Author's response to reviews

Title: A role for subchondral bone changes in the process of osteoarthritis; a micro-CT study of two canine models.

Authors:

Yvonne H Sniekers (y.sniekers@erasmusmc.nl)
Femke Intema (f.intema@umcutrecht.nl)
Floris PJG Lafeber (f.lafeber@umcutrecht.nl)
Gerjo JVM van Osch (g.vanosch@erasmusmc.nl)
Johannes PTM van Leeuwen (j.vanleeuwen@erasmusmc.nl)
Harrie Weinans (h.weinans@erasmusmc.nl)
Simon C Mastbergen (s.mastbergen@umcutrecht.nl)

Version: 2 Date: 27 November 2007

Author's response to reviews: see over
Dear editor,

Thank you for sending us the reviewer’s comments and giving us the opportunity to resubmit a revised version of our manuscript (1491595161558379) by Sniekers et al. to BMC Musculoskeletal Disorders. The comments of the reviewers were very much appreciated to improve our manuscript and present our message in a sound way.

Please find below a detailed listing of our answers (in bold printing) to the reviewer’s comments. Changes made in the revised manuscript are underlined.

We hope that we have adequately addressed all the topics raised by both reviewers and that our revised manuscript is now suitable for publication in. BMC Musculoskeletal Disorders

Yours sincerely,

Yvonne Sniekers
Reviewer's report: Referee 1
Title: A role for subchondral bone changes in the process of osteoarthritis; a micro-CT study of two canine models.
Version: 1 Date: 7 October 2007
Reviewer: Daniel Henri Manicourt
Reviewer's report:
General

Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

Please replace mean by median as you have used non parametric tests

Check that your references fit your statements

By using micro-CT, the Authors have detected a decrease in the thickness and an increase in the porosity of the subchondral bone plate from the operated OA knees in two models of canine experimental osteoarthritis (OA). However, at both 10-week and 20-week post-surgery, the changes in subchondral bone plate reached statistical significance in the model of anterior cruciate ligament transection (ACLT), but not in the groove model. Likewise, operated knees exhibited changes in their trabecular subchondral bone, but these changes were statistically significant in the ACLT model, and not in the groove model.

In general, the paper could be written better. Sometimes, this version is rather difficult to read for someone who is not involved in the field.

We adapted various parts of the manuscript in order to make it more clear.

I have several other comments.
Point 1. Since the Authors have used either 4 or 5 dogs for each experimental condition, the differences between operated and non-operated knees within each experimental group as well as the differences between operated knees of the two experimental groups, if any, have to be “big” to be stated as statistically significant by using non-parametric tests. This point should be more clearly stated at the beginning of the discussion. In the abstract and in the manuscript, the Authors should state that bone changes were (not only) “small” (as they state) but also non-statistically significant since changes can be small and statistically significant.

We are aware that the sample size is small in the groove model, and this leads to non-significant changes even when the effect is big and the direction of the effect is the same (all animals show an increase or decrease). This has been more clearly explained in the discussion, and the sentence in the abstract has been adapted.

Point 2. In table and text. I guess how the Authors have computed a “relative
difference”, but, for the reader, this point should be clearly stated in the section “Methods”. Likewise, please define “delta” in Table 1.

We agree with the reviewer that the term relative difference should be explained. This has been added to the methods section. In the legend of table 1, delta is now defined.

Point 3. Since you have used non parametric statistical tests, you should refer to “median” and not to “mean”.

We used the Wilcoxon signed rank test (also called Wilcoxon matched pairs test) to compare to paired groups. This test is different from the Wilcoxon rank sum test (also called Mann Whitney U test), which compares the median of a group to a hypothetical median. Since we have used the first test, we think that use of the mean value is allowed. Furthermore, the mean value and the median value are almost the same for our parameters, since our data are symmetrically distributed. This is a condition for this test. Moreover, previous published studies, using a similar n-value of animals and same OA models, also use these statistics.

Point 4. In the groove model at 20-week post-surgery, and although they did not reach statistical significance, the mean changes in subchondral plate (thickness and porosity) were greater than changes in trabecular subchondral bone. Do you think that these observations might suggest that subchondral plate changes are more relevant than trabecular subchondral bone changes for the progression of post-traumatic OA disease process?

