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KUMULATIVE PROMOTION

Effekt der medial öffnenden Tibiakopfosteotomie auf die menisko-osteochondrale Einheit im lateralen tibiofemoralen Kompartiment – Untersuchungen im translationalen Schafmodell

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1 Enthaltene Originalarbeiten

In diese kumulative Dissertation sind folgende Originalarbeiten (I-III) eingeschlossen, welche, unter meinem damaligen Namen Raphaela Ziegler, je in der Zeitschrift *Knee Surgery, Sports Traumatology, Arthroscopy (KSSTA)* im *peer reviewed* Verfahren publiziert wurden. Der Anteil und die Tätigkeit der Mitautoren an den einzelnen Publikationen sind im Folgenden detailliert aufgelistet. Alle Mitautoren sind mit einer Veröffentlichung der Originalarbeiten im Rahmen dieser kumulativen Dissertation einverstanden.

I. Effect of open wedge high tibial osteotomy on the lateral compartment in sheep. Part I: Analysis of the lateral meniscus. Madry H, **Ziegler R**, Orth P, Goebel L, Ong MF, Kohn D, Cucchiarini M, Pape D. *Knee Surg Sports Traumatol Arthrosc*. 2013; 21(1):39-48. Impakt-Faktor: 2,837

Autor:	**Beitrag:**
Madry, Henning	Konzeption der Studie, Durchführung der Tierversuche, Analyse und Interpretation der Daten, Ausarbeitung und Überarbeitung des Manuskripts
Ziegler, Raphaela	Datenerhebung, Analyse und Interpretation der Daten, Überarbeitung des Manuskripts, Statistische Analyse
Orth, Patrick	Datenerhebung, Analyse und Interpretation der Daten, Überarbeitung des Manuskripts
Goebel, Lars	Datenerhebung, Analyse und Interpretation der Daten
Ong, Mei Fang	Hilfe bei der statistischen Analyse
Kohn, Dieter	Kritische Durchsicht des Manuskripts
Cucchiarini, Magali	Analyse und Interpretation der Daten, Überarbeitung des Manuskripts
Pape, Dietrich	Konzeption der Studie, Durchführung der Tierversuche, Kritische Durchsicht und Überarbeitung des Manuskripts

II. Effect of open wedge high tibial osteotomy on the lateral tibiofemoral compartment in sheep. Part II: standard and overcorrection do not cause articular cartilage degeneration. **Ziegler R**, Goebel L, Cucchiarini M, Pape D, Madry H. *Knee Surg Sports Traumatol Arthrosc*. 2014; 22(7):1666-77. Impakt Faktor: 3,053

Autor:	**Beitrag:**
Ziegler, Raphaela	Datenerhebung, Analyse und Interpretation der Daten, Ausarbeitung und Überarbeitung des Manuskripts, Statistische Analyse
Goebel, Lars	Datenerhebung, Analyse und Interpretation der Daten
Cucchiarini, Magali	Analyse und Interpretation der Daten, Überarbeitung des Manuskripts
Pape, Dietrich	Durchführung der Tierversuche, Kritische Durchsicht des Manuskripts
Madry, Henning	Konzeption der Studie, Durchführung der Tierversuche, Analyse und Interpretation der Daten, Ausarbeitung und Überarbeitung des Manuskripts

III. Effect of open wedge high tibial osteotomy on the lateral tibiofemoral compartment in sheep. Part III: analysis of the microstructure of the subchondral bone and correlations with the articular cartilage and meniscus. **Ziegler R**, Goebel L, Seidel R, Cucchiarini M, Pape D, Madry H. *Knee Surg Sports Traumatol Arthrosc*. 2015; 23(9):2704-14. Impakt Faktor: 3,097

Autor:	**Beitrag:**
Ziegler, Raphaela	Datenerhebung, Analyse und Interpretation der Daten, Ausarbeitung und Überarbeitung des Manuskripts, Statistische Analyse
Goebel, Lars	Datenerhebung, Analyse und Interpretation der Daten
Seidel, Roland	Kritische Durchsicht des Manuskripts, Hilfe bei der Auswertung der Computertomographie
Cucchiarini, Magali	Analyse und Interpretation der Daten, Überarbeitung des Manuskripts
Pape, Dietrich	Durchführung der Tierversuche, Kritische Durchsicht des Manuskripts
Madry, Henning	Konzeption der Studie, Durchführung der Tierversuche, Analyse und Interpretation der Daten, Ausarbeitung und Überarbeitung des Manuskripts

2 Zusammenfassung

Die Gonarthrose ist eines der häufigsten Krankheitsbilder der Orthopädie. Die medial aufklappende Tibiakopfosteotomie (englisch: *High tibial osteotomy;* HTO) mittels winkelstabilem Plattenfixateur ist das derzeit etablierteste Verfahren zur Therapie der symptomatischen medialen tibiofemoralen Gonarthrose bei Patienten mit Varusfehlstellung. Durch die operative Veränderung der varischen mechanischen Tragachse kommt es konsekutiv zu einer vermehrten Belastung des lateralen tibiofemoralen Kompartimentes. Die vorliegende Arbeit soll die Frage klären, ob diese Lastveränderung durch die medial aufklappende Tibiakopfosteotomie pathologische Veränderungen im intakten lateralen tibiofemoralen Kompartiment indiziert. Speziell untersucht wurden die Effekte auf (1) den Außenmeniskus, (2) den tibialen und femoralen hyalinen Gelenkknorpel, sowie (3) auf den subchondralen Knochen durch etablierte makroskopische, histologische und immunhistochemische Bewertungssysteme, sowie biochemische und CT-morphologische Analysen 6 Monate postoperativ nach Varisierung (Positivkontrolle), Valgus-Standard- und Valgusüberkorrektur im Schafmodell und mit der jeweiligen kontralateralen, nicht osteotomierten Kontrollgruppe verglichen.

In allen Außenmenisken zeigte sich biochemisch eine signifikante Abnahme des DNS- und Proteoglykangehaltes von der zentralen Zone zur peripheren Zone. In der Pars intermedia der peripheren Zone der Außenmenisci der überkorrigierten Gruppe fand sich eine signifikante, 0,7-fache Abnahme des durchschnittlichen DNS-Gehaltes (korrelierend mit der Zellzahl) verglichen mit der Kontrollgruppe ohne HTO. Innerhalb der Femurkondylen lagen weder makroskopische, noch (immun-) histologische degenerative Veränderungen vor. In allen zentralen lateralen Tibiaplateaus (auch in den Kontrollen) wurden makroskopisch, histologisch und immunhistochemisch früharthrotische Veränderungen (Fissuren) gefunden, jedoch niemals in den submeniskalen Knorpelabschnitten, welche im Vergleich zu den nicht von den Menisci bedeckten Kniegelenkknorpel signifikant dünner waren. Ebenso war auch die subchondrale Knochenplatte eines normalen (nicht

osteotomierten) lateralen Tibiaplateaus zentral 3,9-fach dicker als in der submeniskalen Peripherie. Weder Varus- noch Valgus- (Standard- und Über-) Korrektur führten zu Veränderungen in der Dicke der subchondralen Knochenplatte oder der Breite des lateralen Tibiaplateaus. Lediglich in der tibialen subartikulären Spongiosa des lateralen Kompartiments resultierte die weitere Druckerhöhung durch Valgusüberkorrektur in einer vergrößerten spezifischen Knochenoberfläche. Hinsichtlich des physiologischen Aufbaus der osteochondralen Einheit zeigte sich in den zentralen (meniskusunbedeckten) Regionen des Tibiaplateaus eine signifikante Korrelation der Dicke der subchondralen Knochenplatte mit der Dicke des Gelenkknorpels. Im Allgemeinen korrelierte ein höherer Arthrosegrad mit Veränderungen der Mikrostruktur der subchondralen Knochenplatte.

Zusammenfassend zeigen die Daten, dass es nach Standardkorrektur zu keinen signifikanten Veränderungen kommt, eine Überkorrektur jedoch adaptive Veränderungen im Außenmeniskus und der osteochondralen Einheit induziert. Somit erscheint, auf Basis dieser translationalen Studie im Großtiermodell, die medial aufklappende Tibiakopfosteotomie ein sicheres Verfahren zur Therapie der symptomatischen medialen Varus-Gonarthrose.

3 Abstract

Osteoarthritis of the knee is one the common diseases in orthopedic surgery. The medial open wedge high tibial osteotomy (HTO) with a plate fixator is the most established method to treat symptomatic medial femorotibial osteoarthritis (OA) in patients with varus malalignment of the knee. As a result of the valgisation, the weightbearing axis of the leg is shifted towards the lateral compartment and its loading is increased. In this study, the influence of the HTO on the stable lateral tibiofemoral compartiment was analysed. Three experimental groups with biplanar osteotomies of the right proximal tibiae were tested. The first group received treatment with a closing wedge HTO of tibial varus malalignment; the second group received treatment with open wedge HTO resulting in a standard correction; and the third group received an overcorrection resulting in a tibial valgus. Six months post-surgery, the lateral meniscus, as well as the tibiofemoral articular cartilage and the subchondral bone were examined macroscopically, histologically, immunohistochemical, biochemically, and CT-morphologically. The findings were compared to the contralateral, non-osteotomized left knees.

Biochemical evaluations of the lateral menisci revealed a significant decrease in the DNA and the proteoglycan contents from the central (white-white) zone compared with the peripheral (red-red) zone. The red-red zone of the pars intermedia of the lateral menisci that underwent overcorrection showed a significant decrease in mean DNA contents compared with the control knee without HTO. Neither macroscopical nor histological nor immunohistochemical OA changes were found in all lateral femoral condyles in either treatment group. Macroscopical, histological and immunohistochemical OA changes were present in all central lateral tibial plateaus (irrespective of the treatment), but never in regions that were covered by the lateral meniscus. Measurements of the articular cartilage and subchondral thickness of the lateral tibial plateaus revealed that the cartilage, as well as the subchondral bone plate in the central region, was

thicker than in the periphery (covered by the lateral meniscus). Neither valgus (standard- or over-) correction, nor varuscorrection lead to alterations in the thickness of the subchondral bone plate or the width of the lateral tibiaplateau. The thickness of the subchondral bone plate correlated with the thickness of the articular cartilage at the central region of the lateral tibial plateau. From the present data we can conclude that a higher degree of osteoarthritis was in all groups accompanied by a lower bone surface/volume and specific bone surface/total volume ratio, as well as a higher cortical thickness in the subchondral bone plate. Specific topographical relationships exist in the central region between the articular cartilage and subchondral bone plate thickness, and in the submeniscal periphery between the articular cartilage and lateral meniscus, underscoring the importance of their interactions.

In conclusion, these data show that no significant change in the lateral meniscus and the tibiofemoral osteochondral unit occur after standard correction. Interestingly, overcorrection induces adaptive changes in the lateral meniscus and the tibiofemoral osteochondral unit. Thus, based on this translational study in a large animal model, medial opening wedge tibial osteotomy appears to be a safe therapy for symptomatic medial tibiofemoral OA of the varus knee.

4 Hauptteil

4.1 Einleitung

Die Gonarthrose ist eines der häufigsten Krankheitsbilder der Orthopädie. Es wird eine primäre, ohne erkennbare Ursache auftretende Gonarthrose von der sekundären Form, als Folge prädisponierender Faktoren, unterschieden (JEFFRIES, 2019). Die Arthrose beinhaltet hauptsächlich die Degeneration des Gelenkknorpels und der Menisken mit konsekutivem Umbau des subchondralen Knochens, klinisch reflektiert in Gelenkschmerz, häufigem Gelenkerguss, sowie fortschreitender Bewegungseinschränkung (BUCKWALTER et al., 2005; LOESER, 2010; WIELAND et al., 2005). Nach einer initialen Knorpelschädigung kommt es zum progressiven Untergang von Chrondroyzten und Zerstörung der extrazellulären Knorpelmatrix. Die Zerstörung des Knorpels führt zudem zu einer reaktiven Synovitis, welche ihrerseits wiederum den Knorpelabbau fördern kann. Prinzipiell sind alle Bestandteile des Gelenkes betroffen (ANGELE et al., 2016; DUCHOW, KOHN, 2003; MADRY et al., 2011; MADRY et al., 2010).

Risikofaktoren der Gonarthrose sind (uni-) kompartimentale Überlastung durch Beinachsenfehlstellung, Alter, Adipositas, Gelenkinstabilität, Gelenkdysplasien oder erworbene Formstörung der Gelenke wie nach Traumata (z.B. Meniskusläsionen), sowie metabolische und endokrine Erkrankungen (VINCENT et al., 2012). Eine hormonelle Komponente wird aufgrund der erhöhten Prävalenz unter Frauen ebenfalls diskutiert. Interessanterweise ist das mediale Kniegelenkskompartiment deutlich häufiger betroffen als das laterale (ANGELE et al., 2016; JACKSON et al., 2004; MATZIOLIS, RÖHNER, 2015; VINCENT et al., 2012). Die Verlagerung der mechanischen Tragachse ist ein unabhängiger Risikofaktor für das Fortschreiten einer radiologischen Gonarthrose (TANAMAS et al., 2009), hat jedoch *per se* keinen Einfluss auf die Entstehung der Gonarthrose (MADRY et al., 2016).

Die Gonarthrose ist konservativ und operativ therapierbar. Die Wahl der

Therapie richtet sich einerseits nach der Schwere der Symptome, anderseits nach den Ansprüchen des Patienten. Zu den von der *Osteoarthritis Research Society International* (OARSI) empfohlenen (symptomatischen) konservativen Ansätzen zählen nach der konsequente Physiotherapie, Gewichtsreduktion, Schuhranderhöhung, sowie systemisch und/oder intraartikulär gegebene Medikamente, wie beispielsweise systemische oder topische nicht-steroidale Antiphlogistika (MCALINDON et al., 2014) oder intraartikuläre Kortikosteroide.

Die operativen Maßnahmen zielen auf eine Verbesserung der Gelenkmechanik und Reduktion fokaler pathologischer Überlastungen. Hierzu zählt insbesondere die Tibiakopfosteotomie. Bei großflächiger Zerstörung der osteochondralen Einheit ist die Implantation eines endoprothetischen Oberflächenersatzes indiziert (MATZIOLIS, RÖHNER, 2015).

Die medial aufklappende Tibiakopfosteotomie mittels eines winkelstabilen Plattenfixateurs ist ein in der Klinik etabliertes und bewährtes Verfahren zur Behandlung der klinisch häufigen symptomatischen medialen tibiofemoralen Gonarthrose bei Patienten mit einer Varus-Fehlstellung (COVENTRY, 1984; LOBENHOFFER, 2014). Hierbei wird das mediale tibiofemorale Kompartiment entlastet. Dies führt zu konsekutiv zu einer Steigerung des lateralen tibiofemoralen Druckes (FUJISAWA et al., 1979). Der ideale Kandidat zur Therapie der medialen tibiofemoralen Gonarthrose mit varischer Achsfehlstellung mittels Tibiakopfosteotomie hat eine isolierte symptomatische mediale Gonarthrose, ein intaktes laterales tibiofemorales Kompartiment, keine patellofemorale Arthrose, ein fehlendes Streckdefizit, einen optimalen Körpermasseindex (*Body-Mass-Index*, BMI), sowie fehlende Bandinstabilität (GAO et al., 2019; HOFMANN et al., 2009; RAO et al., 2015). Laterale tibiofemorale Gonarthrose sowie ein Zustand nach lateraler Meniskektomie sind Kontraindikationen (HOFMANN et al., 2009). Die klinischen Ergebnisse nach valgisierender Tibiakopfosteotomie sind sehr gut und zeigen sich vor allem in einer verbesserten Lebensqualität bei

geringer Komplikationsrate (BODE et al., 2015; LOBENHOFFER, 2014; MATZIOLIS, RÖHNER, 2015).

Obwohl der Nutzen einer HTO klinisch seit Jahren akzeptiert ist und immer zu einer stärkeren Belastung des lateralen Kompartimentes führt, existieren fast keine Untersuchungen zu ihren strukturellen Effekten vor. Generell ist das tibiofemorale Kompartiment von einem engen topografischen und funktionellen Zusammenspiel zwischen Außenmeniskus und tibiofemoralen Gelenkknorpel gekennzeichnet (GOEBEL et al., 2017). Speziell haben Läsionen des Außenmeniskus klinisch oft sehr problematische Ergebnisse (SERVIEN et al., 2009). Dennoch liegen bislang keine Studien im Tiermodell vor, welche die Folgen der durch die Osteotomie induzierten vermehrten Belastung auf das laterale tibiofemorale Kompartiment differenziert untersuchen. Des Weiteren ist nicht sicher, inwieweit eine Standard- oder Überkorrektur der Beinachse das strukturelle Ergebnis im lateralen Kompartiment beeinflusst.

Ziel der vorliegenden Arbeit war daher zu untersuchen, ob die medial aufklappende Tibiakopfosteotomie abhängig vom Ausmaß der Korrektur zu strukturellen Alterationen im lateralen tibiofemoralen Kompartiment in einem stabilen Kniegelenk führt.

Speziell wurden in der vorliegenden Arbeit folgende vier Hypothesen untersucht:

1. Die medial öffnende Tibiakopfosteotomie führt zu makroskopischen, biochemischen und histomorphologischen Veränderungen im Außenmeniskus.

2. Die Standard- oder Überkorrektur durch die medial öffnende Tibiakopfosteotomie verursacht keine Degeneration des tibiofemoralen Gelenkknorpels im intakten lateralen Kompartiment.

3. Die medial öffnende Tibiakopfosteotomie induziert Veränderungen der Mikrostruktur des subchondralen Knochens im lateralen tibiofemoralen Kompartiment.

4. Spezifische Veränderungen von Außenmeniskus, Gelenkknorpel und subchondralen Knochen korrelieren.

4.2 Darstellung der einzelnen Publikationen

Tiermodell

Allen folgenden Publikationen liegt ein standardisiertes Tiermodell zu Grunde. Hierzu wurden nach Genehmigung des Tierversuchsantrages durch das Thüringische Landesamt (AZ. 22-2684-04-14-005/08) unter Überwachung durch einen Veterinärmediziner 19 ausgewachsene, weibliche Merinoschafe (66,6 ± 5,0 kg) operiert. Randomisiert wurden die Schafe drei experimentelle Gruppen zugeordnet, die jeweils rechts eine Umstellungsosteotomie mit einem winkelstabilen Implantat erhielten. Das jeweilige linke Knie wurde arthrotomiert und diente als Kontrollgruppe.

Gruppe	Korrektur	Zweck	Osteotomietechnik
1	4,5° Valgus	Standardkorrektur	medial aufklappend, biplanar
2	9,5° Valgus	Überkorrektur	medial aufklappend, biplanar
3	4,5° Varus	Positivkontrolle	medial schließend, biplanar
4	Arthrotomie ohne Korrektur	Negativkontrolle	-

Tabelle 1: Untersuchungsgruppen

Eine Arthrose wurde präoperativ radiologisch ausgeschlossen. Postoperativ konnten alle Tiere sofort voll belasten (PAPE, MADRY, 2013). Sechs Monate postoperativ wurden die Außenmenisci und die laterale osteochondrale Einheit entnommen und untersucht.

Mittels Wilcoxon-Rangsummentest wurde die Kontroll- mit der Therapiegruppe verglichen. Um die verschiedenen Untersuchungsgruppen untereinander zu vergleichen wurde der Mann-Whitney-U-Test angewendet. *P*-Werte < 0,05 wurden als signifikant erachtet.

Effekt der medial öffnenden Tibiakopfosteotomie auf das laterale tibiofemorale Kompartiment im Schafmodell. Teil I: Analyse des Außenmeniskus

Ziel dieser Studie war es, zu testen, ob die medial öffnende Tibiakopf-Osteotomie (HTO) zu strukturellen und biochemischen Veränderungen im Außenmeniskus führt.

