Ms. Ref. No.: AANAT1671
Title: Relative Distribution of Quadriceps Head Anatomical Cross-Sectional
Areas and Volumes -Sensitivity to Pain and to Training Intervention
Annals of Anatomy

Decision letter

Dear Dr. Dannhauer,

The reviewers have commented on your above paper. They indicated that it is not
acceptable for publication in its present form.

However, if you feel that you can suitably address the reviewers' comments (included
below), I invite you to revise and resubmit your manuscript.

The comments provided by the reviewers very often considerably help to improve and
strengthen a paper. In addition, the authors' response letter accompanying a revised
version often contains important information which may be regarded as an added
value to the final version of the manuscript. Therefore, Editors decided that the non-
confidential comments of the reviewers and the non-confidential authors' responses
will be published as online supplementary material together with the final version of
an eventually accepted article.

Please carefully address the issues raised in the comments.

If you are submitting a revised manuscript, please also:

a) outline each change made (point by point) as raised in the reviewer comments

AND/OR

b) provide a suitable rebuttal to each reviewer comment not addressed

I look forward to receiving your revised manuscript.

Yours sincerely,

Friedrich Paulsen
Editor-in-Chief
Annals of Anatomy

Reviewers' comments:
Reviewer #2: Overall the study demonstrates interesting results regarding the
anatomical proportions in specific patient groups. However, some concerns exist.

Material and Methods
Did you match the patients? Flowchart?
What do you mean by pain intensity of the painless contra-lateral knee?
What was the range of motion before and after? Free extension?

The data for the study was obtained from your in earlier studies (Sattler 2012 and
Ring-Dimitriou 2009) and now you analysed the ACSAs?

You mentioned that the slices differ between the studies. Please explain more detailed
how you minimized this problem.

The figures should contain the data of all groups. Fig. 1,2 and 3 show one sample
without specific patient details and references.

Figure 4: How can I see the data of the Quadriceps before and after? I guess you mean
the figure displays the difference (before and after).

Please point our the new aspects of this study more detailed and intensive.

Thank you

Authors response letter

Revision

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and Volumes -Sensitivity to Pain and to Training Intervention
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Dear Dr. Dannhauer,

The reviewers have commented on your above paper. They indicated that it is not
acceptable for publication in its present form.

However, if you feel that you can suitably address the reviewers' comments (included
below), I invite you to revise and resubmit your manuscript.

Dear Professor Paulsen,
We would like to thank you very much for the opportunity to revise the above paper.
We outline below how we have addressed each of the comments in itemized form and
hope that these satisfactorily address the reviewer's concerns.
Sincerely
Martina Sattler, Torben Dannhauer and Felix Eckstein

Reviewers' comments:

Reviewer #1:
Author comment: No comments received

Reviewer #2: Overall the study demonstrates interesting results regarding the anatomical proportions in specific patient groups. However, some concerns exist.
Author comment: Thank you for favorably commenting on the interest of the study for publication in Ann Anat.

Material and Methods
Did you match the patients? Flowchart?
Author comment: We are not exactly sure about the question, because the study design did not involve comparison between different groups and patients that needed to be matched.
In the unilateral pain cohort (study cohort #1), we compared the painful with the painless knee in the same person, hence no matching was required.
In the training intervention cohort (study cohort #2), we compared baseline with follow-up values, again in the same subjects.
Hence, in each of both studies (#1 and #2), each participant served as her/his own control and was not compared with another sample or person, so that no matching was needed
We are unsure whether the impression has arisen that we compared the OAI unilateral pain cohort with the training cohort, but this was not the case for any of the analyses. To avoid that possible impression, we have divided the sentence in the method section of the abstract, describing both cohorts, in two:
Author action: The sentence in the abstract: *The relative proportion of quadriceps heads on the total muscle was determined in 48 Osteoarthritis Initiative participants with unilateral pain (65% women; age 45-78y), and that of their volumes in 35 untrained women (45-55y) during 12 week training intervention.*
has been changed to state:
The proportion of quadriceps heads on the total muscle was determined in 48 Osteoarthritis Initiative participants with unilateral pain (65% women; age 45-78y). Quadriceps head volumes were also measured in 35 untrained women (45-55y) before and after 12 week training intervention.
Further, in the material and method section, we now state:
We examined data from two samples cohorts that were previously described in detail, one from the US-based Osteoarthritis Initiative (Eckstein et al., 2014, 2012) that suffered from unilateral frequent knee pain (Sattler et al., 2012), and the other from a 12 week training intervention study performed in Salzburg, Austria (Hudelmaier et al., 2010; Ring-Dimitriou et al., 2009).

