, which shows the
changes in the CSAs for each group (control, sham side, immobilized side) and in
the ratio of the myofiber CSAs of the immobilized side to those of the sham side.
We used the CSAs of the sham side not control side. Because muscle changes
could have been caused by the surgical invasion, e.g., damage or inflammation in
the early phase after surgery, we chose the sham for comparison with the subject
to exclude the influence of surgical procedures. In the Abstract, we revised “The
myofiber CSAs” to “The ratio of the myofiber CSAs to the sham side”, per the
reviewer’s suggestions (Page 4, Lines 6-7).

#3

In methods (p8, lines 3 and 5), the authors used ‘component’. Readers may
not understand well this term because ‘contracture’ was used in the very
similar meaning in other parts in the very same paragraph. Also the authors
used ‘limitation’ in Table 1 but used ‘component’ in results section (p10, lines
14, 15 and 16) in the very similar or the same meaning. The same is true in
Table 2, Fig.2 I, and results (p11, lines 2, 5, 8, 13). The term ‘restriction’ is also
used in the results (p11, lines 10 and 13) and Fig. 3. Is there any difference or
meaning of using different terms?

Reply3

Previous study reported that the components of joint contracture after
immobilization are classified into arthrogenic and myogenic components
(Reference no.11). We termed the contracture caused by “arthrogenic” and
“myogenic” components as “arthrogenic contracture” and “myogenic
contracture,” respectively. We rewrote “restriction” or “components” to
“contracture” (Page 10, Lines 6–10; Page 13, Line 2, 3, 15 and 18; Page 15, Line
16 to Page 16, Line 5; Page 17, Line 1; Figure.2; Figure.3; Table 1).
The “PT or PL limitation” indicates that “the PT or PL components contribute to
the extension limitation of the knee ROM”. We defined these terms (Page 10,
Lines 12-14), so we rewrote the word “limitations” to “components” (Page 13, Lines 10, 13 and 17; Page 16 Lines 6, 7, and 9; Table 2; Figure 2).

# 4

Why did the authors need to discuss the difference between internal and external fixation? (p13, lines 15 -18). Please make this clear.

Reply4

The methods about the kind of fixation not related to the main theme were deleted.

Many studies on contracture involved animal models where joints were immobilized by internal fixation (with plate or sprint), external fixation (with cast, bandage), pinning etc. Many of previous studies used internal fixators. The results of these animal studies have indicated that joint alterations differ especially in terms of the mechanical forces on the cartilage (H. Moriyama, et al., 2008) and surgical invasion. In our study, we used an external fixator constructed from wire and resin. In this sentence, we discussed to indicate that the validity of our method, as it has been found that external fixation can produce similar restriction of knee extension and knee contracture in rats as internal fixation.

# 5.

The authors discussed the weight and volume of muscles in the discussion (p14, lines 14 to 18) with no data. The authors should not discuss anything without showing their data, or the authors need to include relevant references.

Reply5

We rewrote this part of the Discussion (Page 17, Line 18 to Page 18, Line 10). We confirmed the weight of several samples. The timing and number of sampling were diverse in this experiment. Therefore, we could not present the numerical data about muscle mass that the hamstrings were heavier than that of gastrocnemius. We had mentioned about muscle mass based on the basis formula (Reference no.32). We considered that the muscle mass were calculated by using the CSA data which we gained and the muscle length data which obtained based on the cited reference (Reference no.25).
# 6.
The authors discussed lever arms of UL and LL muscles (p15, lines 5 to 12). The only reference is 33, but this is just for medial gastrocnemius muscle. Please add one or two references for hamstrings or on comparison of the two muscles.

Reply6:
We rewrote the Discussion. We added a reference (no.6) to the relationship between the moment arm and sarcomere length with respect to each proximal (hip) and distal (knee) joint in the Frogs’ post thigh region (Page 18, Lines 16-17).

# 7.
The limitations of the study should be summarized in one paragraph.

Reply7:
We added a limitations paragraph (Page 19, Line 12 to Page 20, Line 5).

# 8.
The conclusion should not contain many suggestion or discussion as described (p18, lines 1 to 5).

Reply8:
The text was deleted in the Conclusions, and some part of it was moved to the Discussion (Page 20, Lines 4-5).

Minor essential revisions

# 1.
The authors wrote ‘date’ in Tables 1 and 3. Are these ‘data’? Also, ‘time interval’ should be ‘time point’ under the reviewer’s understanding.

Reply1:
We rewrote the word “date” to “data” (Table 1). Table 3 was deleted.
We rewrote “time interval” to “time point” (Page 13, Lines 1 and 5; Table 1)
The authors used ‘upper leg’ for upper biarticular muscle and ‘lower leg’ for lower biarticular muscle. Is this term common or established? These terminology may be confusing.

Reply2:
Thank you for your suggestion. We rewrote the words “upper limb (UL)” and “lower limb (LL)” to “post thigh (PT)” and “post leg (PL)”, as suggested by Reviewer 1. We confirmed that the words “thigh” and “leg” were defined on MeSH.

In methods (p6, line 1 to 2), the authors described ‘a high-resolution micro-CT scanner was used to confirm knee immobilization. Is that so? Wasn’t CT scan used for measuring the ROM in immobilization?

Reply3:
We agree with your suggestion. We rewrote this sentence as follows: "A high-resolution micro-CT scanner was used to check the insertion site of the wires and view the immobilized leg.” (Page 8, Lines 6-8)

‘U’ is missing at the beginning of the short title in line 2 of page 11.

Reply4: We rewrote “L” to “PT” and “LL” to “PL,” as suggested by Reviewer 1 (Page 13, Line 8).

The authors described ‘among all samples’ in results (p12, lines 6 and 13). However, the authors used only two samples in each group for morphological analysis (p5, lines 17 to 18). The word ‘all’ is seemingly an exaggeration.

Reply5: We rewrote “all” to “both” (Page 14, Line 12; Page 15, Line 1).