Die Außenmenisci wurden durch ein makroskopisches Bewertungssystem semiquantitativ evaluiert (Punktzahl 0 = gesunder Meniskus, Punktzahl 8 = zerstörter Meniskus). Zur biochemischen Evaluation wurden Proben der peripheren (rot-roten) und zentralen (weiß-weißen) Zone des vorderen, mittleren und hinteren Drittels der Menisci entnommen. Der DNS-, Proteoglykan- und Gesamtproteingehalt wurde per Hoechst 33258, DMMB und Bradford-Test bestimmt (MADRY et al., 2001; ORTH et al., 2012c). Zur histologischen Untersuchung der Menisken erfolgte die Paraffineinbettung und anschließende Färbung der 5 µm dicken Schnitte mittels Hämatoxylin-Eosin-Färbung und Safranin-O-Echtgrün, sowie Typ-I-Kollagen-Immunhistochemie (CUCCHIARINI et al., 2007; KAMBIC, MCDEVITT, 2005; MADRY et al., 2003; MADRY et al., 2005).

Die Daten zeigten makroskopisch schlechtere numerische Ergebnisse für überkorrigierte Achsen als für Knie mit Standardkorrektur oder der induzierten Varus-Fehlstellung im Vergleich zu den kontralateralen Knien, ohne statistische Signifikanz zu erreichen ($P > 0,15$). Auch mikroskopisch fand sich kein signifikanter Unterschied innerhalb der verschiedenen Therapiegruppen. Biochemisch war eine signifikante Abnahme des DNS- und Proteoglykangehaltes von der zentralen Zone zur peripheren Zone in allen Menisken zu dokumentieren. In der Pars intermedia der peripheren Zone der Außenmenisci der überkorrigierten Gruppe zeigte sich eine signifikante Abnahme des durchschnittlichen DNS-Gehaltes (korrelierend mit der Zellzahl) um das 0,7-fache verglichen mit der Kontrollgruppe ohne HTO ($P = 0,012$). Ein Vergleich des Proteoglykangehaltes aller Bereiche

und Zonen der Außenmenisci ließ keine signifikanten Unterschiede erkennen.

Schlussfolgernd zeigten die Daten, dass die medial öffnende HTO zu keinen wesentlichen makroskopischen Veränderungen im Außenmeniskus sechs Monate postoperativ führt. Die Daten zeigten weiterhin, dass von allen untersuchten Parametern lediglich die Zellzahl nach einer Überkorrektur in der peripheren Zone des mittleren Drittels signifikant abnimmt (MADRY et al., 2013).

Effekt der medial öffnenden Tibiakopfosteotomie auf das laterale tibiofemorale Kompartiment im Schafmodell. Teil II: Standard- und Überkorrektur verursachen keine Gelenkknorpeldegeneration

Mit dieser Studie sollte die Frage geklärt werden, ob die Tibiakopfosteotomie zu strukturellen Veränderungen des Gelenkknorpels im lateralen tibiofemoralen Kompartiment führt.

Die makroskopische Evaluation erfolgte mittels eines modifizierten Bewertungssystems nach Outerbridge (OUTERBRIDGE, 1961). Es wurden Gesamtpunktwerte von 0 (normaler Knorpel) bis 4 (maximaler Knorpelschaden) vergeben. Zur quantitativen Evaluation des Knorpelschadens erfolgte die Unterscheidung in absolute und relative betroffene Knorpelfläche, sowie die Einteilung des lateralen Tibiaplateaus in 9 gleichgroße Quadranten und damit auch in die 3 Zonen Eminentia, zentral und peripher (submeniskal). Die Knorpeldicke wurde an 2 standardisiert definierten Punkten gemessen, einer submeniskal, der andere zentral im sicher nicht vom Außenmeniskus bedeckten Bereich. Auch in dieser Studie erfolgte die histologische Untersuchung nach Einbettung des lateralen Tibiaplateaus und der lateralen Femurkondyle in Paraffin und Färben der 4 µm Schnitte mittel Hämatoxylin-Eosin-Färbung, Safranin-O-Echtgrün und Typ-II-Kollagen-Immunhistochemie (CUCCHIARINI et al., 2007; HEILIGENSTEIN et al., 2011; MADRY et al., 2003; MADRY et al., 2005). Zur Auswertung wurden etablierte Arthrose-Bewertungssysteme der OARSI und nach Mankin verwendet (LITTLE et al., 2010; MANKIN et al., 1971).

In allen lateralen Tibiaplateaus (auch in den Kontrollen) wurden makroskopisch und (immun-) histologisch früharthrotische Veränderungen (Fissuren) gefunden. Diese fanden sich jedoch niemals in den submeniskalen Knorpelabschnitten, sondern immer zentral an den Knorpelflächen, welche niemals, oder nur teilweise vom Außenmeniskus bedeckt sind. Bezüglich der absoluten und relativen von degenerativen

Veränderungen betroffenen Fläche ließ sich kein Unterschied zwischen den verschiedenen Behandlungsgruppen feststellen. Die Messung der Knorpeldicke des lateralen Tibiaplateaus zeigte, dass der nicht vom Meniskus bedeckte zentrale Gelenkknorpel signifikant dicker ist, als der submeniskale Knorpel, ähnlich wie beim Menschen (THAMBYAH et al., 2006). Ein Unterschied zwischen den verschiedenen Behandlungsruppen und den Kontrollen fand sich nicht. Innerhalb der Femurkondylen lagen weder makroskopische, noch histologisch, oder immunhistochemisch detektierbare, degenerative Veränderungen vor.

Zusammenfassend zeigten die Daten, dass die medial öffnende Tibiakopf-Osteotomie, sowohl bei einer Standard- als auch bei einer Überkorrektur in einem ansonsten gesundem Knie keine negative Arthrose-induzierende Auswirkungen auf den Gelenkknorpel des lateralen tibiofemoralen Kompartimentes hat (ZIEGLER et al., 2014).

Effekt der medial öffnenden Tibiakopfosteotomie auf das laterale tibiofemorale Kompartiment im Schafmodell. Teil III: Analyse der Mikrostruktur des subchondralen Knochens und Korrelation mit dem Gelenkknorpel und Meniskus

Im Rahmen dieser Publikation (ZIEGLER et al., 2015) erfolgte zunächst die Analyse der Auswirkungen der medial öffnenden Tibikopfosteotomie auf die Mikrostruktur des subchondralen Knochens. Zudem wurde die Hypothese getestet, dass spezielle topographischen Korrelationen zwischen dem subchondralen Knochens, Gelenkknorpel und dem Außenmeniskus bestehen.

Die histologische Evaluation der Tibiaplateaus erfolgte per Goldner-Trichrom-Färbung (ORTH et al., 2012a; ZIEGLER et al., 2014). Alle linken und rechten Tibiaplateaus der Schafe der 3 Untersuchungsgruppen (n = 19) wurden durch eine Philips Brilliance 16-slice Computertomographie (CT) analysiert. Um eine standardisierte Auswertungsmöglichkeit zu schaffen, wurden 2 spezifische „*Volumes of interest*“ (VOI) festgelegt (ORTH et al., 2012b). VOI 1 enthielt ausschließlich die subchondrale Knochenplatte des lateralen Tibiaplateaus. VOI 2 enthielt die subartikuläre Spongiosa distal der Knochenplatte. Anschließend erfolgte die Erhebung differenzierter Parameter getrennt nach den beiden festgelegten VOI. Außerdem wurde die Dicke der subchondralen Knochenplatte an jeweils 2 standardisierten Punkten gemessen, wovon einer sicher nicht vom Meniskus bedeckt und der andere submeniskal lagen.

Weder Varus- noch Valgus- (Standard- und Über-) Korrektur führten zu Veränderungen in der Dicke der subchondralen Knochenplatte oder der Breite des lateralen Tibiaplateaus. Lediglich in der tibialen subartikulären Spongiosa des lateralen Kompartiments führte die weitere Druckerhöhung durch Valgusüberkorrektur zu einer vergrößerten spezifischen Knochenoberfläche (Knochenoberfläche-Knochenvolumen, BS/BV) verglichen mit der Varuskontrolle.

Die Korrelationsanalysen identifizierten auch spezifische topographische Beziehungen zwischen Variablen der submeniskalen und zentralen Region des lateralen tibiofemoralen Kompartimentes. Ebenso wie der submeniskale Gelenkknorpel deutlich dünner als der zentrale Gelenkknorpel war, wie in der vorangegangenen Studie gezeigt wurde, war auch die subchondrale Knochenplatte eines normalen (nicht osteotomierten) lateralen Tibiaplateaus zentral 3,9-fach dicker als in der submeniskalen Peripherie. Die Dicke der zentralen Knochenplatte korrelierte mit der Dicke des zentralen Gelenkknorpels. Interessanterweise fand sich im Allgemeinen eine Korrelation eines höheren Arthrosegrades mit Veränderungen der Mikrostruktur der subchondralen Knochenplatte.

Die wichtigste Erkenntnis der vorliegenden Studie war, dass weder Varus- noch Valgus- (Standard- und Über-) Korrektur zu Veränderungen der Dicke der subchondralen Knochenplatte in einem ansonsten stabilen Knie im Schafmodell führten. Ebenso beeinflussten weder erhöhte, noch verringerte Lasten, auch nach Valgus-Überkorrektur, nach 6 Monaten, nicht die Breite des lateralen Tibiaplateaus (ZIEGLER et al., 2015).

4.3 Diskussion

Die vorliegenden drei zusammenhängenden Publikationen untersuchen systematisch die Folgen der medial öffnenden Tibiakopfosteotomie als operative Therapiemaßnahme der medialbetonten Gonarthrose auf das laterale tibiofemorale Kompartiment.

Im Außenmeniskus führte die Valgus-Überkorrektur (9,5°) zu einem Abfall der Zellzahl (DNS-Gehalt) in der rot-roten Zone der Pars intermedia. Diese signifikante Reduktion der Zellzahl lässt eine Adaptation der rot-roten Zone der Pars intermedia an den erhöhten Druck im lateralen Kompartiment vermuten.

Der Gelenkknorpel wies bei allen untersuchten Gelenken, unabhängig der vorausgegangenen Behandlung, bereits degenerative Veränderungen auf, welche durch Über- oder Unterkorrektur nicht beeinflusst wurden. Dass diese Veränderungen jedoch regelmäßig an den Stellen auftraten, an denen die Gelenkfläche nie von Meniskus bedeckt ist, belegt die wichtige Rolle des Meniskus als „Stoßdämpfer“ und Lastverteiler (ODGAARD et al., 1989). Gleichzeitig passt sich der Gelenkknorpel, genauso wie der subchondrale Knochen, auf die erhöhte Druckbelastung an den nicht durch Meniskus bedeckten Arealen durch zunehmende Dicke an (KUMAR et al., 2013; MCNAMARA et al., 2013; SPAHN et al., 2013).

Weiterhin korrelierten arthrotische Veränderungen in der submeniskalen Region mit einer schlechteren Matrixanfärbbarkeit des Außenmeniskus nach 6 Monaten *in vivo;* ein Hinweis auf den engen Zusammenhang von Menikusschaden mit Gonarthrose (HUNTER et al., 2006; VERDONK et al., 2016). Studien, die den lateralen tibiofemoralen Gelenkknorpel nach Tibiakopfosteotomie arthroskopisch und durch Magnetresonanztomographie untersuchten, zeigten keine Veränderungen (PARKER et al., 2011; SPAHN et al., 2012). Ist der Schutz des dünneren submeniskalen Gelenkknorpels durch den Meniskus nicht mehr gewährleistet, wie z.B. durch einen Meniskusriss oder nach Menisketomie,

ist der Knorpel gefährdet (FINE et al., 1995). Little et al. beschreiben eine deutliche Beschädigung des Femur- und Tibiaplateaugelenkknorpels bereits sechs Monate nach Meniskektomie (LITTLE et al., 1997). Im Gegensatz zur partialen Entfernung des Innenmeniskus, geht die teilweise Entfernung des Außenmeniskus mit einem höheren Risiko eines Knorpelschadens einher (BEAUFILS et al., 2006). Dies unterstreicht die Anfälligkeit des lateralen Kompartimentes für Knorpelschäden im Vergleich zum medialen. Darüber hinaus ist ein Meniskusschaden hochgradig mit Arthrose assoziiert (BHATTACHARYYA et al., 2003) und pathologische Veränderungen im Meniskus prognostizieren Knorpelverlust bei symptomatischer Gonarthrose (ENGLUND et al., 2016; HUNTER et al., 2006). Bick et al. fanden bei Patienten keine morphologischen Veränderungen des Außenmeniskus nach Valgus-Überkorrektur. Jedoch zeigten sich magnetresonanztomographisch signifikant fortschreitende degenerative Veränderungen per Stoller-Klassifikation in allen Bereichen des Meniskus (BICK et al., 2018).

Das signifikant größere Knochenoberflächen-zu-Volumen Verhältnis in der subartikulären Spongiosa nach Valgus-Überkorrektur im lateralen Kompartiment deutet an, dass der trabekuläre subchondrale Knochen sich an die erhöhte Belastung zunächst durch die Vergrößerung seiner spezifischen Knochenoberfläche anpasst. Dies zeigt die enge Verbundenheit des subchondralen Knochens mit der Entstehung einer Arthrose (DE GIROLAMO et al., 2016; MADRY et al., 2010). Solch eine Anpassung des subchondralen Knochens an unterschiedliche Belastungen wurden auch in klinischen CT-osteoabsorptiometrischen Untersuchungen (MADRY et al., 2013) nach Tibiakopfosteotomie bei Patienten mit Varus-Fehlstellung gefunden.

Interessanterweise traten die signifikanten Veränderungen sowohl im Meniskus, als auch in der subartikulären Spongiosa nur in der Gruppe der überkorrigierten Tiere auf. Bei einer Standardkorrektur ließen sich keine signifikanten strukturellen Veränderungen feststellen. Ist der Druck jedoch

zu groß, wie es etwa bei einer Überkorrektur durch eine valgisierende Tibiakopfosteotomie der Fall ist, kommt es zu Kompensationsmechanismen, welche auf eine gute Anpassung des lateralen tibiofemoralen Kompartiments an die Druckerhöhung schließen lassen.

Buckwalter *et al.* zeigten, dass der humane Gelenkknorpel einem Anpressdruck von mehr als 25 MPa nicht standhalten kann (BUCKWALTER et al., 2006). Untersuchungen von Suero *et al*. ergaben eine deutliche Veränderung der Biomechanik im menschlichen Kniegelenk nach einer Überkorrektur (SUERO et al., 2015). Des Weiteren fanden Tuskada *et al*. keinen positiven Effekt auf den medialen Gelenkknorpel nach einer Überkorrektur im Vergleich zu einer Standardkorrektur (TSUKADA, WAKUI, 2017).

Die hier festgestellten Korrelationen deuten auf einen linearen Zusammenhang zwischen individuellen Variablen des subchondralen Knochens, des Gelenkknorpels und des Meniskus hin. In einer klinischen Studie von Cicuttini *et al*. fand sich ein signifikanter Zusammenhang zwischen abnehmendem Volumens des Tibiaknorpels und einer Valgusdeformität (CICUTTINI et al., 2004). Ding *et al*. zeigten an Patienten, dass der subchondrale Tibiaplateauknochen quantitativ mit der Schwere der Gonarthrose in Verbindung zu bringen ist und auch die Notwendigkeit einer Kniegelenksprothese voraussagen kann, unabhängig von radiologischen Veränderungen (DING et al., 2007). Ebenso ist eine Achsabweichung ein unabhängiger Risikofaktor für die Entstehung und das Fortschreiten der radiologischen Gonarthrose (ANGELE et al., 2016; BROUWER et al., 2007; MADRY et al., 2011; TANAMAS et al., 2009).

Zusammenfassend unterstreichen diese Ergebnisse die wichtige Rolle des tibialen subchondralen Knochens als eine aktive Komponente, die zur Anpassung an die unterschiedlichen Anforderungen eines Kniegelenkes fähig ist. Diese Ergebnisse untermauern auch die enge Beziehung

zwischen Veränderungen im subchondralen Knochen und Arthrose (HENROTIN et al., 2012; ORTH et al., 2013).

Das laterale tibiofemorale Kompartiment ist somit als eine Einheit verschiedener Komponenten zu sehen, welche jede für sich auf die Druckerhöhung nach valgisierender Tibiakopfosteotomie reagieren kann. Liegen jedoch zusätzliche pathologische Faktoren wie ein Meniskusschaden, fokale Knorpeldefekte oder bereits vorbestehende Gonarthrose vor, ist die Gefahr des Versagens dieser Kompensationsmechanismen und somit des Fortschreitens einer Gonarthrose erhöht (SPAHN et al., 2006).

Die medial aufklappende HTO bei Standardkorrektur ist daher ein sicheres Verfahren zur Behandlung der medial betonten Varus-Gonarthrose in einem ansonsten gesunden Knie und führt nur bei Überkorrektur zu adaptiven physiologischen Veränderungen im lateralen tibiofemoralen Kompartiment.

4.4 Ausblick

Zukünftige Studien müssen einerseits frühe Veränderungen, andererseits Langzeitveränderungen untersuchen. Ebenso gilt es, von den Daten dieser Studie in einem nicht-arthrotischen Modell ausgehend, die kompartimentale Auswirkung von Begleitpathologien wie fokalen Knorpelschadens, einer unikompartimentalen Gonarthrose oder definierter Außenmeniskusläsion zu studieren. Damit können unsere grundlegenden Kenntnisse der Arthroseentstehung im Zusammenhang mit Achsfehlstellungen erweitert werden (MADRY et al., 2014).

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5 Originalarbeiten

I. Madry H, **Ziegler R**, Orth P, Goebel L, Ong MF, Kohn D, Cucchiarini M, Pape D. Effect of open wedge high tibial osteotomy on the lateral compartment in sheep. Part I: Analysis of the lateral meniscus. Knee Surg Sports Traumatol Arthrosc. 2013; 21(1):39-48

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KNEE

Effect of open wedge high tibial osteotomy on the lateral compartment in sheep. Part I: analysis of the lateral meniscus

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Abstract
Purpose To evaluate whether medial open wedge high tibial osteotomy (HTO) results in structural and biochemical changes in the lateral meniscus in adult sheep.
Methods Three experimental groups with biplanar osteotomies of the right proximal tibiae were tested: (a) closing wedge HTO resulting in 4.5° of tibial varus, (b) open wedge HTO resulting in 4.5° of tibial valgus (standard correction) and (c) open wedge HTO resulting in 9.5° of valgus (overcorrection), each of which was compared to the contralateral knees with normal limb axes. After 6 months, the lateral menisci were macroscopically and microscopically evaluated. The proteoglycan and DNA contents of the red–red and white–white zones of the anterior, middle and posterior third were determined.
Results Semiquantitative macroscopic and microscopic grading revealed no structural differences between groups. The red–red zone of the middle third of the lateral menisci of animals that underwent overcorrection exhibited a significant 0.7-fold decrease in mean DNA contents compared with the control knee without HTO ($P = 0.012$). Comparative estimation of the DNA and proteoglycan contents and proteoglycan/DNA ratios of all other parts and zones of the lateral menisci did not reveal significant differences between groups.
Conclusion Open wedge HTO does not lead to significant macroscopic and microscopic structural changes in the lateral meniscus after 6 months in vivo. Overcorrection significantly decreases the proliferative activity of the cells in the red–red zone of the middle third in the sheep model.