What do you mean by pain intensity of the painless contra-lateral knee?
Author comment: There are several classification systems of knee pain that are being used in context of knee osteoarthritis. The most frequently applied is that of pain frequency in the past year. This classification system relies on the answers of the participants with regard to the 3 below questions:
- Frequent knee pain = pain, aching or stiffness no most days of at least one month in the past 12 months
- Infrequent knee pain = pain, aching or stiffness in the past 12 months, but not on most days of a month
- No pain = no pain aching or stiffness in the past 12 months

The patients studied here, all answered to have had frequent pain in one knee, and no pain in the other (contra-lateral) knee over the past year, and hence they fulfilled our preset inclusion criterion.

Other pain subscales in OA are more related to pain intensity (NRS) or to pain during function (WORMS), usually during the past 7 days. In the description of the sample, we had reported the answers given by the above participants to these alternative grading system, which may not be 100% consistent with the answers given to the pain frequency questions given above.

We understand, however, that this information may be slightly confusing, and in fact these data have been previously reported in the paper published by Sattler et al. in 2012. We refer to this paper, and we have now eliminated these additional information on pain intensity and function pain from the text to avoid misunderstanding

Author action: The following statements in the method section have been deleted:
The painful knees also displayed greater pain intensity (numerical rating scale = 3.7±2.6) than the contra-lateral painless knees (0.8±2.3), with 10 corresponding to the worst pain the participant could imagine. The pain subscale WOMAC score (Western Ontario and McMaster Universities, range 0-20, with 20 being the worst) was also greater in the frequently painful (4.0±3.5) than in the painless knees (1.1±2.4) (Sattler et al., 2012).

What was the range of motion before and after? Free extension?
Author comment: We assume that the reviewer asks whether range of motion = ROM (in the knee) was measured before and after training intervention (2nd cohort with training intervention). ROM measurements were not part of the exams conducted during the study and we therefore are unfortunately unable to provide these.
We are not real clear about the question pertaining to free extension?

The data for the study was obtained from your in earlier studies (Sattler 2012 and Ring-Dimitriou 2009) and now you analysed the ACSAs?
Author comment: We did analyze the ACSAs of the total quadriceps muscle in the two previous studies. The purpose of the current study was to analyze, in detail, the relative proportions of the quadriceps heads (i.e. the vastus medialis, intermedius, lateralis, and rectus femoris), and their relative proportion under various conditions (pain, training intervention) has not been previously studied. This is explained in the objective statement at the end of the introduction:
We previously reported that, in patients with the same grade of bilateral radiographic knee osteoarthritis, quadriceps ACSAs and isometric strength were significantly smaller in limbs with frequent knee pain, relative to a contralateral reference knee without pain (Sattler et al., 2012). We further reported that a supervised 12 week training intervention in untrained perimenopausal women (Ring-Dimitriou et al., 2009) involved a statistically significant increase in quadriceps ACSAs and volume (Hudelmaier et al., 2010). However, it is currently unknown whether, and if yes to what extent, there is a differential response of the quadriceps heads. In the current study, we therefore aimed to explore the relative distribution of the heads (i.e. the VL, VIM, VM, RF) to total quadriceps anatomical cross-sectional area (ACSA) and volume, and their individual response to pain and to training intervention.

Author action: To clarify potential ambiguity, we now start the subject description with the following sentence: *We examined data from two cohorts that were previously*

described in detail, one from the US-based Osteoarthritis Initiative (Eckstein et al., 2014, 2012) that suffered from unilateral frequent knee pain (Sattler et al., 2012), and the other from a 12 week training intervention study performed in Salzburg, Austria (Hudelmaier et al., 2010; Ring-Dimitriou et al., 2009).

You mentioned that the slices differ between the studies. Please explain more detailed how you minimized this problem.