The authors agree with the referee that the very consistent and large subchondral plate changes may be more relevant than the trabecular changes. Although it was already stated in the discussion section that ‘it seems that the trabecular bone changes are not directly related to the changes in subchondral plate and cartilage’ (page 17), we think this statement should be extended. Therefore we adjusted this paragraph.

Point 5. Section “Discussion”, the paragraph 4 dealing with osteophytosis could be somewhat rewritten in order to have a greater impact.

We have rewritten the paragraph and added a reference concerning the location of osteophytes in order to give it more impact as suggested by the reviewer.

Point 6. Section “Discussion”, paragraph 7. You state that the trabecular subchondral changes of the operated OA knee observed in the ACLT model are secondary to disuse. If it so, how do you conciliate your statement with the study of Behets et al (J Bone Miner Res, 2004, 19: 1821-1826) who, by using a pQCT, showed that most of subchondral bone changes were observed in the medial compartment of the tibia? What makes OA in the ACLT model is not disuse, but rather dramatic changes in the joint biomechanics concentrating a lot of biomechanical forces (compression and torsion) on the medial tibial plateau.


First, we want to make clear that we don’t think that all the osteoarthritic changes are caused by disuse, only the trabecular bone changes may be (partly) a result of disuse. We used a region in the metaphysis containing trabecular bone to verify this. We also studied a region in the diaphysis to check whether potential disuse would also affect the cortical bone. Since we did not find differences in the diaphyseal region, we think that cortical bone and thus also the subchondral plate are not affected by disuse. We have extended this explanation in the discussion section to clarify this. In addition, we did not find any difference between the medial and lateral side of the tibia for both the cartilage and bone parameters. As we don’t know the loading pattern of the dogs or bone data of a control region further away from the joint in these animals, it is hard to make a comparison between the data of Behets and our own study. Furthermore, factors such as weight of the animal (our animals were 1.5 to 3 years old, weighing 10-15 kg, Behets animals were 4 years old, but no weight was mentioned), and post-surgical exercise program may be of influence on the changes found. These could all be factors which might explain the differences between the study of Behets and our own data.

Point 7. The Authors should be very careful with cited references as some of their contentions do not always correspond exactly to the findings made in cited papers. In the section “Background”, paragraph 2, line 2, the Authors state: “In human studies, an increase in trabecular bone volume fraction and trabecular thickness was found (3-5), as well as an increase in cortical subchondral plate thickness (6)”. First, the study of Pelletier et al. (ref 6) is not devoted to human OA, but to canine experimental OA where the important subchondral bone resorption can be counteracted by Licofenac. Second, while Bobinac et al. (ref 4) indeed report an increase in the trabecular bone volume fraction in the subchondral bone of human OA knees at very late stages of the disease process, Chappard et al. (ref 5) clearly show that there is a marked difference between, on the one hand, OA bone still covered by its overlying articular cartilage and, on the other hand, OA bone having lost its overlying cartilage tissue, at least for human hip OA. Indeed, for instance, values found for both trabecular bone fraction and trabeculae thickness were higher in subchondral bone from OA femoral heads having lost their overlying articular cartilage than in subchondral bone from OA femoral heads still having their overlying cartilage tissue. However, in subchondral bone from OA femoral heads still having their articular cartilage, values found for trabecular bone volume fraction and trabeculae thickness were similar to values found in osteoporotic femoral heads without OA !!

We apologize that we have cited the wrong reference and we have corrected this. Furthermore, we agree that the data from Chappard et al are more complicated than presented. We have adapted this part of the background section, as suggested by the reviewer.
Reviewer's report: Referee 2
Title: A role for subchondral bone changes in the process of osteoarthritis; a micro-CT study of two canine models.
Version: 1 Date: 19 October 2007
Reviewer: David Holdsworth
Reviewer's report:
Reviewer's report: “A role for subchondral bone changes in the process of osteoarthritis: a micro-CT study of two canine models,” Sniekers et al.

Summary:
This Research Article describes an investigation of subchondral bone changes in two canine models of OA. Micro-CT and histology are used to investigate bone architecture and cartilage histology. Significant differences are observed between the “groove” and “ACLX” models in the dog.