Keywords HTO · Histology · Type I collagen · Lateral meniscus · Macroscopic evaluation · Proteoglycan content · DNA content

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Introduction

High tibial osteotomy (HTO) is a useful therapy for symptomatic medial femorotibial osteoarthritis in patients with varus malalignment of the knee [5, 38]. Particularly for younger and physically active patients with medial knee osteoarthritis, HTO is an excellent alternative to knee

arthroplasty [52]. Here, the weight-bearing axis of the leg is shifted away from the medial compartment with the aim of reducing excessive loading and pain and improving joint function. Consequently [1], the load distribution between the medial and lateral compartments of the knee is altered [50]. Loading is transferred towards the lateral tibiofemoral compartment—especially when a valgus overcorrection is performed—while loading of the medial compartment is decreased [1]. Interestingly, its consequences on the tibiofemoral cartilage have been described in cadaver studies focussing on pressure distribution [1] and in patients after HTO determining the distribution of mineralization of the tibial plateau by CT-osteoabsorptiometric investigations [28, 32]. In addition, effects of differences in load distribution on articular cartilage proteoglycans were assessed in models of below-knee amputation or femur valgus osteotomy in guinea pigs [53] or in cartilage samples obtained from patients undergoing total knee replacement [35]. Here, increased load was associated with a decreased proteoglycan concentration [53]. Although these studies found morphological and biochemical changes in the cartilage in relation to loading [35, 53], the influence of increased loading on the lateral meniscus remains unknown.

Here, we hypothesized that medial open wedge HTO results in structural and biochemical changes in the lateral meniscus in a preclinical sheep model and that these changes predominantly occur after overcorrection.

Materials and methods

Study design

Three experimental groups with medial osteotomies of the right tibiae were tested in sheep: (a) a closing wedge HTO resulting in 4.5° of tibial varus (unloaded control), (b) an open wedge HTO resulting in 4.5° of tibial valgus (standard correction) and (c) an open wedge HTO resulting in 9.5° of tibial valgus (overcorrection), each of which was compared to the contralateral left knees that received an arthrotomy only. Six months postoperatively, the animals were killed, and the macroscopic appearance of the lateral meniscus was scored. The menisci underwent histological and immunohistological analysis. The red–red and white–white zones of the anterior, middle and posterior third of the lateral menisci were evaluated for proteoglycan and DNA contents.

Animal experiments

Animal experiments were conducted under an Institution Animal Studies Committee–approved protocol. Animals had a weight of 66.6 ± 5.0 kg. Radiographs were taken prior to the experiments to exclude osteoarthritis. Biplanar osteotomies of the proximal tibiae were carried out using an anteromedial approach with an oscillating saw, leaving the contralateral cortical bone intact as described elsewhere [39]. The osteotomies underwent gradual opening using flat chisels, resulting in standardized openings [37]. A small stature TomoFix plate fixator (Synthes, Tuttlingen, Germany) was applied. The following experimental groups with medial osteotomies of the right tibiae were tested: (a) closing wedge HTO resulting in 4.5° of tibial varus (range, 2.0–6.0; unloaded control; $n = 5$), (b) open wedge HTO resulting in 4.5° of tibial valgus (range, 2.0–7.5°; valgus standard correction; $n = 5$), and (c) open wedge HTO resulting in 9.5° of valgus (range, 7.5–13.0°; overcorrection; $n = 9$), each of which was compared to the contralateral left knees that received an arthrotomy only. Postoperatively, all animals were immediately allowed full weight-bearing. Six months postoperatively, the animals were killed, and the lateral menisci were evaluated.

Table 1 Semiquantitative macroscopic meniscus score

Parameter	Point value
Colour of meniscus	
Normal	0
Abnormal	1
Presence of meniscal lesion	
Normal inner meniscal rim	0
Fibrillation of inner meniscal rim	1
Radial/horizontal tear	2
Complex tear	3
Central meniscal degeneration	
No	0
Yes	2
Insertion (bone and joint capsule)	
Intact	0
Not intact	2
Average total score range	0–8

Semiquantitative macroscopic score for the evaluation of the lateral menisci. The resulting inverse macroscopic score ranges from 0 points (normal meniscus) to 8 points (maximal meniscal damage)

Macroscopic analysis

The macroscopic appearance of the lateral meniscus was evaluated using a newly developed score (Table 1), which grades colour (0–1), quality of the inner meniscal rim (0–3), central meniscal degeneration (0–2) and meniscal insertion (0–2).

Histological and immunohistochemical analyses

Menisci were fixed in 4 % phosphate-buffered formalin. Paraffin-embedded coronal [19] sections (5 μm) were

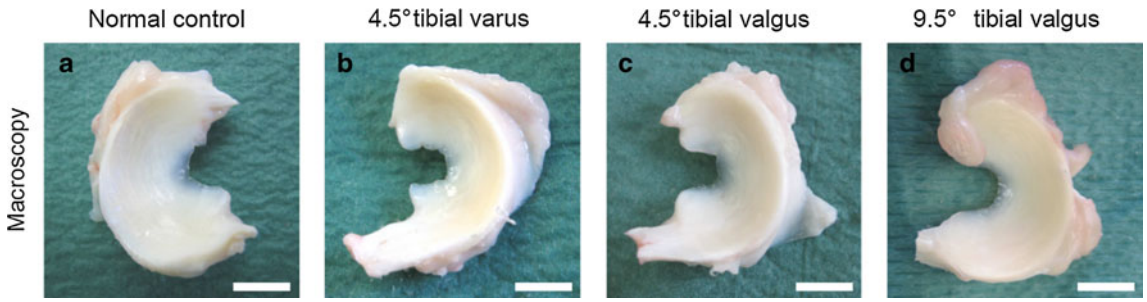

Fig. 1 Macroscopic analysis of the lateral menisci following high tibial osteotomy. Semiquantitative grading revealed a trend towards worse scores for overcorrected knees with increased pressure load on the lateral meniscus (9.5° tibial valgus; **d**) than for knees with standard (4.5° tibial valgus; **c**) or varus correction (**b**; $P > 0.15$). *Images* were selected from menisci having a macroscopic rating equal to the mean score for its respective treatment group. *Scale bars* 5.0 mm

stained with haematoxylin and eosin to detect cells and safranin O/fast green to detect proteoglycans according to the routine histological protocols [8, 25, 26]. Histological analysis was performed in a blinded fashion as described by Pauli et al. [40] with the modification that matrix staining was inversely scored. Immunostaining for collagen type I was performed as described [20]. All immunoreactivities were assessed as follows: 0, no immunoreactivity; 1, significantly weaker immunoreactivity; 2, moderately weaker immunoreactivity; 3, similar immunoreactivity; 4, stronger immunoreactivity compared with the positive control (sheep subchondral bone). A total of 114 sections were scored.

Biochemical analyses

For biochemical evaluations, samples were taken from the red–red zone and the white–white zone of the anterior, middle and posterior third of the menisci. Proteoglycan contents were measured by binding to the DMMB dye, and DNA contents were monitored using Hoechst 33258 as previously described [29, 34]. All data were normalized to the protein contents, as determined using a Bradford assay (Pierce, Rockford, IL, USA). Measurements were performed with a GENios spectrophotometer/fluorometer (Tecan, Crailsheim, Germany). A total of 38 lateral menisci were processed.

Statistical analysis

Mean values and SD were assessed for all evaluated criteria. A Wilcoxon signed-rank test was applied to compare the treatment versus control groups (SPSS, version 17.0; Chicago, IL). P values <0.05 were considered statistically significant.

Results

Six months postoperatively, all osteotomies healed uneventfully. Semiquantitative macroscopic evaluation of the lateral menisci (Fig. 1) revealed a trend towards worse scores for overcorrected knees (2.9-fold; n.s.) than for knees that received a standard correction (1.4-fold; n.s.) or a varus correction (1.3-fold; n.s.) compared with the

Table 2 Semiquantitative macroscopic evaluation of the lateral menisci

	4.5° tibial varus			4.5° tibial valgus			9.5° tibial valgus		
	Unloaded control			Standard correction			Overcorrection		
	Control	HTO	*P* value	Control	HTO	*P* value	Control	HTO	*P* value
Colour	0.2 (0.4)	0.2 (0.4)	n.s.	0.2 (0.5)	0.2 (0.5)	n.s.	0.2 (0.5)	0.3 (0.5)	n.s.
Meniscal lesion	0.4 (0.4)	0.6 (0.6)	n.s.	0.8 (1.1)	1.2 (0.8)	n.s.	1.2 (0.8)	0.9 (0.8)	n.s.
Central degeneration	0 (0)	0 (0)	n.s.	0 (0)	0 (0)	n.s.	0 (0)	0 (0)	n.s.
Insertion	0 (0)	0 (0)	n.s.	0 (0)	0 (0)	n.s.	0 (0)	0 (0)	n.s.
Average total score	0.6 (0.9)	0.8 (1.0)		1.0 (1.6)	1.4 (1.3)		1.4 (1.3)	1.1 (1.3)	
P value	n.s.			n.s.			n.s.		

Semiquantitative macroscopic evaluation of the lateral menisci using the inverse score described in Table 1. Three experimental groups were tested in the right knees of adult sheep: closing wedge HTO resulting in 4.5° of tibial varus, open wedge HTO resulting in 4.5° of tibial valgus (standard correction) and open wedge HTO resulting in 9.5° of valgus (overcorrection), each of which was compared to the contralateral left knees

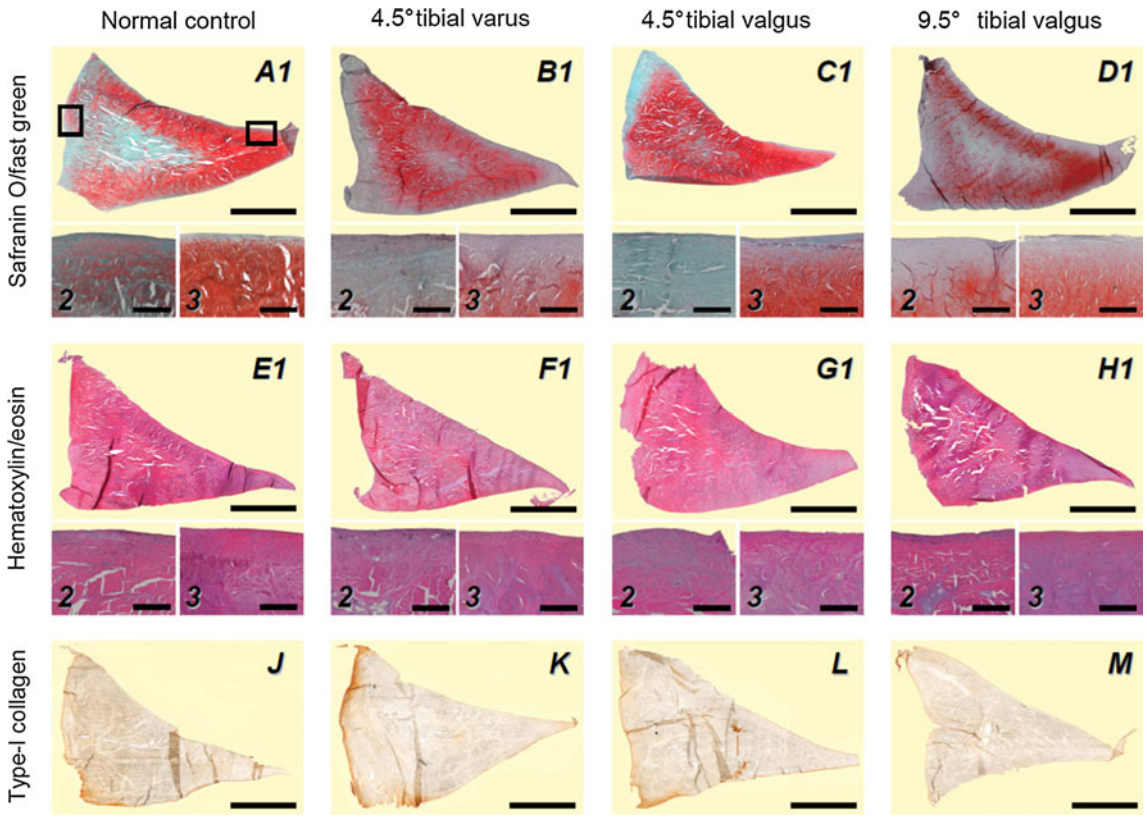

Fig. 2 Histological evaluation of changes in the middle thirds of the lateral menisci following high tibial osteotomy. *Large images* **A1–H1** and **j–k** show coronal cross-sections of the middle third (pars intermedia) of the entire lateral meniscus, whereas small images display magnified views of the red–red zone (**A2–H2**) and the white–white zone (**A3–H3**). *Rectangles* in image **A1** represent the standardized locations for analysis of the red–red (*left*, vertical) and white–white (*right*, horizontal) zones. According to safranin O/fast green staining (**a–d**), proteoglycan distribution (coloured in *red*) was unaffected by the change in limb axis and the resulting alteration in pressure load on the lateral menisci following the different tibial osteotomies. Haematoxylin/eosin staining **e–h** did not reveal variations in cell density or cellular organization between the three treatment groups (**F1–F3**, **G1–G3**, **H1–H3**) and the normal controls (**E1–E3**). Likewise, none of the treatments provoked changes in the immunoreactivity to a monoclonal mouse anti-human type I collagen IgG (**j–m**) in the lateral menisci. Sections were taken from menisci having a histological rating equal to the mean score for its respective treatment group. *Scale bars* 2.0 mm (**A1–H1** and **j–m**) and 0.2 mm (**A2–H2** and **A3–H3**)

Table 3 Semiquantitative microscopic evaluation of the lateral menisci

	4.5° tibial varus			4.5° tibial valgus			9.5° tibial valgus		
	Unloaded control			Standard correction			Overcorrection		
	Control	HTO	*P* value	Control	HTO	*P* value	Control	HTO	*P* value
Surface									
Femoral side	1.2 (1.1)	1.2 (0.8)	n.s.	1.6 (1.5)	0.8 (0.8)	n.s.	1.2 (1.0)	1.1 (1.4)	n.s.
Tibial side	0.6 (0.4)	0.6 (0.5)	n.s.	0.6 (0.9)	1.0 (0.7)	n.s.	2.1 (1.1)	1.0 (1.2)	n.s.
Inner border	2.8 (0.4)	1.6 (1.1)	n.s.	2.2 (1.1)	1.2 (1.1)	n.s.	2.1 (1.1)	1.9 (1.3)	n.s.
Cellularity	1.6 (0.9)	1.4 (1.3)	n.s.	1.2 (1.1)	0.6 (0.9)	n.s.	1.0 (1.0)	0.9 (1.1)	n.s.
Matrix organization	3.0 (0.0)	3.0 (0.0)	n.s.	3.0 (0.0)	2.8 (0.4)	n.s.	2.8 (0.4)	2.9 (0.3)	n.s.
Matrix staining	1.6 (0.5)	2.0 (0.7)	n.s.	1.8 (0.4)	1.6 (0.5)	n.s.	1.8 (0.4)	1.8 (0.7)	n.s.
Average total score	10.8 (1.9)	9.8 (2.6)	n.s.	10.4 (2.6)	8.0 (2.3)	n.s.	11.0 (2.8)	9.6 (3.6)	n.s.

Semiquantitative microscopic evaluation of the lateral menisci using the score described by Pauli et al. [39] with the modification that matrix staining was inversely scored. Three experimental groups were tested in the right knees of adult sheep: closing wedge HTO resulting in 4.5° of tibial varus, open wedge HTO resulting in 4.5° of tibial valgus (standard correction) and open wedge HTO resulting in 9.5° of valgus (overcorrection), each of which was compared to the contralateral left knees

Table 4 Immunohistochemical analysis of type I collagen

	4.5° tibial varus			4.5° tibial valgus			9.5° tibial valgus		
	Unloaded control			Standard correction			Overcorrection		
	Control	HTO	*P* value	Control	HTO	*P* value	Control	HTO	*P* value
Type I collagen immunoreactivity	0.7 (0.6)	1.5 (0.7)	n.s.	1.3 (0.6)	0.6 (0.5)	n.s.	0.8 (1.0)	1.0 (0.0)	n.s.

Type I collagen immunoreactivity in coronal sections of the red–red and white–white zones of the pars intermedia of the lateral menisci was compared with immunoreactivity of the ovine subchondral bone, used as a positive control. Immunoreactivity was scored as follows: 0, no immunoreactivity; 1, significantly weaker immunoreactivity; 2, moderately weaker immunoreactivity; 3, similar immunoreactivity; 4, stronger immunoreactivity compared with controls. Data are given as mean ± SD

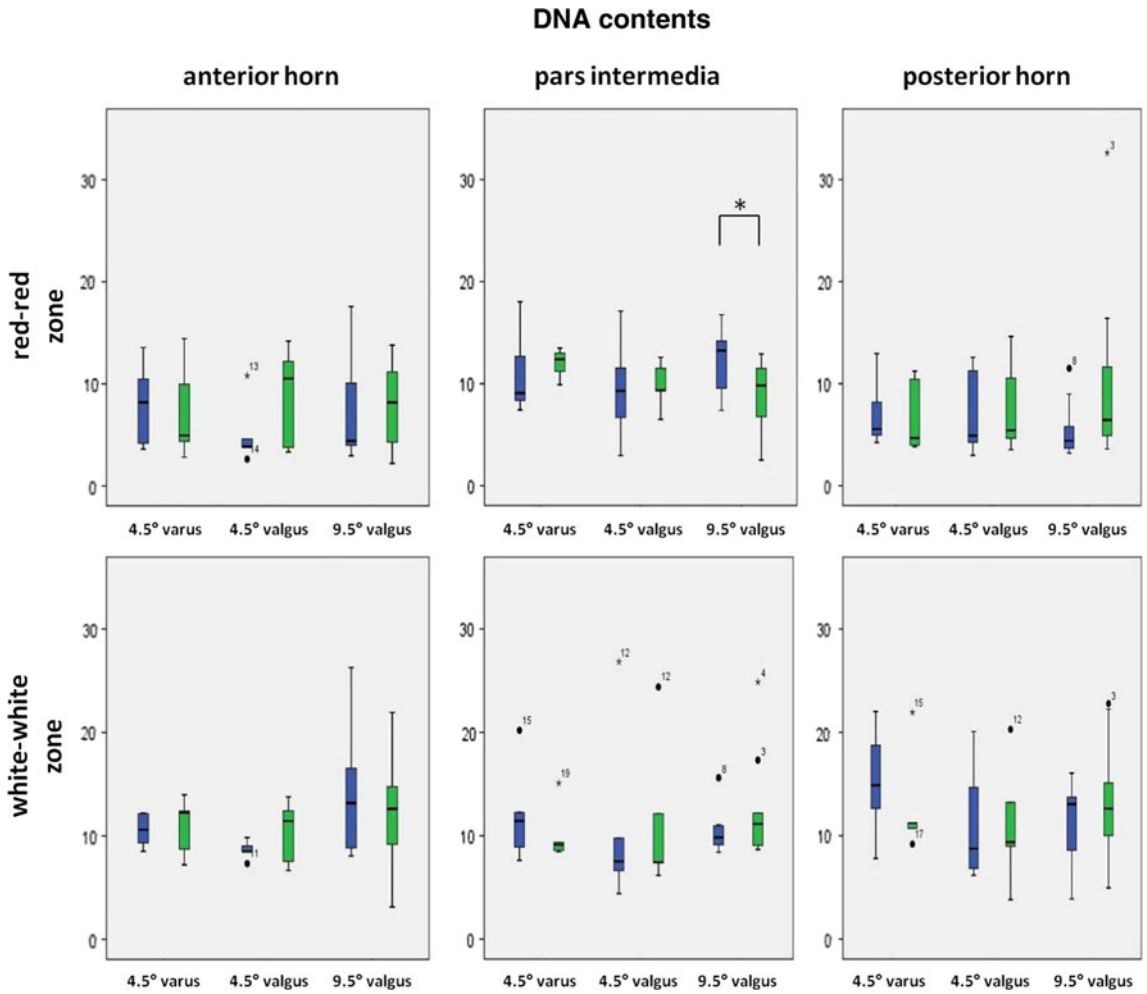

Fig. 3 DNA contents (μg DNA/mg total protein) of the different zones of the lateral menisci. *Blue boxes* control (*left* knees). *Green boxes* HTO groups (*right* knees): **a** 4.5° varus: unloaded control. **b** 4.5° valgus: standard correction. **c** 9.5° valgus: overcorrection. *Bottom* and *top* of the *boxes* show the 25th and 75th percentile; the median is shown as a *black band* in the *box*. *Whiskers* are defined as the lowest value still within 1.5 interquartile range (IQR) of the lower quartile and the highest value still within 1.5 IQR of the upper quartile

unoperated contralateral control without reaching statistical significance (Table 2).