Author comment: The slices do not really differ between the studies:

In the first study (unilateral pain), we selected one slice for analysis that was located at 31% of the femoral length, since the acquisition did not cover the entire thigh. Please note that the comparison was made between both limbs so that a slight variation of the slice position, had it occurred, would not have an effect on the results, as the slice covered both thighs at the same time, and the focus was on comparing both sides with each other, not one participant with the other.

In the second study (training intervention), the entire thigh was imaged, and we examined quadriceps head volumes in the entire distal third of the muscle, where they were straight forward to separate. Hence, no particular slice was selected.

The figures should contain the data of all groups. Fig. 1,2 and 3 show one sample without specific patient details and references.

Author comment:

Figure 1 shows the image acquisition protocol used by the Osteoarthritis Initiative and how we have segmented the quadriceps heads. This is a figure explaining the method and does not contain any results; hence we can only show and example. Based on the above request, however, we have added demographic information for the participant shown.

Author action: We have now added to the figure legend the following information:

The images are from a 57 year old women, with a body height of 167.5 cm and a body weight of 49.3 kg (BMI 17.6).

Figure 2 showed a reconstruction of the muscle volumes as measures in the 2nd cohort (the training study), but this figure is not absolutely needed, and we have hence eliminated it.

Figure 3 shows the results on the proportion of the quadriceps heads from proximal to distal and is only meant to give a visual impression of the relative volume taken by each of them. The quantitative data, with mean values and standard deviations, are presented in the text:

The total volume of the quadriceps in the region of interest (distal third of the thigh between the femoral neck and the quadriceps tendon) was $354\pm50cm^3$; the proportion taken by the VM was 36±3.1%, that by the VL 29±3.5%, that by the VIM 30±2.3%, and that by the RF 4.8±1.2%. In the most proximal slice of the region of interest, the relative proportions of ACSAs were 25±3.0% for the VM, 36±3.2% for the VL, 31±3.0% for the VIM, and 8.1±1.7% for the RF. This distribution was different at the most distal slice in the region of interest, where it amounted to 49±5.6% for the VM, 19±4.9% for the VL, 30±4.0% for the VIM, and 1.8±0.5% for the RF. A representative example of the change in the relative contribution of the quadriceps heads to the total quadriceps ACSA from proximal to distal is shown in Figure 3.

Author action: We have added to the figure legend the following information:

The results are from a 50 year old women, with a body height of 170.0 cm and a body weight of 57.0 kg (BMI 19.7).

Figure 4: How can I see the data of the Quadriceps before and after? I guess you mean the figure displays the difference (before and after).

Author comment: Yes, correct, the graph shows the difference between baseline and follow-up (i.e. before and after training intervention). The graph hence corresponds with the statistical approach, by which values were compared within each participant (before/after) across the 35 subjects (paired t-test), and not by comparing mean values across the group before and after the intervention (non-paired test)

Author action: To avoid potential confusion, we have clarified this approach in the figure legend (now Figure 3):

Bar graphs showing the mean difference in muscle volume between baseline and follow-up (i.e. before and after 12 week training intervention) in percent (%) (i.e. the within-person changes) across the 35 participants: Quad. = quadriceps femoris in the distal third of the region of interest, where the quadriceps heads could be well differentiated; VM = vastus medialis; VL = vastus lateralis; VIm = vastus intermedius; RF = rectus femoris. The error bars show the standard deviation.

Please point our the new aspects of this study more detailed and intensive.

Author comment and action:

We now state in the 2nd sentence of the abstract: *This is the first study to explore the relative distribution of quadriceps heads anatomical cross-sectional areas (ACSA) and volumes, and their response to pain and to training intervention.*

In the introduction, we have stated: *However, it is currently unknown whether, and if yes to what extent, there is a differential response of the quadriceps heads.*

The background again which this question may be important, also has been highlighted throughout the introduction.

At the beginning of the discussion, we clearly state: *To our knowledge, this is the first study to explore the relative distribution of the heads to total quadriceps anatomical cross-sectional area (ACSA) and volume, and their response to pain and to training intervention.*

Thank you

We would like to thank the reviewer for the comments, and we hope we have addressed them satisfactorily.