This paper addresses an important topic; the questions posed are novel and quite well-defined. The methods are sound, and (with a minor exception related to the details of the CT experiments) sufficient details are provided so that others could replicate the study. The discussion and conclusions are well-balanced. The title and abstract are appropriate.

The paper is very well written, with a succinct style and few errors. I am happy to recommend publication, with only a few revisions, as described below.

Major Compulsory Revisions:
1/ The only revision I would like to see is quite straightforward, related to the description of the micro-CT acquisition and analysis on pages 8 and 9. It would be helpful to have more details about the micro-CT acquisition parameters, so that this study could be replicated by others. Important parameters include the kVp, the mA, the exposure time, and the number of views. In addition, it would probably be more accurate to say that the scan was acquired “with isotropic voxel spacing of 18 µm,” rather than refer to spatial resolution (unless spatial resolution has been measured independently).

We agree that the above-mentioned parameters are important to be able to replicate this study. We have added these parameters to the methods section (page 8 and 9). Indeed it is more accurate to refer to voxel size instead of resolution. We have changed this in the methods section (page 8).

Minor Essential Revisions:
1/ Background, page 4, paragraph 3: The point about previous animal models showing either increase or decrease in subchondral bone is important. It is possible that, in some cases, the confusion may result from the time at which the bone density was measured. This was certainly the case in the rabbit ACLX model studied by Battiste et al., where the subchondral density fell to a minimum at 8 weeks post-surgery, and then rose again at 12 weeks. Is it possible for the
authors to elaborate a bit on this point, with respect to previous studies? The timing of the observation of BMD could be very important in an animal OA model. The referee states correctly that time of measurement can be very important and that some parameters may react in a biphasic manner. We have adjusted this part of the background section.

2/ With respect to the data analysis (page 10), could the authors provide some additional clarification? Why was a non-parametric test used? Did the data fail the test for normality, or was it just because of the small sample size? Why change from one-sided test to two-sided test for cartilage and bone, respectively? Note that I am not disputing the correctness of the decisions, but it might help the reader to clarify why these choices were made.

We used a non-parametric test because the sample size is small. The bone changes were tested two sided because the bone changes were not studied before in the groove model, so we did not know in which direction (increase or decrease) the changes would evolve. However, the cartilage parameters were studied before in previous experiments in which we always found an increase in Mankin score and a decrease in GAG content as a result of OA development. We have added a few sentences to the methods section to clarify this.

3/ Related to the discussion, page 15, second paragraph: The finding that “Since the subchondral bone changes in the tibia cannot be caused directly by the grooves, we believe that these changes are part of the osteoarthritic process” is interesting and potentially significant. Can the authors provide any speculation about how this can come about, mechanistically? Could it be due to a change in joint loading? Changes in the local environment of the synovial fluid? If there is any likely explanation for this observation, it could be included here.

We agree that this is an interesting observation, and that speculation about the mechanism behind it will add value to this part of the discussion. We have added such a speculation to the discussion section.

Discretionary Revisions:
1/ Abstract; page 2: Consider replacing “… and architecture of subchondral plate” with “… and architecture of the subchondral plate”
   The word ‘the’ has been added.

2/ page 9, third last line: change “… of which bone volume was calculated...” to “… of which bone volume fraction was calculated...”

In the metaphyseal region described in this part of the methods section, a cylinder was selected which contained only trabecular bone. The bone volume (BV, not the bone volume fraction) of this cylinder was calculated, since the total volume (TV) of the cylinder was the same in each bone. Therefore, the bone volume fraction (BV/TV) would not give more information than bone volume (BV).
3/ page 14, last line: add a period (full stop) to this sentence.
A full stop has been added.

4/ page 16, end of first paragraph: with respect to the location of osteophytes, it may be useful to compare the findings in this paper with those of Batiste in a rabbit ACLX model, also studied with micro-CT.
Indeed, the location of the osteophytes shown in the figures in the paper of Batiste et al appears to correspond to the location of the osteophytes in our models. We thank the referee for pointing this out and added a remark on this in the discussion section.

5/ related to Figure 3: It is difficult to follow the anatomical orientation of the sections presented here. Perhaps some additional labels could be included, to clarify the orientation within the animal? Otherwise, an accompanying “cartoon” could be used to show the orientation of the sections.
Labels indicating anterior (a) and posterior (p) have been added to figure 3 and are explained in the legend of figure 3.