Microscopic analysis of the lateral menisci showed no significant differences between groups with respect to surface, cellularity, matrix organization, matrix staining and the average total histological score (Fig. 2a–h; Table 3).

Immunoreactivity to type I collagen was located in the femoral and tibial superficial zones of the menisci and also in central parts of the lateral menisci (Fig. 2j–m). No

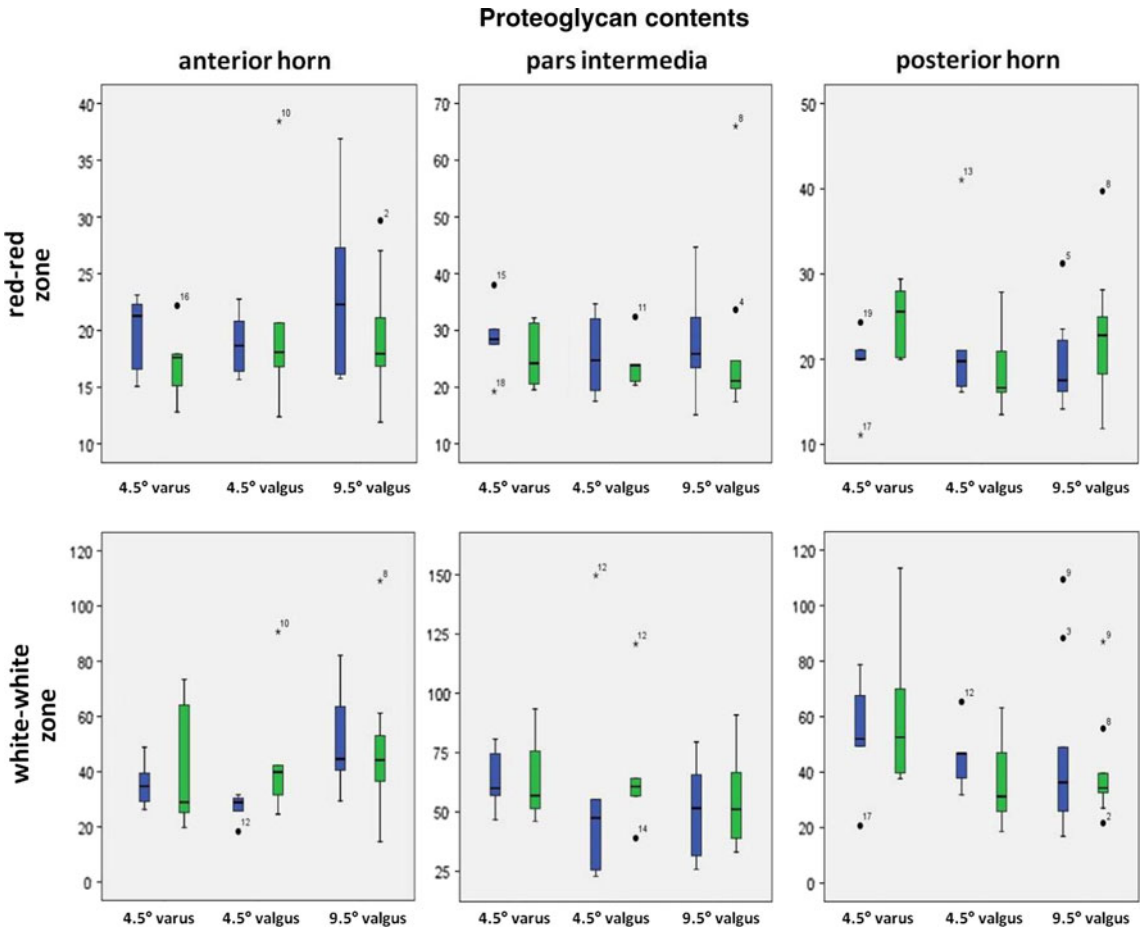

Fig. 4 Proteoglycan contents (μg proteoglycans/mg total protein) of the different zones of the lateral menisci. *Blue boxes* control (*left* knees). *Green boxes* HTO groups (*right* knees): **a** 4.5° varus: unloaded control. **b** 4.5° valgus: standard correction. **c** 9.5° valgus: overcorrection. *Bottom* and *top* of the *boxes* show the 25th and 75th percentile; the median is shown as a *black band* in the *box*. *Whiskers* are defined as the lowest value still within 1.5 interquartile range (IQR) of the lower quartile and the highest value still within 1.5 IQR of the upper quartile

difference in immunoreactivity to type I collagen was seen between the four groups (Table 4).

Biochemical evaluations revealed a significant decrease in the DNA and the proteoglycan contents from the white–white zone compared with the red–red zone in all menisci (Fig. 3). Between the experimental groups, there were no differences in the proteoglycan contents in each zone of the menisci (Fig. 4). However, the lateral menisci of animals that underwent overcorrection (9.5° of valgus) exhibited a significant 0.7-fold decrease in mean DNA contents in the red–red zone of the middle third (pars intermedia) of the samples compared with the control knee without HTO (12.3 ± 3.3 and 8.5 ± 3.8 μg DNA/mg total protein, respectively; $P = 0.012$) (Fig. 3). Interestingly, the proteoglycan contents of this region were also reduced compared with control condition, although without reaching statistical significance (Fig. 4). The proteoglycans-to-DNA ratios remained unchanged (Fig. 5). Comparative estimation of the proteoglycan contents of all other parts and zones of the lateral menisci did not reveal significant differences between groups.

Discussion

The main finding of the present study was that open wedge HTO was not associated with significant macroscopic and microscopic structural changes in the lateral meniscus 6 months after surgery in the preclinical sheep model. Standard correction (with 4.5° tibial valgus) does not lead to mid-term morphological alterations and differences in the DNA and proteoglycan content of the lateral menisci. Overcorrection (with 9.5° tibial valgus) significantly reduced cell numbers in the middle third of the red–red zone of the lateral menisci, as indicated by the reduced DNA contents compared with control knees without HTO, but does not lead to detectable mid-term morphological alterations after 6 months. Interestingly, this effect was not

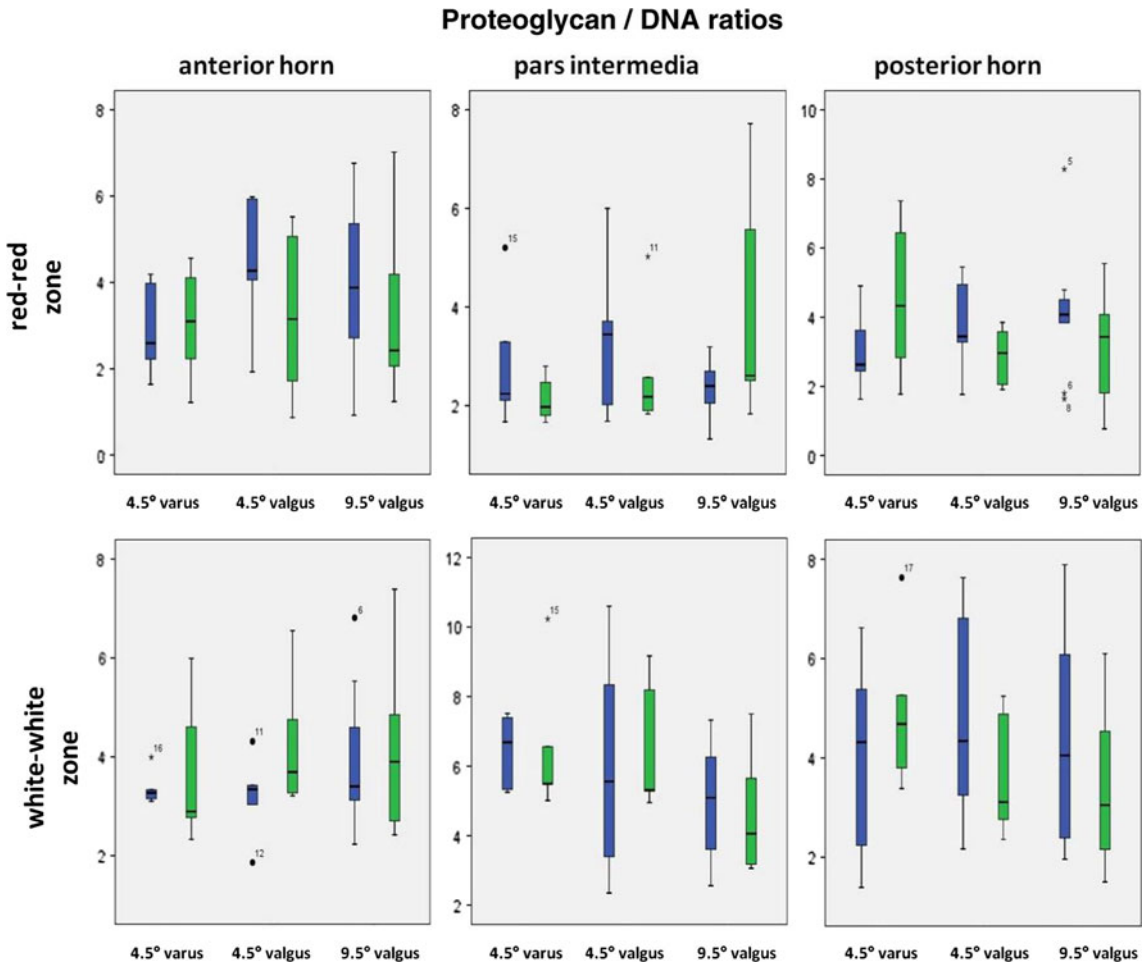

Fig. 5 Proteoglycan/DNA ratios (μg proteoglycans/μg DNA) of the different zones of the lateral menisci. *Blue boxes* control (*left* knees). *Green boxes* HTO groups (*right* knees): **a** 4.5° varus: unloaded control. **b** 4.5° valgus: standard correction. **c** 9.5° valgus: overcorrection. *Bottom* and *top* of the *boxes* show the 25th and 75th percentile, the median is shown as a *black band* in the *box*. *Whiskers* are defined as the lowest value still within 1.5 interquartile range (IQR) of the lower quartile and the highest value still within 1.5 IQR of the upper quartile

associated with inferior proteoglycan contents in this region. It is possible that the increased lateral contact pressure as a result of overcorrection may lead to structural changes in the red–red zone of the lateral meniscus in the long-term.

The menisci are indispensable components of the knee joint, as they allow for maximum congruence between the incongruent surfaces of the flattened tibial plateau and curved femoral condyles, have energy dissipating capacities and distribute loads [12, 51]. A meniscal tear can lead to knee osteoarthritis, but knee osteoarthritis can also lead to a spontaneous meniscal tear [9, 10, 31]. The subsequent increase in contact pressure and compression over time induces the development of osteoarthritis [23],

The lateral meniscus [3] is of specific importance, since the peak contact stress and maximum shear stress in the articular cartilage increased 200 % more after a lateral than a medial meniscectomy under axial femoral compressive loads, as shown in a three-dimensional finite element model of the human tibiofemoral joint [41]. The delicate balance between the lateral meniscus and the articular cartilage [15] is underlined by the clinical observation that cartilage lesions proceed much faster after lateral than after medial meniscectomy and that the clinical outcomes of lateral meniscectomy are significantly worse than after medial meniscectomy [4, 17, 24, 43]. Loss of meniscal tissue, such as resulting from injury or partial meniscectomy, significantly alters the biomechanical environment of the knee joint [15]. A recent clinical study on the effect of microfracture and medial open wedge HTO in patients with varus knee osteoarthritis demonstrated that those with injury of the medial meniscus have a higher likelihood of later undergoing total knee arthroplasty than patients without meniscal damage [44].

While it has been shown that meniscal resection leads to a disturbance of the contact between tibial and femoral cartilage [22], the mid-term effect on the lateral meniscus of an increase in loading following HTO has not, to the best of our knowledge, been demonstrated to date. When the knee is normally aligned, the centre of pressure passes

slightly through the medial side of the knee [36]. Varus malalignment abnormally distributes the load towards the medial tibiofemoral compartment [46]. Similarly, valgus malalignment increases load in the lateral compartment. The effect of axial malalignment and subsequent overload on the articular cartilage and the subchondral bone has been well described [2, 14, 15, 45]. Despite this clear correlation, little is known on the effect of axial malalignment on the lateral meniscus. The data of the present study show that in the sheep model, no significant macroscopic and microscopic structural changes occur in the lateral meniscus at 6 months after surgery.

This study also supports the finding of region-specific differences within the lateral meniscus [16, 30, 49]. The red–red and white–white zone of the sheep meniscus express different patterns of genes [11, 48], and the central part of the meniscus of sheep is more cartilaginous (e.g. containing more glycosaminoglycans) than the peripheral part [7, 13]. Of note, we found a significant decrease in cell proliferation in the red–red zone of the middle third in overcorrected knees compared with non-operated control knees. This suggests an inhibitory effect of the increased compression on the lateral compartment including the meniscus as a result of the valgus overcorrection. Ex vivo, it has already been proposed that the dynamic compressive behaviour of human meniscus correlates with its extra-cellular matrix composition [6]. When meniscus tissue explants in radial confinement were subjected to in vitro compressive overload, cell lysis increased with peak injury force and loading rate. In contrast, the content and release of glycosaminoglycans, together with mechanical properties, did not significantly vary with loading rate. Also, after 9 days in vitro, the tissue displayed little to no macroscopic damage [6]. These results, together with the present in vivo findings, indicate that meniscal cell damage may be present without immediate physical or compositional changes in meniscal tissue [33]. Whether this plays a role in the development of early osteoarthritis [27] remains to be determined.

Many in vitro studies have demonstrated that the homoeostatic balance between collagen biosynthesis and catabolism of meniscal cells is altered by static and dynamic compression and that the biosynthetic response of the meniscus to mechanical stimuli is regulated, in part, at the transcriptional level [17, 18, 47]. The data of the present study suggest that a standard correction (with 4.5° tibial valgus) does not lead to detectable mid-term morphological alterations and differences in the DNA and proteoglycan content of the lateral menisci in all of the six meniscal regions evaluated.

These findings contradict the hypothesis that medial open wedge HTO results in structural and biochemical changes in the lateral meniscus in a preclinical sheep model. Thus, from the viewpoint of this preclinical large animal model, this indicates that such standard correction is safe. The data of the present study also show that overcorrection (with 9.5° tibial valgus) does not lead to detectable mid-term morphological alterations, but to a significant decrease in the DNA content of the middle third of the red–red zone of the lateral menisci. This result supports the hypothesis that overcorrection leads to biochemical changes in the lateral meniscus. Consequently, it warrants further long-term studies to determine whether this reduction in the cell numbers will translate to structural changes of the meniscus.

Limitations of this study include the different lateral meniscus morphology of sheep compared with humans [7, 42]. Although the sheep is an important experimental model for meniscal repair, tissue engineering and regeneration [21], the degree of postoperative weight-bearing in quadruped animals is difficult to control. Moreover, this study did not evaluate possible pre-existing (e.g. surgically induced) lateral meniscal lesions, as sometimes present in the clinical situation.

Conclusion

Valgization following HTO with a subsequent increase in pressure load does not result in major mid-term macroscopic and microscopic changes in the lateral meniscus in sheep at 6 months postoperatively.

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Conflict of interest None.

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Effect of open wedge high tibial osteotomy on the lateral tibiofemoral compartment in sheep. Part II: standard and overcorrection do not cause articular cartilage degeneration

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Abstract
Purpose To evaluate whether medial open wedge high tibial osteotomy (HTO) results in structural changes in the articular cartilage in the lateral tibiofemoral compartment of adult sheep.
Methods Three experimental groups received biplanar osteotomies of the right proximal tibiae: (a) closing wedge HTO (4.5° of tibial varus), (b) opening wedge HTO (4.5° tibial valgus; standard correction), and (c) opening wedge HTO (9.5° of valgus; overcorrection), each of which was compared to the contralateral knees that only received an arthrotomy. After 6 months, the macroscopic and microscopic characteristics of the articular cartilage of the lateral tibiofemoral compartment were assessed.
Results The articular cartilage in the central region of the lateral tibial plateau in sheep had a higher safranin O staining intensity and was 4.6-fold thicker than in the periphery (covered by the lateral meniscus). No topographical variation in the type-II collagen immunoreactivity was seen. All lateral tibial plateaus showed osteoarthritic changes in regions not covered by the lateral meniscus. No osteoarthritis was seen in the peripheral submeniscal regions of the lateral tibial plateau and the lateral femoral condyle. Opening wedge HTO resulting in both standard and overcorrection was not associated with significant macroscopic and microscopic structural changes between groups in the articular cartilage of the lateral tibial plateau and femoral condyle after 6 months in vivo.
Conclusion Opening wedge HTO resulting in both standard and overcorrection is a safe procedure for the articular cartilage in an intact lateral tibiofemoral compartment of adult sheep at 6 months postoperatively.

Keywords HTO · Articular cartilage · Lateral compartment · Tibial plateau · Femoral condyle · Macroscopic evaluation · Osteoarthritis · Histology · Type-II collagen

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Introduction

High tibial osteotomy (HTO) is indicated for symptomatic medial tibiofemoral osteoarthritis in patients with varus malalignment of the knee [58]. As a result of the valgisation, the weightbearing axis of the leg is shifted towards the lateral compartment and its loading is increased [3], positively correlating with the position of the axis in the frontal plane [1]. Recently, no significant macroscopic and microscopic structural changes in the lateral meniscus were noted at 6 months after opening wedge HTO in an ovine model in vivo [32]. Here, overcorrection decreased the proliferative activity of the cells in the red–red zone of the

middle third of the lateral meniscus [32]. Also, morphological and biochemical changes of osteoarthritic articular cartilage in relation to loading have been reported in guinea pigs and patients [45, 61]. Moreover, a history of partial lateral meniscectomy is associated with a high rate of cartilage defects in the lateral compartment, highlighting the susceptibility of the lateral compartment to cartilage damage [41]. In patients, no articular cartilage damage was seen radiographically in the lateral tibiofemoral compartment after standard correction HTO [18]. Although HTO is well established [13, 53], its effects on the tibiofemoral cartilage structure remain largely unknown.

Based on a previous study, it was hypothesized that medial open wedge HTO leads to structural changes in the articular cartilage of the lateral tibiofemoral compartment, predominantly occurring following overcorrection after 6 months in vivo.

Materials and methods

Study design

As previously reported in detail [32], three experimental groups with medial osteotomies of the right tibiae were established in the hind limbs of sheep applying a small stature TomoFix plate fixator (Synthes, Tuttlingen, Germany): (a) closing wedge HTO (resulting in 4.5° of tibial varus; unloaded control), (b) opening wedge HTO (resulting in 4.5° of tibial valgus; standard correction), and (c) an opening wedge HTO (resulting in 9.5° of tibial valgus; overcorrection), each of which was compared to the contralateral left knee (receiving only an arthrotomy). Six months postoperatively, the macroscopic and microscopic characteristics of the articular cartilage of the lateral compartment were assessed.

