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

Summary:
The authors studied the relationship between muscle atrophy and elasticity. Atrophy is measured by decreased myofiber CSA and length and elasticity is measured by knee ROM.

They report more atrophy in post thigh c/w contralateral sham-operated.

They found more restriction of post thigh muscles c/w post leg muscles 4:1.

Opinion
This is a revised manuscript. The authors have gone over the manuscript and responded to comments from 2 reviewers. They have added sections to their manuscript, and graphs are improved. This is a worthy research effort apparently using good methods for measuring ROM and myofiber CSA. Unfortunately, after a second submission, there remain a large number of issues that would prevent recommending this report as a solid and polished contribution; mainly in the areas of hypothesis-driven research, data interpretation and discussion of the importance and relevance of the results. Despite a translation company reviewing the manuscript, many sentences remain scientifically and/or grammatically difficult to interpret.

The rationale and relevance of studying the relationship between atrophy and elasticity is underdeveloped. Then Objective 1 departs from the relationship the investigators want to study. Finally, the conclusion of the study in the abstract makes no mention of the relationship between atrophy and elasticity. This leaves this reviewer wondering how much hypothesis-driven was this investigation.

General comments:
The measure of elasticity using knee ROM has a number of drawbacks.

Results with n=2 are interpreted as preliminary.

Table 1 Why does arthrogenic + myogenic not add up to total contracture?

How do the investigators explain the negative myogenic contractures figures at 8 and 16 weeks?
Table 2: How do the investigators explain that the PT component of the myogenic contracture is always larger than the total myogenic contracture in Table 1?

Still very difficult to read e.g.; “Many studies have examined muscle changes after immobilization and compared muscles adhere to the same single segment [12,15,27].” Sometimes, train of thought is hard to follow e.g.; in the intro the link between fiber type change and restriction in ROM used to justify their study is surprising, and more so since they did not study fiber type. Or incomplete e.g.; “To measure identical direction and tension, force gauge (DS2 series, Imada, Japan) was used, as previously described [27] with modifications.” Which modifications?

P15 the smaller CSA of medial gastroc sham surgery cast doubt that this measure is important for myogenic component of joint contracture, maybe only related to the surgery or limited by the power of the study (n=2).

Discussion P16 l16-18 “Atrophy was accompanied by decreased muscle elasticity [5], which may have been caused by an increase in the amount of intramuscular connective tissue [14,15] and collagen synthesis [9,13,29].” The authors measured none of elasticity, connective tissue or collagen synthesis. Discussion is overall again difficult to read and unfocused, authors should reiterate why this is important and applications from their results. See specific comments.

Specific comments:
Abstract: conclusion does not mention atrophy
Intro p6 line 8 change leg for lower limb
Objective 1 is poorly worded
Methods: The direction of pull is not specified.
The sequence of ROM testing may have damaged the PL muscles.
P10 line 15 and others replace the word avulsion
P10 line 15 relative to the GAIN in ROM
P11 the calculation for arthrogenic contracture is not provided
P11 lines 3-6 upper what? Lower what?
P11 line 17 how were they calculated? Need detailed methods.
P13 lines 8-10: what does this sentence mean? Differed from what?
P13 Results lines 1-3: the 2 sentences appear to contradict each other.
PP14-15 CSA is not of muscles but of mean fiber size.
Discussion P16 l1-16 these repeat the result section.
P16 l4-5 Can you clarify the meaning of this sentence
P17 l13-15 Unreferenced and speculative statement
P18 l10-14 Would be helpful if you provided length of your specimen
P19 l2-3 This sentence lacks a verb, its meaning is unknown
P20 lines 12-14 this sentence is vague and of uncertain meaning
P21 l1 spell out post

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Not suitable for publication unless extensively edited

**Statistical review:** Yes, and I have assessed the statistics in my report.

**Declaration of competing interests:**
'I declare that I have no competing interests'