Animal experiments, macroscopical, histological, and immunohistochemical analyses

Animal experiments were essentially conducted as previously described [32]. The lateral tibial plateaus and sagittal sections of the lateral femoral condyles were stained with India ink [36]. Macroscopic appearance was evaluated using a modified Outerbridge score [46] based on the close similarity between the areas covered by cartilage of the human patella and the ovine tibial plateau. A value of 0 was given for normal cartilage, 1 for cartilage with softening and swelling, 2 for fissures on the surface <127 mm^2, 3 when fissures on the surface were >127 mm^2, and 4 when subchondral bone was exposed. The resulting macroscopic score ranges from 0 points (normal cartilage) to 4 points (maximal cartilage damage). The absolute and relative area of the tibial articular cartilage affected by osteoarthritic changes was determined with a standardized Cartesian grid projected on to the articular surface (Fig. 1). To distinguish the different regions, the lateral tibial plateau was divided into 9 individual quadrants based on the maximum width and length of the lateral tibial plateau divided into three equal partitions. Measurements were made from digitalized macroscopic images using an image analysis system consisting of a mounted digital camera and a computer with analysis software programme (analySIS docu 5.0, Olympus Soft Imaging Solutions, Münster, Germany). Histological processing was essentially performed as previously described [17]. All tibial plateaus were sectioned perpendicular to the surface in the coronal plane at the level of the tibial eminentia, representing quadrants IV (tibial spine), V (cartilage partially covered by the lateral meniscus), and VI (cartilage completely covered by the lateral meniscus) (shown in Fig. 1 as a horizontal white line). All lateral femoral condyles were sectioned perpendicular to the surface in the coronal plane at the level of the weightbearing area. Paraffin-embedded sections

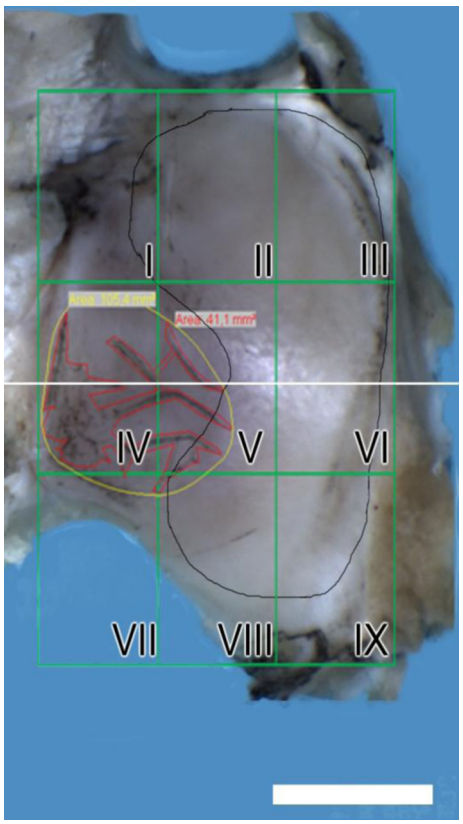

Fig. 1 Macroscopic view of an ovine lateral tibial plateau stained with India ink with the osteoarthritic defect outlined for measurements. *Red line* absolute defect area, *yellow line* relative defect area (perimeter), *green lines* dimensions and quadrants of the standardized grid, *black line* footprint of the lateral meniscus, *white line* coronal section through the tibial plateau perpendicular to the surface for histological processing. Quadrants are labelled with *Roman numerals*. *Scale bar* 10 mm

Table 1 Quantitative evaluation of the lateral tibial plateau

	4.5° tibial varus			4.5° tibial valgus			9.5° tibial valgus		
	Unloaded control			Standard correction			Overcorrection		
	Control	HTO	*P* value	Control	HTO	*P* value	Control	HTO	*P* value
Absolute osteoarthritic defect area (mm^2)									
Quadrant I	3.8 (5.4)	3.3 (5.7)	n.s.	7.7 (9.3)	8.9 (7.8)	n.s.	3.5 (5.6)	4.0 (4.4)	n.s.
Quadrant II	2.9 (2.8)	1.1 (1.8)	n.s.	2.8 (4.2)	0.4 (0.7)	n.s.	1.6 (2.2)	3.2 (4.6)	n.s.
Quadrant III	0.0 (0.0)	0.0 (0.0)	n.s.	0.0 (0.0)	0.0 (0.0)	n.s.	0.0 (0.0)	0.0 (0.0)	n.s.
Quadrant IV	28.1 (24.6)	27.0 (22.6)	n.s.	38.8 (20.1)	38.1 (25.4)	n.s.	26.1 (24.0)	21.3 (14.5)	n.s.
Quadrant V	22.6 (11.0)	20.9 (18.3)	n.s.	15.8 (5.5)	12.0 (5.0)	n.s.	14.7 (9.9)	16.0 (10.0)	n.s.
Quadrant VI	0.0 (0.0)	0.0 (0.0)	n.s.	0.0 (0.0)	0.0 (0.0)	n.s.	0.0 (0.0)	0.0 (0.0)	n.s.
Quadrant VII	13.8 (12.1)	15.2 (9.6)	n.s.	5.2 (7.2)	9.5 (6.8)	n.s.	3.2 (4.6)	6.9 (12.1)	n.s.
Quadrant VIII	3.0 (3.8)	6.1 (9.9)	n.s.	1.1 (1.5)	2.2 (3.3)	n.s.	7.1 (14.8)	1.2 (1.7)	n.s.
Quadrant IX	0.0 (0.0)	0.0 (0.0)	n.s.	0.0 (0.0)	0.0 (0.0)	n.s.	0.0 (0.0)	0.0 (0.0)	n.s.
Total absolute area	74.1 (41.2)	71.6 (46.2)	n.s.	71.5 (32.9)	70.5 (33.1)	n.s.	51.7 (40.2)	52.6 (34.4)	n.s.
Relative osteoarthritic defect area (mm^2)									
Quadrant I	8.4 (7.7)	5.3 (7.7)	n.s.	14.3 (16.3)	13.4 (11.0)	n.s.	7.2 (8.5)	9.2 (7.7)	n.s.
Quadrant II	9.3 (6.8)	2.2 (3.3)	n.s.	6.8 (8.2)	1.5 (1.0)	n.s.	3.0 (3.9)	6.4 (6.5)	n.s.
Quadrant III	0.0 (0.0)	0.0 (0.0)	n.s.	0.0 (0.0)	0.0 (0.0)	n.s.	0.0 (0.0)	0.0 (0.0)	n.s.
Quadrant IV	44.6 (27.6)	48.3 (28.6)	n.s.	60.4 (19.9)	58.7 (20.5)	n.s.	36.5 (32.0)	42.4 (24.4)	n.s.
Quadrant V	43.8 (13.5)	37.9 (20.1)	n.s.	36.4 (10.0)	31.0 (9.5)	n.s.	30.9 (11.0)	35.4 (20.2)	n.s.
Quadrant VI	0.0 (0.0)	0.0 (0.0)	n.s.	0.0 (0.0)	0.0 (0.0)	n.s.	0.0 (0.0)	0.0 (0.0)	n.s.
Quadrant VII	18.0 (13.3)	19.4 (12.4)	n.s.	8.2 (7.8)	13.6 (7.9)	n.s.	5.8 (7.2)	9.0 (12.9)	n.s.
Quadrant VIII	5.5 (5.6)	8.9 (13.8)	n.s.	2.8 (3.2)	4.4 (5.8)	n.s.	4.4 (7.2)	3.6 (5.1)	n.s.
Quadrant IX	0.0 (0.0)	0.0 (0.0)	n.s.	0.0 (0.0)	0.0 (0.0)	n.s.	0.0 (0.0)	0.0 (0.0)	n.s.
Total relative area	129.5 (49.8)	122.2 (52.0)	n.s.	128.9 (39.6)	122.6 (27.4)	n.s.	87.7 (52.9)	106.0 (50.4)	n.s.

Quantitative evaluation of osteoarthritic changes in the lateral tibial plateau. Shown are the original values of the defect areas and the *P* values. Data are given as mean ± SD

n.s. not statistically significant

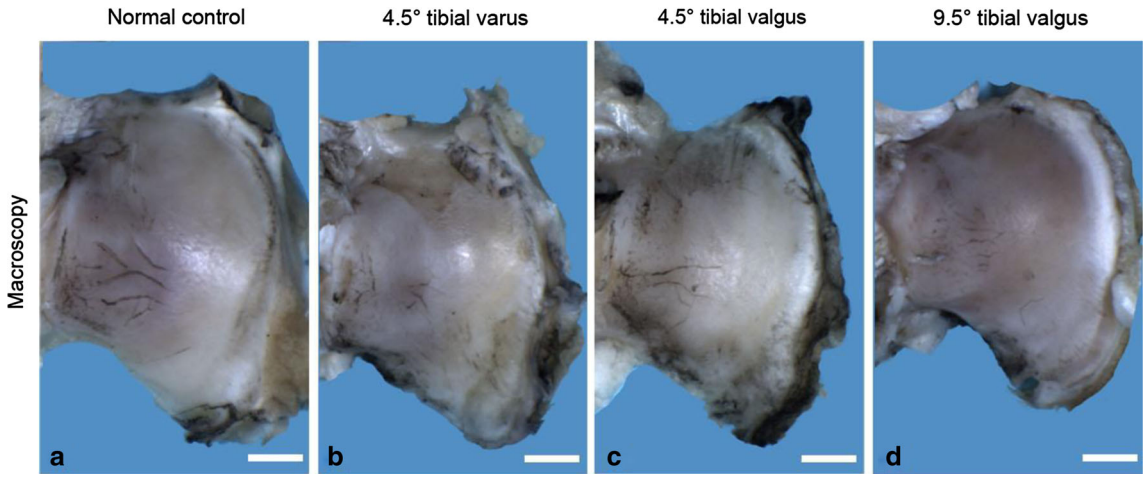

Fig. 2 Macroscopic view of lateral tibial plateaus stained with India ink (submeniscal regions are right in images **a**–**d**). *Scale bars* 5 mm

(4 µm) were stained with haematoxylin and eosin and safranin O/fast green according to routine histological protocols [7, 29, 30]. Histological analysis was performed by a blinded observer applying the OARSI [28] and Mankin scores [33]. For all scoring, the tibial plateaus were divided into three parts (eminentia, central, peripheral region) in a standardized manner, based on the grid applied for macroscopic evaluation. The thickness of the cartilage of the lateral tibial plateau was

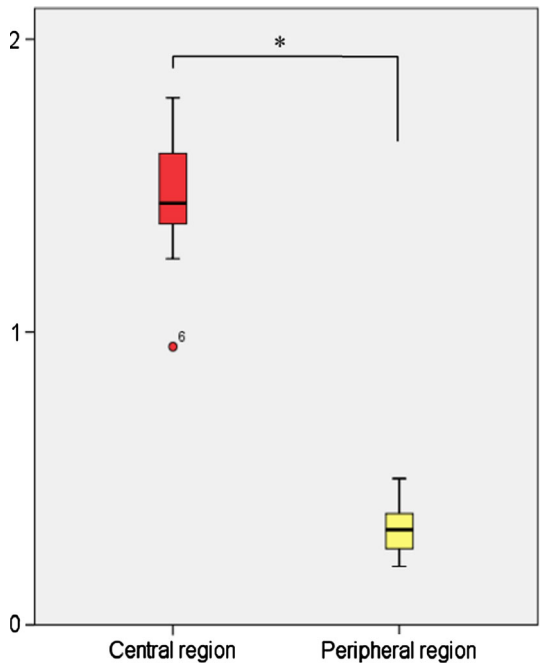

Fig. 3 Boxplot of the thickness (mm) of the articular cartilage of the lateral tibial plateau of the *left* knees. *Red box* central region (i.e. not covered by the lateral meniscus). *Yellow box* peripheral (submeniscal) region. *Bottom* and *top* of the *boxes* show the 25th and 75th percentile, the median is shown as a *black band* in the *box*. Whiskers are defined as the lowest value still within 1.5 interquartile range (IQR) of the *lower quartile*, and the highest value still within 1.5 IQR of the *upper quartile*. *$P < 0.001$

determined at two standard points that were defined to be at 25 and 75 % of its entire width (corresponding to quadrants IV and VI, respectively; Fig. 1), measured from its lateral end, using digitalized microscopic images of safranin O/fast green sections as described above, including the calcified cartilage to the cement line. As a pilot analysis revealed no differences in the distribution of the immunoreactivity in the deep zone in all groups, samples from the deep zone of normal sheep were selected as positive controls for type-II collagen. Sheep subchondral bone served as a negative control. Immunostaining for collagen type-II was performed mostly as described [20]. All immunoreactivities were assessed as follows: 0: no immunoreactivity; 1: weaker immunoreactivity; 2: similar immunoreactivity; 3: stronger immunoreactivity compared with the positive control. A total of 342 regions in 114 histological sections were scored.

Statistical analysis

Accuracies of all measurements were determined using calibration with a standard. Results of all analyses are given with the same accuracy as the methods. To enhance test–retest reliability, interpretations were performed with 3 slides per knee, allowing for high intraobserver agreement [44]. Mean values and standard deviations were assessed for all evaluated criteria. A Wilcoxon signed-rank test was applied to compare the treatment versus control groups (SPSS, version 20.0; Chicago, IL). To compare the different groups, the Mann–Whitney U test was used. P values < 0.05 were considered statistically significant.

Results

Osteoarthritic changes were present in all lateral tibial plateaus, irrespective of the treatment. They occurred in a distinct pattern, with osteoarthritic changes always present in the central and inner middle quadrants (IV, V), regions that are never (IV) or only partially (V) covered by the lateral meniscus (Fig. 1). In marked contrast, the peripheral three quadrants (III, VI, IX)—areas always covered by the lateral meniscus—were never affected by macroscopic osteoarthritic changes (Table 1). There was no significant difference in the absolute and relative area affected by osteoarthritic changes in all of the 9 individual quadrants between groups (Table 1; Fig. 2). Approximately 13 % (absolute area) of the lateral tibial plateaus in animals who underwent closing wedge HTO (4.5° of varus) showed osteoarthritic changes, not significantly different from the 13 % in animals who underwent standard correction (4.5° of valgus) and the 10 % in animals who underwent overcorrection (9.5° of valgus) (all $P > 0.05$; Fig. 2). Likewise, the relative areas of overall osteoarthritic changes in unloaded tibial plateaus were of 23 % in animals who underwent standard correction and of 20 % in animals who underwent overcorrection (all $P > 0.05$) (Fig. 2). Macroscopic evaluation of the lateral tibial plateau revealed mean Outerbridge scores between 2.3 and 2.6, with no significant differences between groups.

Measurements of the articular cartilage thickness of the tibial plateaus revealed that in control knees, the cartilage in the central region was 4.6-fold thicker than in the periphery (covered by the lateral meniscus) ($P < 0.001$; Fig. 3). No significant difference was found when the cartilage thickness in the central and peripheral region was compared between the four treatment groups (data not shown). Interestingly, individual histological scores for surface structure were always worse in the central region compared with the periphery of all left lateral tibial plateaus (Table 2; $P < 0.001$). Safranin O staining intensity was always stronger in the central region than in the periphery (Table 2; $P < 0.001$). No significant differences were found between all treatment groups for all evaluated individual and total areas with respect to the individual parameters and the average total Mankin and OARSI scores (Figs. 4, 5, 6; Table 2; data of the Mankin score are not shown). An analysis of immunoreactivity to type-II

Table 2 Semiquantitative microscopic evaluation of the lateral tibial plateau according to the OARSI score

	4.5° tibial varus			4.5° tibial valgus			9.5° tibial valgus		
	Unloaded control			Standard correction			Overcorrection		
	Control	HTO	*P* value	Control	HTO	*P* value	Control	HTO	*P* value
Structure									
Eminentia region	2.2 (1.4)	0.7 (0.9)	n.s.	0.6 (0.8)	2.0 (1.5)	n.s.	1.8 (1.5)	2.7 (1.9)	n.s.
Central region	1.4 (1.1)	2.9 (1.6)	n.s.	2.9 (1.8)	1.6 (1.2)	n.s.	2.9 (2.1)	2.2 (1.9)	n.s.
Peripheral region	0.1 (0.2)	0.1 (0.2)	n.s.	0.1 (0.2)	0.1 (0.1)	n.s.	0.5 (0.4)	0.4 (0.5)	n.s.
Mean score	1.2 (1.0)	1.2 (1.4)	n.s.	1.2 (1.5)	1.2 (1.0)	n.s.	1.7 (1.2)	1.8 (1.2)	n.s.
Chondrocyte density									
Eminentia region	0.0 (0.0)	0.0 (0.0)	n.s.	0.0 (0.0)	0.0 (0.0)	n.s.	0.0 (0.0)	0.0 (0.0)	n.s.
Central region	0.0 (0.0)	0.0 (0.0)	n.s.	0.0 (0.0)	0.1 (0.1)	n.s.	0.0 (0.0)	0.0 (0.0)	n.s.
Peripheral region	0.0 (0.0)	0.0 (0.0)	n.s.	0.0 (0.0)	0.0 (0.0)	n.s.	0.1 (0.2)	0.2 (0.4)	n.s.
Mean score	0.0 (0.0)	0.0 (0.0)	n.s.	0.0 (0.0)	0.0 (0.0)	n.s.	0.0 (0.0)	0.0 (0.0)	n.s.
Cell cloning									
Eminentia region	0.0 (0.0)	0.0 (0.0)	n.s.	0.0 (0.0)	0.0 (0.0)	n.s.	0.0 (0.0)	0.1 (0.2)	n.s.
Central region	0.0 (0.0)	0.0 (0.0)	n.s.	0.0 (0.0)	0.0 (0.0)	n.s.	0.0 (0.0)	0.0 (0.0)	n.s.
Peripheral region	0.3 (0.3)	0.2 (0.3)	n.s.	0.1 (0.1)	0.5 (0.4)	n.s.	0.2 (0.4)	0.5 (0.6)	n.s.
Mean score	0.1 (0.2)	0.1 (0.1)	n.s.	0.0 (0.1)	0.2 (0.3)	n.s.	0.1 (0.1)	0.2 (0.3)	n.s.
Safranin O staining									
Eminentia region	2.5 (1.6)	2.0 (1.4)	n.s.	2.5 (1.0)	2.2 (1.0)	n.s.	3.0 (1.5)	2.7 (1.4)	n.s.
Central region	0.7 (0.4)	0.9 (0.6)	n.s.	1.0 (0.0)	1.3 (0.6)	n.s.	1.1 (0.8)	1.3 (0.7)	n.s.
Peripheral region	3.6 (0.5)	3.5 (0.5)	n.s.	3.2 (0.3)	3.4 (0.4)	n.s.	3.3 (0.7)	3.1 (0.8)	n.s.
Mean score	2.3 (1.5)	2.1 (1.3)	n.s.	2.2 (1.1)	2.3 (1.1)	n.s.	2.5 (1.2)	2.4 (1.0)	n.s.
Tidemark									
Eminentia region	0.5 (0.5)	0.5 (0.5)	n.s.	0.8 (0.4)	0.8 (0.4)	n.s.	0.4 (0.5)	0.5 (0.8)	n.s.
Central region	0.6 (0.6)	0.6 (0.6)	n.s.	0.8 (0.4)	1.2 (0.4)	n.s.	0.7 (0.7)	0.9 (1.1)	n.s.
Peripheral region	0.6 (0.6)	0.9 (0.7)	n.s.	0.1 (0.1)	0.8 (1.3)	n.s.	1.3 (1.0)	1.2 (1.2)	n.s.
Mean score	0.6 (0.0)	0.7 (0.2)	n.s.	0.6 (0.4)	0.9 (0.2)	n.s.	0.8 (0.5)	0.9 (0.3)	n.s.
Sum									
Eminentia region	5.3 (2.7)	2.5 (1.7)	n.s.	3.9 (0.9)	5.0 (2.3)	n.s.	4.9 (2.8)	5.5 (3.1)	n.s.
Central region	2.6 (1.4)	4.5 (1.4)	n.s.	4.7 (2.2)	4.1 (2.2)	n.s.	4.8 (1.9)	4.1 (1.8)	n.s.
Peripheral region	4.6 (1.0)	4.7 (1.1)	n.s.	3.5 (0.4)	4.7 (1.9)	n.s.	5.3 (1.6)	5.4 (2.3)	n.s.
Total score	4.2 (1.3)	3.9 (0.5)	n.s.	4.0 (1.0)	4.6 (1.9)	n.s.	5.0 (1.5)	5.0 (1.8)	n.s.

Semiquantitative microscopic analysis of the articular cartilage of the lateral tibial plateau according to the OARSI score. Shown are the original values and *P* values. Data are given as mean ± SD. Normal cartilage receives a score of 0, maximal osteoarthritic cartilage a score of 25

n.s. not statistically significant

collagen in the articular cartilage of the lateral tibial plateau revealed a similar staining pattern between the central region and the periphery (covered by the lateral meniscus) (Fig. 7; Table 3). There were no significant differences in the immunoreactivity to type-II collagen between the four treatment groups for the three individual (eminentia, central, peripheral) and combined areas in both zones (superficial and middle zone).

No macroscopic osteoarthritic changes were found in all lateral femoral condyles in either treatment group, rendering a measurement of absolute and relative osteoarthritic surfaces unfeasible (Fig. 8). Microscopic analysis of the lateral femoral condyle by applying the Mankin and OARSI scores showed no significant differences between groups with respect to individual parameters and the average total histological score (Table 4; data of the Mankin score are not shown). An analysis of immunoreactivity to type-II collagen in the lateral femoral condyle showed no significant differences between groups in both zones (Fig. 9; Table 5).

Discussion

The data reveal that the articular cartilage in the central region of the lateral tibial plateau in sheep is much thicker

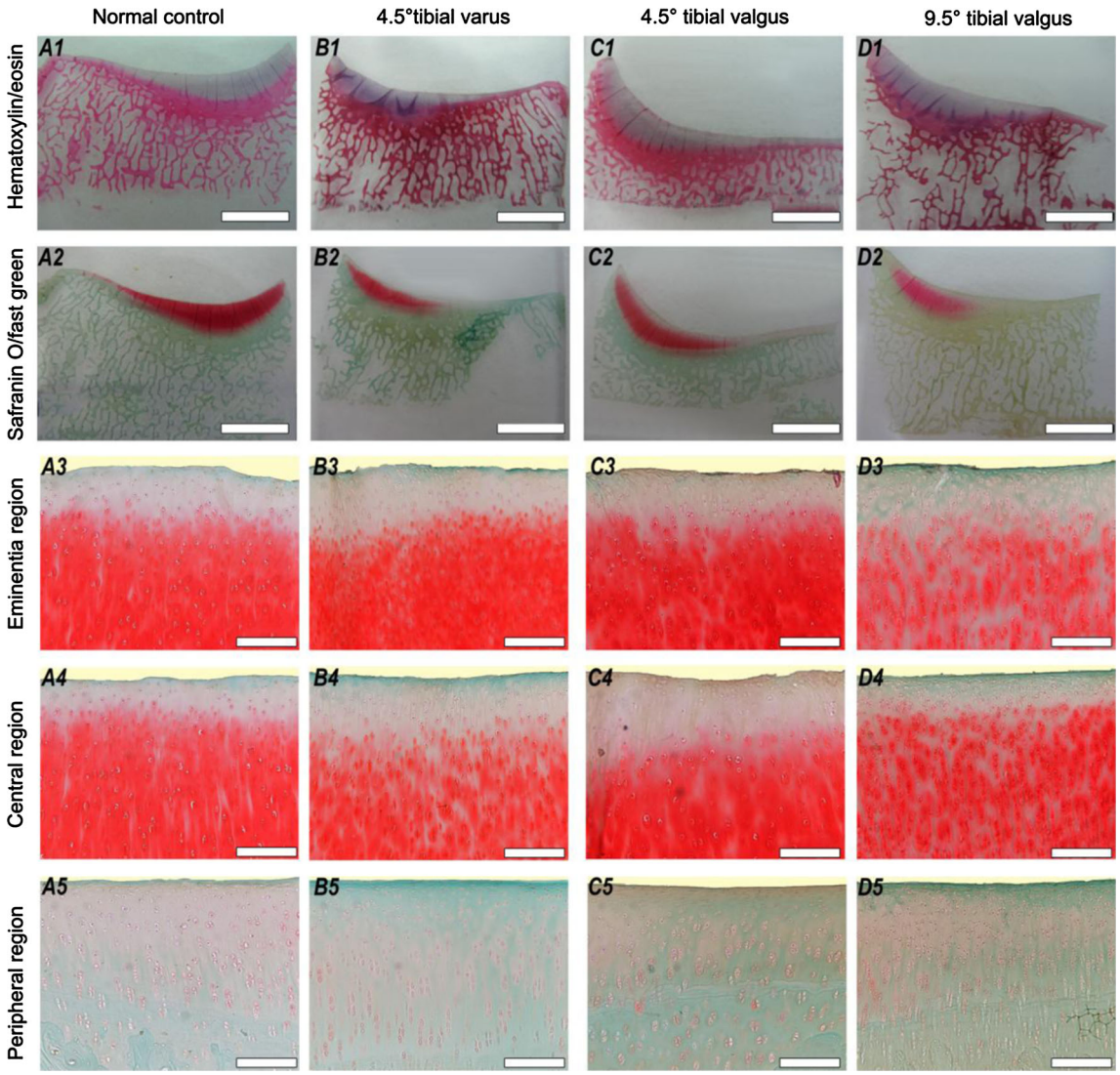

Fig. 4 Histological evaluation of lateral tibial plateaus. Images A1–D1 and A2–D2 show coronal cross sections of the entire lateral tibial plateau (submeniscal regions are left in images A1, A2 and right in images B1–D1, B2–D2), whereas images A3–D5 display magnified views of the eminentia (A3–D3), the not meniscus-covered central (A4–D4) and the peripheral (submeniscal) region (A5–D5) of the lateral tibial plateau. According to safranin O/fast green staining (A1–D1 and A3–D5), proteoglycan distribution (coloured in red) was unaffected by the change in limb axis and the resulting alteration of pressure load on the lateral tibial plateau following the different tibial osteotomies. Haematoxylin/eosin staining (A2–D2) did not reveal variations in cell density or cellular organization between the three treatment groups (B1–B5, C1–C5, D1–D5) and the normal controls (A1–A5). Scale bars: 5 mm (A1–D2) and 200 μm (A3–D5)

than the cartilage in the periphery which is covered by the lateral meniscus. Similarly, the safranin O staining intensity is stronger in the central than in the submeniscal peripheral regions. In contrast, there is no region-specific difference in type-II collagen immunoreactivity. The lateral tibial plateau shows a consistent pattern of osteoarthritic changes that are predominantly located in the inner middle and the central quadrant, regions that are never or only partially covered by the lateral meniscus. The peripheral submeniscal regions of the lateral tibial plateau and the lateral femoral condyle are not affected by such changes. The key finding of the present study is that opening wedge HTO resulting in both standard and overcorrection is not associated with significant macroscopic and microscopic structural changes in the cartilage of the lateral tibial plateau and femoral condyle after 6 months in vivo, contradicting the initial hypothesis.

Similar differences in the morphological characteristics of normal articular cartilage exist also in human tibial plateaus [26, 56, 60]. The human submeniscal cartilage is 50 % thinner than the cartilage not covered by the lateral meniscus [60], and has different biomechanical properties [14, 56, 60, 62]. Yet, its thickness is related to the mechanical stress applied, suggesting that the thinner

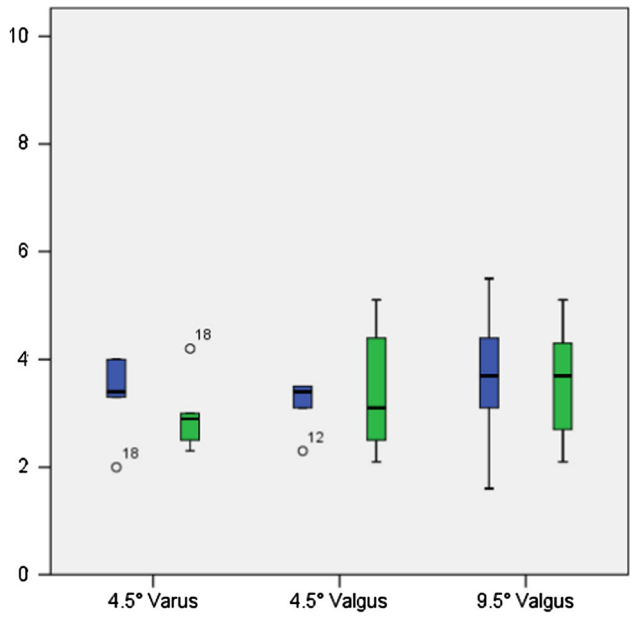

Fig. 5 Boxplot of the Mankin score of the articular cartilage of the lateral tibial plateaus. *Blue boxes* control (*left* knees). *Green boxes* HTO groups (*right* knees): **a** 4.5° varus: unloaded control. **b** 4.5° valgus: standard correction. **c** 9.5° valgus: overcorrection. *Bottom* and *top* of the *boxes* show the 25th and 75th percentile, the median is shown as a *black band* in the *box*. Whiskers are defined as the lowest value still within 1.5 interquartile range (IQR) of the *lower quartile*, and the highest value still within 1.5 IQR of the *upper quartile*

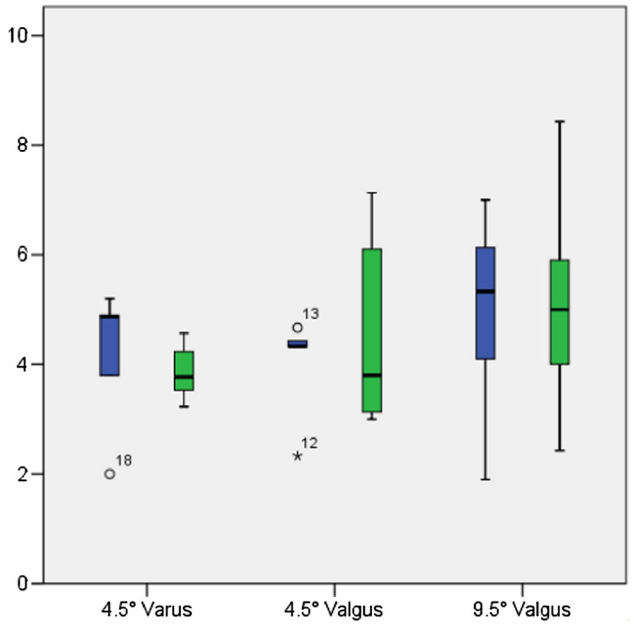

Fig. 6 Boxplot of the OARSI score of the articular cartilage of the lateral tibial plateaus. *Blue boxes* control (*left* knees). *Green boxes* HTO groups (*right* knees): **a** 4.5° varus: unloaded control. **b** 4.5° valgus: standard correction. **c** 9.5° valgus: overcorrection. *Bottom* and *top* of the *boxes* show the 25th and 75th percentile, the median is shown as a *black band* in the *box*. Whiskers are defined as the lowest value still within 1.5 interquartile range (IQR) of the *lower quartile*, and the highest value still within 1.5 IQR of the *upper quartile*

cartilage is stiffer in the region where it is shielded by the meniscus, and load is distributed over a larger area [40]. Once this protection diminishes—for example, after a meniscal tear—the cartilage is at high risk [12]. Radial tears of the lateral meniscus lead to unfavourable biomechanical properties of the cartilage which are not different from those occurring after partial lateral meniscectomy [5], and are further worsened by meniscectomy [4, 10, 11, 23, 34]. Interestingly, a history of partial lateral meniscectomy is associated with a higher rate of cartilage defects (25 %) than a history of partial medial meniscectomy (7 %), suggesting that the lateral compartment is more vulnerable to cartilage damage after partial meniscectomy than the medial compartment [41]. On the other hand, the central regions of the tibial plateaus and the eminentia, which supports mediolateral stability [25, 35], are thicker and have a lower stiffness [60]. Here, the tibiofemoral cartilage contact points are located [8, 25]. As safranin O staining of cartilage reflects its proteoglycan content, the stronger staining intensity suggests that areas not covered by the lateral meniscus contain more proteoglycans than the submeniscal parts [54]. The large proteoglycans bind the tissue water and interact with the collagen network, thus influencing resilience of cartilage [22, 38]. Interestingly, no changes were apparent in the distribution of the immunostaining for type-II collagen. Type-II collagen integrity is of major importance for the cartilage biomechanical function [24]. Previously, changes in collagen organization and proteoglycan content were observed after meniscectomy in the ovine stifle joint [4, 43]. Thus, the altered loading as applied here without meniscectomy did not alter the content of this key cartilage matrix molecule.

Osteoarthritic changes including lesser histological scores for surface structure were always present in the inner middle and central quadrants (IV, V), regions that are never or only partially covered by the lateral meniscus. Little et al. also observed more structural abnormalities in regions of ovine tibial plateaus not protected by the meniscus than elsewhere [27]. In contrast, peripheral submeniscal regions of the lateral tibial plateau were never affected by osteoarthritis. A recent study in patients with radiographically normal lateral compartments who underwent total knee arthroplasty for medial osteoarthritis showed some evidence of osteoarthritis in the lateral compartment, without distinguishing regions of the tibial plateau [37]. Human samples from donors ageing from 62 to 70 years had normal cartilage in regions covered by the meniscus [60]. Previously, it has been demonstrated that patterns of density distribution depend on the mechanical axis [39]. Yet, the relationship between malalignment and the onset of knee osteoarthritis remains unclear [59], although an association between malalignment and the

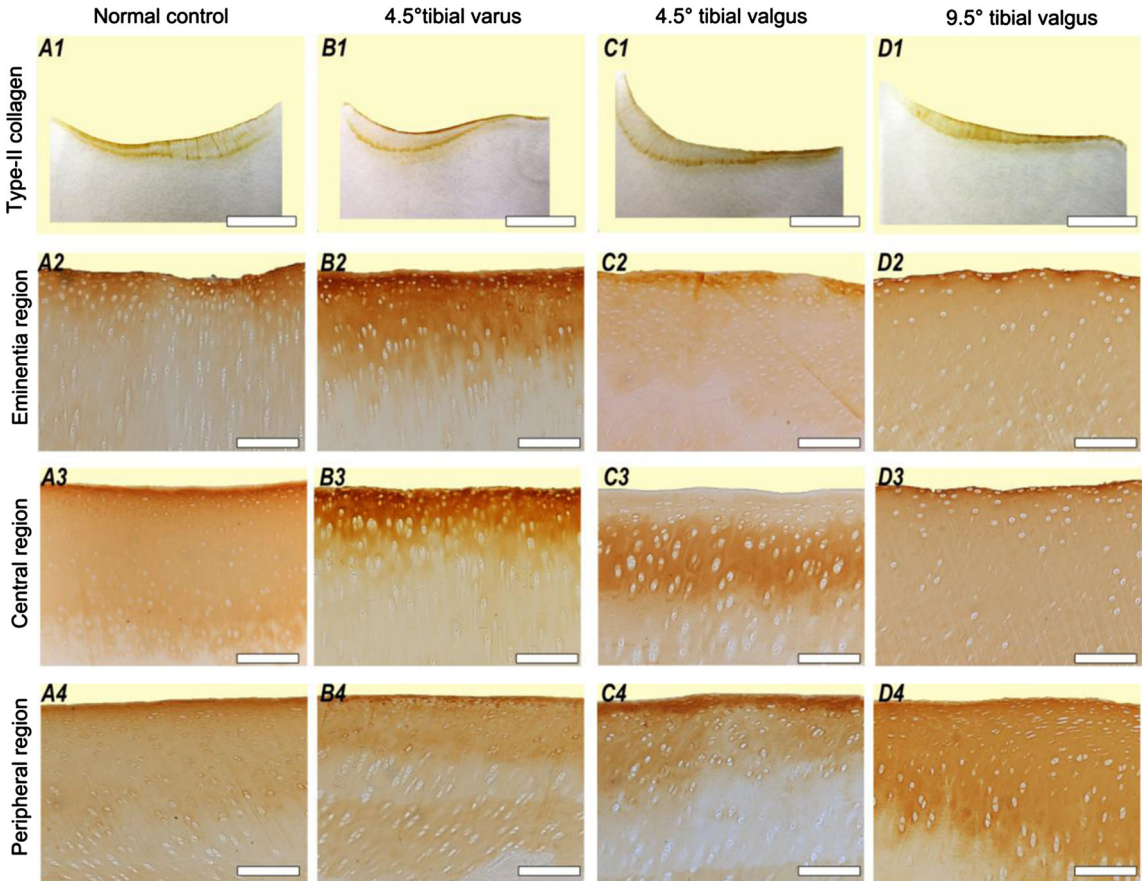

Fig. 7 Histological evaluation of the lateral tibial plateaus. Images **a1–d1** show coronal cross sections of the entire lateral tibial plateau (submeniscal regions are *left* in image **a1** and right in images **b1–d1**) whereas images **a2–d4** display magnified views of the eminentia (**a2–d2**), the not meniscus-covered central (**a3–d3**) and the peripheral (submeniscal) region (**a4–d4**) of the lateral tibial plateau. *Scale bars* 5 mm (**a1–d1**) and 200 μm (**a2–d4**)

Table 3 Immunohistochemical analysis of type-II collagen of the lateral tibial plateau

	4.5° tibial varus			4.5° tibial valgus			9.5° tibial valgus		
	Unloaded control			Standard correction			Overcorrection		
	Control	HTO	*P* value	Control	HTO	*P* value	Control	HTO	*P* value
Surface zone									
Eminentia region	2.8 (0.4)	2.2 (0.8)	n.s.	2.8 (0.4)	2.6 (0.5)	n.s.	2.7 (0.5)	2.6 (0.5)	n.s.
Central region	2.8 (0.4)	2.6 (0.5)	n.s.	2.6 (0.5)	2.6 (0.5)	n.s.	2.6 (0.7)	2.6 (0.7)	n.s.
Peripheral region	3.0 (0.0)	2.6 (0.5)	n.s.	2.4 (0.5)	2.8 (0.4)	n.s.	2.6 (0.5)	2.4 (0.7)	n.s.
Total score	2.9 (0.3)	2.5 (0.4)	n.s.	2.6 (0.4)	2.7 (0.3)	n.s.	2.6 (0.4)	2.5 (0.6)	n.s.
Deeper zones									
Eminentia region	2.0 (0.0)	2.4 (0.5)	n.s.	2.4 (0.5)	2.0 (0.0)	n.s.	2.3 (0.5)	2.2 (0.4)	n.s.
Central region	2.2 (0.4)	2.6 (0.5)	n.s.	2.6 (0.5)	2.4 (0.5)	n.s.	2.3 (0.5)	2.3 (0.5)	n.s.
Peripheral region	2.4 (0.5)	2.2 (0.4)	n.s.	2.2 (0.4)	2.8 (0.4)	n.s.	2.3 (0.5)	2.4 (0.5)	n.s.
Total score	2.2 (0.2)	2.4 (0.3)	n.s.	2.4 (0.4)	2.4 (0.1)	n.s.	2.3 (0.3)	2.3 (0.3)	n.s.
Average total score	2.5 (0.2)	2.4 (0.3)	n.s.	2.5 (0.4)	2.5 (0.2)	n.s.	2.5 (0.3)	2.4 (0.4)	n.s.

Type-II collagen immunoreactivity in coronal sections of the lateral tibial plateau was compared with immunoreactivity of the ovine transitional zone, used as a positive control. Immunoreactivity was scored as follows: 0: no immunoreactivity (as subchondral bone); 1: weaker immunoreactivity; 2: similar immunoreactivity; 3: stronger immunoreactivity (always compared with the positive control). Shown are the original values and *P* values. Data are given as mean ± SD

n.s. not statistically significant

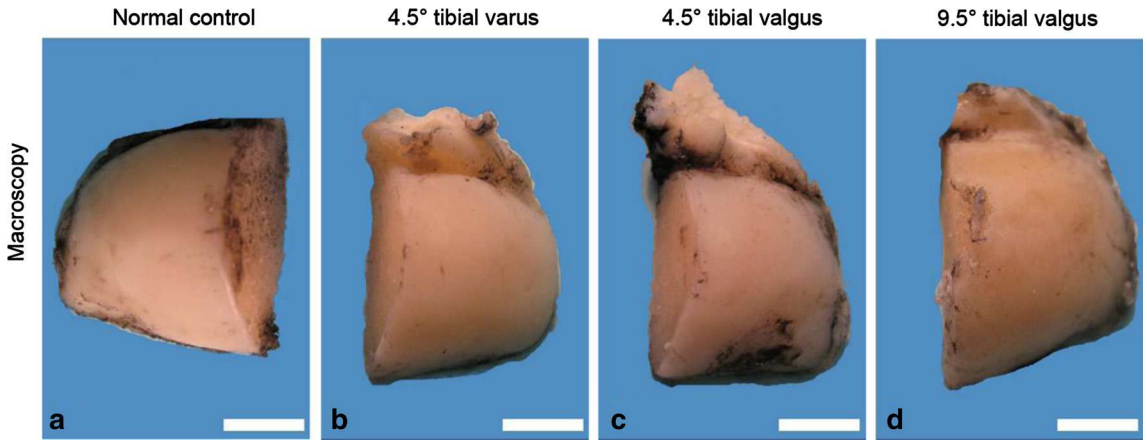

Fig. 8 Macroscopic view of the lateral femoral condyles stained with India ink. Semiquantitative grading revealed no significant differences between the three osteotomy groups. *Scale bars* 5 mm

Table 4 Microscopic analysis of the lateral femoral condyle according to the OARSI score

	4.5° tibial varus			4.5° tibial valgus			9.5° tibial valgus		
	Unloaded control			Standard correction			Overcorrection		
	Control	HTO	*P* value	Control	HTO	*P* value	Control	HTO	*P* value
Structure	0.0 (0.0)	0.0 (0.0)	n.s.	1.0 (1.4)	0.0 (0.0)	n.s.	0.0 (0.0)	0.0 (0.0)	n.s.
Chondrocyte density	0.0 (0.0)	0.0 (0.0)	n.s.	0.5 (0.7)	0.0 (0.0)	n.s.	0.0 (0.0)	0.0 (0.0)	n.s.
Cell cloning	0.0 (0.0)	0.0 (0.0)	n.s.	0.0 (0.0)	0.0 (0.0)	n.s.	0.0 (0.0)	0.0 (0.0)	n.s.
Safranin O staining	0.0 (0.0)	0.0 (0.0)	n.s.	0.5 (0.7)	0.3 (0.4)	n.s.	1.0 (0.0)	1.5 (0.7)	n.s.
Tidemark	0.5 (0.7)	1.0 (0.0)	n.s.	0.5 (0.7)	0.3 (0.4)	n.s.	1.0 (0.0)	1.0 (0.0)	n.s.
Total score	0.5 (0.3)	1.0 (0.0)	n.s.	2.5 (2.1)	0.5 (0.7)	n.s.	2.0 (0.0)	2.5 (0.3)	n.s.

Microscopic analysis of the articular cartilage of the lateral femoral condyle according to the OARSI score. Shown are the original values and *P* values. Data are given as mean ± SD. Normal cartilage receives a score of 0, maximal osteoarthritic cartilage a score of 25

n.s. not statistically significant

development of radiographic osteoarthritis exists for obese patients [6]. Moreover, malalignment is a risk factor for progression of early osteoarthritis [16, 31].

The overriding finding was that increased loading following both standard and overcorrection does not lead to differences in the macroscopic and microscopic appearance of the articular cartilage of the lateral tibial plateau and femoral condyle at 6 months postoperatively in the sheep model. Both standard and overcorrection were induced because the extent of the shift in the weightbearing axis remains unclear, especially with the advent of new plate fixators with high primary stability [3]. Of note, the ovine lateral tibial plateau is susceptible to osteoarthritis induced by lateral meniscectomy. Six months after meniscectomy—an identical time point as presented here—cartilage of both the lateral femoral condyles and the tibial plateaus showed the most pronounced osteoarthritic changes [27]. Also, the correlation of loss of cartilage volume with knee osteoarthritis is well established in patients [49–51]. Recently, no changes in the lateral articular cartilage were detected arthroscopically [57] and by delayed gadolinium-enhanced magnetic resonance imaging of cartilage after HTO [48, 52]. The lack of osteoarthritic changes and the similar chondrocyte densities in the lateral articular cartilage at 6 months postoperatively suggest that opening wedge HTO resulting in both standard and overcorrection is a safe procedure for the intact lateral articular cartilage without concomitant lateral meniscal lesions in a stable knee. In our previous study that focussed on the lateral meniscus, overcorrection significantly reduced cell numbers in the middle third of the red–red zone of the lateral menisci, but did not lead to morphological changes. In the context of the data from the present study, this first suggests that these minor changes were induced by overcorrection only, and that the resulting increased lateral contact pressure primarily leads to structural changes in the red–red zone of the lateral meniscus.

Second, these data indicate that such changes are not present in the cartilage of the lateral compartment. Placed in the focus of the previous data on the effect of open

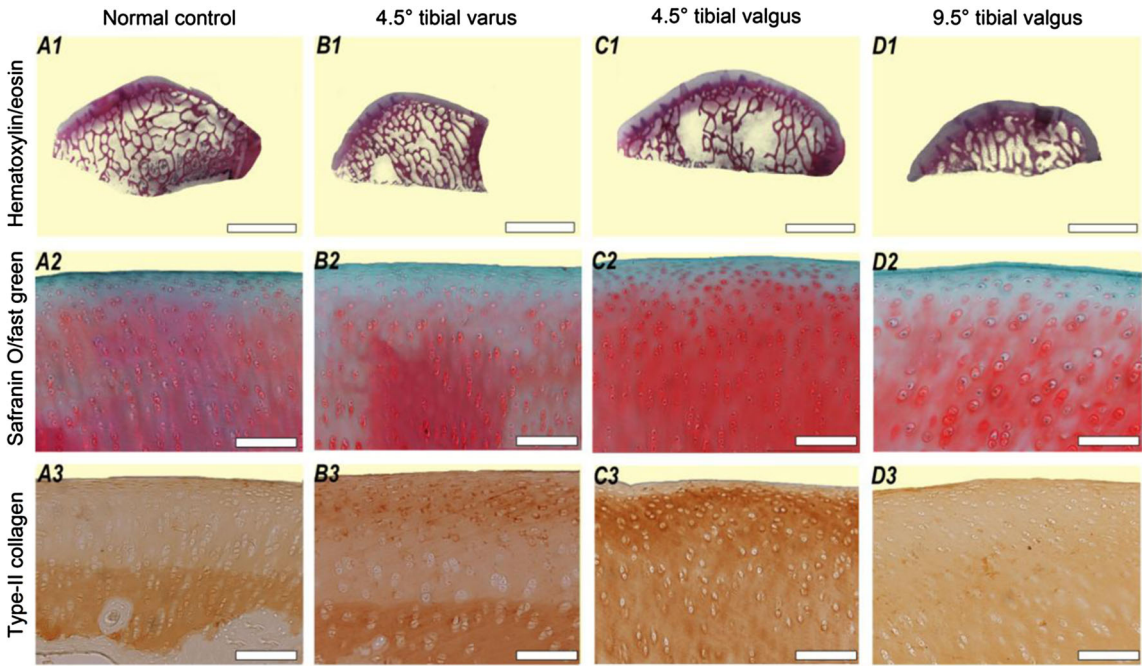

Fig. 9 Histological evaluation of changes in the lateral femoral condyles. Images **a1–d1** show coronal cross sections of the lateral femoral condyle whereas images **a2–d3** display magnified views. Haematoxylin/eosin staining (**a1–d1**) did not reveal variations in cell density or cellular organization between the three treatment groups (**b1–b3**, **c1–c3**, **d1–d3**) and the normal controls (**a1–a3**). According to safranin O/fast *green* staining (**a2–d2**), proteoglycan distribution (coloured in *red*) was unaffected by the change in limb axis and the resulting alteration of pressure load on the lateral tibiofemoral unit following the different tibial osteotomies. Likewise, none of the treatments provoked changes in the immunoreactivity to a monoclonal mouse anti-human type-II collagen IgG (**a3–d3**) in the lateral femoral condyle. *Scale bars* 5 mm (**a1–d2**) and 200 μm (**a1–d3**)

Table 5 Immunohistochemical analysis of type-II collagen of the lateral femoral condyle

	4.5° tibial varus			4.5° tibial valgus			9.5° tibial valgus		
	Unloaded control			Standard correction			Overcorrection		
	Control	HTO	*P* value	Control	HTO	*P* value	Control	HTO	*P* value
Surface zone	2.0 (0.0)	2.0 (0.0)	n.s.	2.0 (0.0)	2.0 (0.0)	n.s.	2.0 (0.0)	1.9 (0.2)	n.s.
Deeper zones	2.0 (0.0)	2.0 (0.0)	n.s.	2.0 (0.0)	2.0 (0.0)	n.s.	2.0 (0.0)	2.0 (0.0)	n.s.
Average total score	2.0 (0.0)	2.0 (0.0)	n.s.	2.0 (0.0)	2.0 (0.0)	n.s.	2.0 (0.0)	2.0 (0.1)	n.s.

Type-II collagen immunoreactivity in coronal sections of the lateral femoral condyle was compared with immunoreactivity of the ovine transitional zone, used as a positive control. Immunoreactivity was scored as follows: 0: no immunoreactivity (as to subchondral bone); 1: weaker immunoreactivity; 2: similar immunoreactivity; 3: stronger immunoreactivity (always compared with the positive control). Shown are the original and *P* values. Data are given as mean ± SD

n.s. not statistically significant

wedge HTO on the ovine lateral meniscus [32], and on the reported accelerated degeneration of discoid lateral menisci in patients [21], the alterations of the cartilage seen in the present study are comparably less pronounced. This is in line with the data on compressive stresses in the tibia of porcine knees in neutral, varus, and valgus alignments [15], and suggests that the articular cartilage contributes less to transmit loads than the meniscus. Since, from the clinical standpoint, effects of lateral meniscal lesions are much more severe than in the medial compartment [19, 55], the intact lateral meniscus appears to be more resilient. Future studies are needed to shed more light on the effects of HTO when meniscal [21] or articular cartilage defects [42] are present in the lateral compartment. Limitations of this study include the choice of quadruped sheep that has a different knee resting position in flexion and a smaller range of motion without full extension [2], resulting in a different loading of the sheep knee in about 35° extension

deficit [47], while human knees are loaded in full extension [9], the lack of a biochemical analysis, and the absence of correlating these findings with clinical parameters.

Altogether, the data broaden the scientific base to support HTO as an excellent option to therapeutically address medial tibiofemoral osteoarthritis in patients with varus malalignment.

Conclusion

Opening wedge HTO resulting in both standard and overcorrection is a safe procedure for the articular cartilage in an intact lateral tibiofemoral compartment of adult sheep at 6 months postoperatively.

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Conflict of interest None.

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III. **Ziegler R**, Goebel L, Seidel R, Cucchiarini M, Pape D, Madry H. Effect of open wedge high tibial osteotomy on the lateral tibiofemoral compartment in sheep. Part III: analysis of the microstructure of the subchondral bone and correlations with the articular cartilage and meniscus. Knee Surg Sports Traumatol Arthrosc. 2015; 23(9):2704-14.

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EXPERIMENTAL STUDY

Effect of open wedge high tibial osteotomy on the lateral tibiofemoral compartment in sheep. Part III: analysis of the microstructure of the subchondral bone and correlations with the articular cartilage and meniscus

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Abstract
Purpose First, to evaluate whether medial open wedge high tibial osteotomy (HTO) induces alterations of the microstructure of the lateral tibial subchondral bone plate of sheep. Second, to test the hypothesis that specific correlations exist between topographical structural alterations of the subchondral bone, the cartilage and the lateral meniscus.
Methods Three experimental groups received biplanar osteotomies of the right proximal tibiae: (a) closing wedge HTO (4.5° of tibial varus), (b) opening wedge HTO (4.5° tibial valgus; standard correction) and (c) opening wedge HTO (9.5° of valgus; overcorrection), each of which was compared to the non-osteotomised contralateral proximal tibiae. After 6 months, subchondral bone structure indices were measured by computed tomography. Correlations between the subchondral bone, the articular cartilage and the lateral meniscus were determined.
Results Increased loading by valgus overcorrection led to an enlarged specific bone surface (BS/BV) in the subarticular spongiosa compared with unloading by varisation. The subchondral bone plate was 3.9-fold thicker in the central region of the lateral tibial plateau than in the submeniscal periphery. Its thickness in the central region significantly correlated with the thickness of the articular cartilage. In the submeniscal region, such correlation did not exist. In general, a higher degree of osteoarthritis (OA) correlated with alterations of the subchondral bone plate microstructure. OA of the submeniscal articular cartilage also correlated with worse matrix staining of the lateral meniscus.
Conclusion Osteoarthritis changes are associated with alterations of the subchondral bone plate microstructure. Specific topographical relationships exist in the central region between the articular cartilage and subchondral bone plate thickness, and in the submeniscal periphery between and the articular cartilage and lateral meniscus. From a clinical perspective, the combined follow-up data from this and the previous two investigations suggest that open wedge valgus HTO is a safe procedure for the lateral compartment to manage medial osteoarthritis of the knee with varus malalignment in the short term.

Keywords HTO · Subchondral bone · Articular cartilage · Tibial plateau · Osteoarthritis · Meniscus · Histology

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Introduction

High tibial osteotomy (HTO) is an excellent option [10, 16, 35] to address medial tibiofemoral osteoarthritis (OA) in patients with varus malalignment [15, 22]. Preceding work investigated the effect of both increased and decreased loading on the lateral tibiofemoral compartment with a focus on the meniscus [20] and the cartilage [38] in a preclinical model [32] in vivo. These studies showed that valgus standard and overcorrection do not cause lateral articular cartilage degeneration [38] and did not induce significant structural changes in the lateral meniscus after 6 months [20].

The subchondral bone plays an important role in joint homeostasis [17, 19]. It is involved at an early stage [18] in the development of OA [34], with changes occurring at the onset of cartilage degradation [1]. Moreover, meniscal lesions are associated subchondral bone and articular cartilage alterations, underscoring the importance to study possible interactions [11]. From a clinical perspective, it is important to know whether HTO is safe procedure for the subchondral bone of the lateral compartment, as possible effects of both increased or decreased loading remain largely unknown. This study therefore first evaluated whether medial open wedge HTO induces alterations of the microstructure of the subchondral bone plate of sheep. Second, the hypothesis was tested that specific and significant correlations exist between topographical structural changes in the subchondral bone, the articular cartilage and the meniscus.

Materials and methods

As reported before in detail [20], 19 skeletally mature Merino sheep were randomly distributed to three experimental groups receiving biplanar [31] osteotomies: (a) closing wedge HTO resulting in 4.5° of tibial varus (unloaded control, $n = 5$), (b) opening wedge HTO resulting in 4.5° of tibial valgus (standard correction, $n = 5$) and (c) opening wedge HTO resulting in 9.5° of valgus (overcorrection, $n = 9$) in the right hind limbs. Using a bilateral research design, the contralateral left knees served as internal controls [30]. Postoperatively, all animals were immediately allowed full weight-bearing with no cage restrictions. Six months postoperatively, morphological characteristics of the subchondral bone plate and the subarticular spongiosa of the lateral tibial plateau were assessed using computed tomography (CT) and correlated with the previously established grade of osteoarthritis (OA) in the cartilage [38] and changes of the meniscus [20].

Animal experiments and histological analysis

Animal experiments [7, 20] and histological analysis employing Goldner's trichrome staining to sections of the lateral tibial plateaus [27, 38] were essentially conducted as described previously. Experiments were approved by the Landesamt für Verbraucherschutz, Freistaat Thüringen, Germany (ID number of the approval: 14-005/08).

Image acquisition by a computed tomography (CT) scanner

All left and right tibial plateaus (19 animals, 38 tibial plateaus) were analysed with a Philips Brilliance 16-slice computed tomography (CT) scanner (Philips, Eindhoven, The Netherlands). For the acquisition of the 16-bit X-ray shadow transmission images (approximately 150 images/specimen), the tube voltage was set at 120 kV and the current was set at 200 mA. As kernel filter, an edge-enhancing ultra-high filtering was applied. Coronal images were created with an in-plane matrix size of $1{,}024 \times 1{,}024$ pixels and a field of view of 6×6 cm^2 resulting in an in-plane spatial resolution of 58.5×58.5 μm^2. Slice thickness was set to 0.65 mm. Image stacks were then exported as "dicom" archives for further evaluation. Further image analysis was performed using the Skyscan "CT Analyser" software version 1.9.3.0 (Bruker microCT, Kontich, Belgium), developed to analyse μCT images. All "dicom" archives were converted to ".bmp" image stacks to enable processing of the images with the "CT Analyser" software. Thresholding levels of grey values were set to 100 and 255 for segmentation of binary images.

Standardisation of volumes of interest

To reproducibly evaluate the subchondral bone plate and the subarticular spongiosa, two standardised volumes of interest (VOI) were defined for the lateral tibial plateau (Fig. 1): the VOI "subchondral bone plate" contains exclusively the whole subchondral bone plate of the lateral tibial plateau from the lateral rim to the intercondylar eminence, while "subarticular spongiosa" was located distal to the VOI "subchondral bone plate". A safety distance of several voxels was adhered to in order to preclude a possible overlapping of the VOIs. The lower end of the VOI was defined through the growth plate. Regions with sclerotic changes caused by the drill holes from the fixation screws of the medially located osteotomy plates were always excluded without affecting the analysis. For each VOI, 30 consecutive images were analysed.

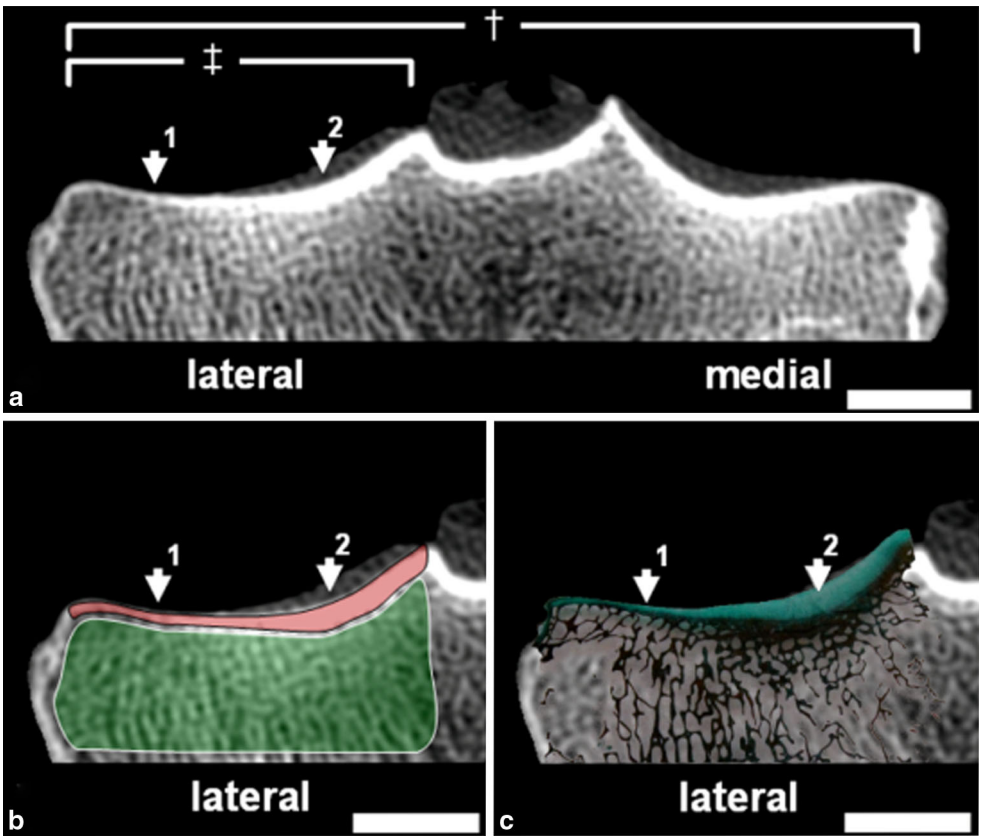

Fig. 1 Representative coronal CT images of a sheep tibial plateau. **a** The total tibial plateau width(*dagger*) and the width of the lateral tibial plateau (*double dagger*) are indicated. *Numbered arrows* (**a**–**c**) show the position where the thickness of the peripheral (submeniscal) subchondral bone plate (*arrowhead 1*) and the thickness of the central subchondral bone plate (without meniscal coverage; *arrowhead 2*) were measured. **b** Standardised regions of interest for the evaluation of subchondral bone changes in the lateral tibial plateau following HTO. Two standardised volumes of interest (VOI) were defined on CT images. Red-shaded area indicates the VOI "subchondral bone plate", and green-shaded area indicates the VOI "subarticular spongiosa". **c** Presents a CT image merged with an image of a histological section stained with Goldner's trichrome of the lateral sheep tibial plateau, demonstrating the topographical relationship between subchondral bone plate and articular cartilage thickness. *Scale bar* 10 mm

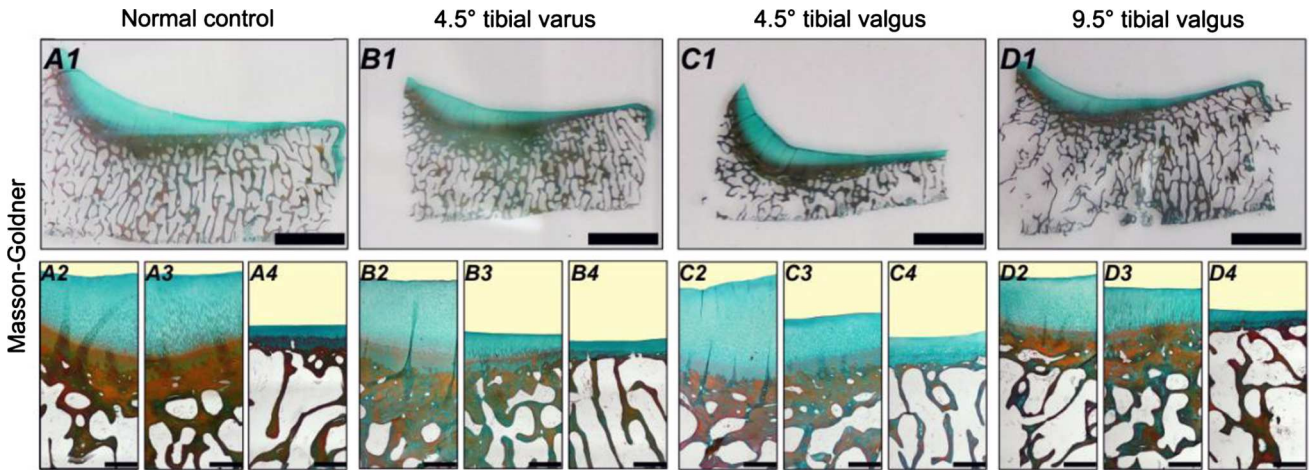

Fig. 2 Histological evaluation of lateral tibial plateaus (*left* side of each picture: intercondylar eminence; *right* side of each picture: submeniscal region). Images **a1**–**d1** show coronal cross sections of the entire lateral tibial plateau. Images below display magnified views of the eminentia (**a2**, **b2**, **c2**, **d2**), the (not meniscus covered) central (**a3**, **b3**, **c3**, **d3**) and the peripheral submeniscal region (**a4**, **b4**, **c4**, **d4**) of the lateral tibial plateau. Goldner's trichrome staining did not reveal qualitative differences between the three treatment groups (**b1**–**b4**, **c1**–**c4**, **d1**–**d4**) and the normal controls (**a1**–**a4**). *Scale bars* 5 mm (**a1**–**d1**) and 500 μm (**a2**–**d4**)

CT analysis of bone structure indices

Following the definition of a total of 74 VOIs, the following bone structure indices were determined: for "subchondral bone plate", per cent bone volume (BV/TV, %); specific bone surface/volume ratio (BS/BV, 1/mm); bone surface density (BS/TV, 1/mm); and cortical thickness (Ct.Th, mm). For the VOI "subarticular spongiosa", per cent bone volume (BV/TV, %); specific bone surface/volume ratio (BS/BV, 1/mm); bone surface density (BS/TV, 1/mm); trabecular thickness (Tb.Th, mm); trabecular separation (Tb.Sp, mm); trabecular pattern factor (Tb.Pf, 1/mm); trabecular number (Tb.N, 1/mm); structure model index (SMI, -/-); degree of anisotropy (DA, -/-); and fractal dimension (FD, -/-) were defined. Additionally, the thickness of the subchondral bone plate at 2 distinct points, the width of the lateral as well as the entire tibial plateau were measured. In 3 representative coronal CT images, the lateral tibial plateau was indexed at 25 and 75 % of the lateral tibial plateau width (measured from its lateral end) and the thickness of the subchondral bone plate was measured. The 25 % measurement corresponds to region of the tibial plateau covered by the lateral meniscus (submeniscal peripheral region), while the 75 % measurement (central region) complies with the tibial plateau without meniscal coverage. Comparisons were performed between the different treatment groups and between a treatment group and its untreated internal control.

Correlation analysis

Data sets were correlated with the corresponding data. In detail, bone structure indices of the subchondral bone plate and subarticular spongiosa, as well as the thickness of the submeniscal and central region of the subchondral bone plate, as determined by CT, were correlated with the data from the semiquantitative histological scoring systems developed by Little et al. [13] and Mankin et al. [21] and with the cartilage thickness. According to the graduation of the subchondral bone plate, the cartilage thickness was determined on histological sections at 25 and 75 % of the width of the lateral tibial plateau, measured from its lateral end. Cartilage thickness at these two points was also correlated with the data from the Little and Mankin scores (Fig. 1).

Statistical analysis

Data are expressed as mean value ± standard deviation (95 % confidence interval). Sample size calculation was based according to a previous statistical investigation [30]. For comparison of bone structure indices between different treatment groups, the Mann–Whitney U test was applied. The Wilcoxon signed-rank test was used for comparisons between treatment group and its internal control. Spearman's ρ was applied for correlation between CT and histological parameters. P values <0.05 were considered statistically significant. All calculations were made with SPSS (version 20.0, SPSS Inc, Chicago, Illinois, USA) or XLSTAT (version 2012.5.01; Addinsoft, Brooklyn, New York, USA) and Excel 2007 (Microsoft, Redmond, Washington, USA).

Results

Analysis of the subchondral bone microstructure of the different HTO groups

The subchondral bone plate was 3.9-fold thicker in the central region than in the submeniscal periphery of a normal lateral tibial plateau ($P < 0.001$; Figs. 2, 3). There were no significant differences between thickness of the subchondral bone plate in the central and the submeniscal region between the different treatment groups. Analysis of the thickness of the subchondral

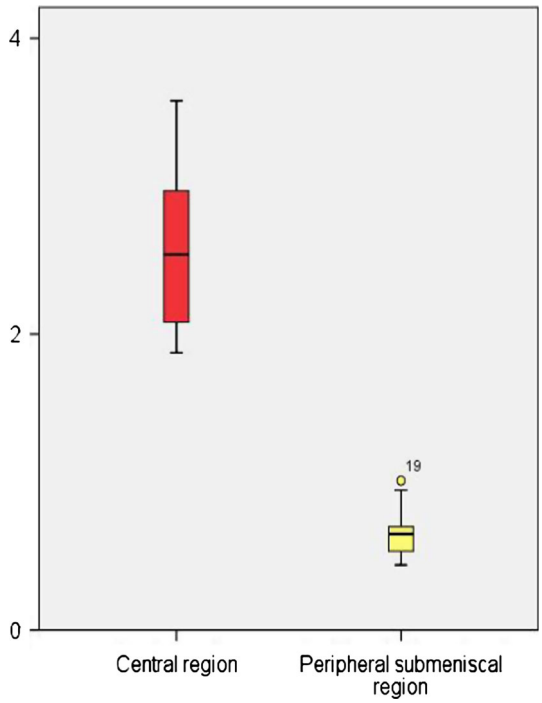

Fig. 3 Boxplot of the thickness (mm) of the subchondral bone plate of the lateral tibial plateau of the *left* knees. *Red box* central region (i.e. not covered by the lateral meniscus). *Yellow box* peripheral (submeniscal) region. *Bottom* and *top* of the boxes show the 25th and 75th percentile, and the median is shown as a *black band* in the *box*. Whiskers are defined as the lowest value still within 1.5 interquartile range (IQR) of the lower quartile, and the highest value still within 1.5 IQR of the upper quartile. *$P < 0.001$. Unit of the y axis = mm

Table 1 CT morphological subchondral bone parameters of the lateral tibial plateau

	4.5° tibial varus			4.5° tibial valgus			9.5° tibial valgus		
	Unloaded control			Standard correction			Overcorrection		
	Control	HTO	*P* value	Control	HTO	*P* value	Control	HTO	*P* value
Entire tibial plateau									
Entire width (mm)	49.3 (7.9)	50.3 (2.2)	n.s.	52.9 (1.2)	52.7 (1.7)	n.s.	52.4 (3.3)	51.3 (3.0)	n.s.
Width of the lateral tibial plateau (mm)	22.0 (2.7)	21.3 (2.4)	n.s.	22.2 (1.3)	20.7 (3.2)	n.s.	21.6 (2.7)	22.4 (1.9)	n.s.
Subchondral bone plate									
BV/TV (%)	99.6 (0.2)	99.4 (0.5)	n.s.	98.6 (1.8)	99.7 (0.2)	n.s.	99.3 (0.5)	99.5 (0.4)	n.s.
BS/BV (1/mm)	4.6 (0.4)	4.7 (0.6)	n.s.	4.9 (2.2)	4.2 (0.7)	n.s.	4.3 (0.9)	4.2 (1.1)	n.s.
BS/TV (1/mm)	4.6 (0.4)	4.7 (0.6)	n.s.	4.8 (2.0)	4.2 (0.7)	n.s.	4.3 (0.9)	4.2 (1.0)	n.s.
Ct.Th, (mm)	0.9 (0.2)	0.9 (0.2)	n.s.	1.0 (0.3)	1.0 (0.2)	n.s.	1.1 (0.2)	1.0 (0.2)	n.s.
Central thickness	2.9 (0.4)	2.4 (0.8)	n.s.	2.6 (0.8)	2.3 (0.5)	n.s.	2.4 (0.4)	2.3 (0.4)	n.s.
Peripheral thickness (submeniscal)	0.8 (0.1)	0.7 (0.1)	n.s.	0.6 (0.1)	0.7 (0.1)	n.s.	0.6 (0.1)	0.7 (0.1)	n.s.
Subarticular spongiosa									
BV/TV (%)	93.8 (4.9)	88.6 (5.9)	n.s.	82.1 (6.0)	85.3 (9.3)	n.s.	79.6 (7.1)	80.4 (10.5)	n.s.
BS/BV (1/mm)	3.3 (0.8)	4.0 (0.9)*	n.s.	4.4(0.7)	4.4(1.1)	n.s.	5.2 (1.1)	5.3 (1.0)*	n.s.
BS/TV (1/mm)	3.0 (0.6)	3.5 (0.6)	n.s.	3.6 (0.3)	3.7 (0.5)	n.s.	4.1 (0.6)	4.2 (0.5)	n.s.
Tb.Pf (1/mm)	−4.2 (2.4)	−4.8 (0.6)	n.s.	−4.3 (1.3)	−3.7 (0.7)	n.s.	−3.6 (0.9)	−3.8 (1.4)	n.s.
SMI	−2.6 (1.9)	−2.2 (0.9)	n.s.	−2.0 (1.1)	−1.6 (0.3)	n.s.	−1.3 (0.6)	−1.3 (0.8)	n.s.
Tb.Th (mm)	1.2 (0.2)	1.0 (0.2)	n.s.	0.9 (0.1)	0.9 (0.2)	n.s.	0.8 (0.2)	0.8 (0.2)	n.s.
Tb.N (1/mm)	0.8 (0.1)	1.0 (0.2)	n.s.	0.9 (0.1)	1.0 (0.2)	n.s.	1.1 (0.1)	1.1 (0.2)	n.s.
Tb.Sp (mm)	0.2 (0.0)	0.3 (0.0)	n.s.	0.3 (0.0)	0.3 (0.0)	n.s.	0.3 (0.0)	0.3 (0.1)	n.s.
DA	0.8 (0.1)	0.8 (0.1)	n.s.	0.7 (0.1)	0.8 (0.1)	n.s.	0.7 (0.1)	0.8 (0.1)	0.016
FD	2.3 (0.1)	2.3 (0.1)	n.s.	2.4 (0.1)	2.3 (0.1)	n.s.	2.4 (0.1)	2.4 (0.1)	n.s.

Shown are the original values and the *P* values. Data are given as mean ± standard deviation. Comparison of the different HTO groups reveals a significant difference in the BS/BV of the subarticular spongiosa between the unloaded control and the overcorrected group (* $P = 0.03$)

n.s. not statistically significant

bone plate at 25 % (submeniscal) and 75 % (central) width of the lateral tibia did not reveal significant differences between HTO and contralateral knees and between HTO groups (Table 1).

In the subchondral bone plate, comparison of HTO with contralateral knees and between the different HTO groups did not reveal significant differences in bone structure indices. In the subarticular spongiosa, an increased loading by valgus overcorrection led to a 1.33-fold significantly increased specific bone surface (BS/BV) compared with unloading by varisation ($P = 0.03$; Table 1).

Correlation of the thickness of the subchondral bone plate with the thickness of the articular cartilage

The thickness of the subchondral bone plate correlated significantly with the thickness of the articular cartilage at the central region of the lateral tibial plateau ($P = 0.02$; Table 2; Fig. 3). No significant correlation between the thickness of the subchondral bone plate and the thickness of the cartilage existed in the submeniscal (peripheral) region of the lateral tibial plateau ($P < 0.05$; Table 2; Fig. 3).

Table 2 Correlation of the thickness of the subchondral bone plate with the thickness of the articular cartilage of the lateral tibial plateau in the peripheral (submeniscal) and the central region

	Thickness of the subchondral bone plate			
	Central region		Peripheral (submeniscal) region	
	ρ	*P*	ρ	*P*
Thickness of the articular cartilage				
Central region	0.388	0.020	–	–
Peripheral (submeniscal) region	–	–	0.213	n.s.

Shown are the Spearman ρ coefficients and the *P* values

n.s. not statistically significant

Table 3 Correlations of the regional [i.e. central and peripheral (submeniscal) region] Mankin and the Little score with the regional subchondral bone plate and articular cartilage thickness of the lateral tibial plateau

	Mankin score										Little score									
	Structure		Chondrocytes		Safranin O Staining		Tidemark		TOTAL		Structure		Chondrocyte density		Cell cloning		Matrix staining		Tidemark		TOTAL	
	ρ	P	ρ	P	ρ	P	ρ	P	ρ	P	ρ	P	ρ	P	ρ	P	ρ	P	ρ	P	ρ	P
Peripheral (submeniscal) region																						
Subchondral bone plate	0.02	n.s.	0.042	n.s.	0.154	n.s.	0.199	n.s.	0.199	n.s.	0.024	n.s.	0.042	n.s.	0.196	n.s.	0.196	n.s.	0.311	n.s.	0.283	n.s.
Articular cartilage	−0.105	n.s.	−0.211	n.s.	−0.055	n.s.	−0.115	n.s.	−0.156	n.s.	−0.1	n.s.	−0.211	n.s.	−0.152	n.s.	−0.075	n.s.	0.157	n.s.	−0.012	n.s.
Central region																						
Subchondral bone plate	−0.024	n.s.	0.022	n.s.	0.038	n.s.	−0.099	n.s.	0.002	n.s.	0.105	n.s.	0.022	n.s.	0.000	n.s.	0.144	n.s.	−0.005	n.s.	0.131	n.s.
Articular cartilage	0.306	n.s.	0.057	n.s.	−0.241	n.s.	−0.36	0.032	0.078	n.s.	0.27	n.s.	0.057	n.s.	0.000	n.s.	−0.343	0.041	−0.491	0.003	−0.066	n.s.

Shown are the Spearman ρ coefficient and the P values

n.s. not statistically significant

Correlation of the thickness of the subchondral bone plate and the articular cartilage with osteoarthritis

No correlation existed between the thickness of the subchondral bone plate of both the submeniscal and the central lateral tibial plateau and the histological parameters of OA. Correlation of articular cartilage thickness in the central region of the lateral tibial plateau with OA changes revealed a significant relationship between loss of matrix staining in the Little score and tidemark doubling in both Mankin and Little scores (Table 3). No correlation existed in the region where the lateral tibial plateau is covered by the meniscus.

Correlation of subchondral bone structure with osteoarthritic cartilage changes

For the subchondral bone plate, a significant association between the parameters "structure" and "total osteoarthritis score" of both the Little and Mankin scoring systems and Ct.Th was revealed by correlation analysis. An inverse correlation was found between these histological parameters and BS/BV and BS/TV (Tables 4, 5).

In the subarticular spongiosa, the parameter "chondrocytes" of the Mankin score and the parameters "chondrocyte density" and "cell cloning" of the Little score significantly correlated with the parameter Tb.Sp. and DA. An inverse relationship was found for BV/TV and "chondrocytes" of Mankin, respectively, "chondrocyte density" of the Little score (Tables 4, 5).

Correlation of changes in the submeniscal articular cartilage with changes of the lateral meniscus

Correlation analysis of the parameter "total OA score" of the Mankin scoring system of the submeniscal region and proteoglycan and proteoglycan/DNA content of the lateral meniscus did not reveal significant correlations (Data not shown). Correlation analysis of changes in the submeniscal articular cartilage with changes of the lateral meniscus revealed a significant association between the parameters "total OA score" of the Mankin scoring system and "matrix staining" of the lateral meniscus. No correlations were seen between other parameters (Table 6).

Discussion

The most important finding of the present study was that neither varus nor valgus (both standard and over) correction led to alterations in subchondral bone plate thickness in an otherwise stable knee in sheep. Likewise, increased or decreased loads, even following valgus overcorrection, did not influence the width of the lateral tibial plateau after

Table 4 Overview of the significant ($P < 0.05$) correlations of the histological scoring (Mankin and OARSI score) with the CT parameters of the subchondral bone plate and the subarticular spongiosa of the lateral tibial plateau

	Mankin score					Little score					
	Structure	Chondrocytes	Safranin O staining	Tidemark	Total	Structure	Chondrocyte density	Cell cloning	Matrix staining	Tidemark	Total
Subchondral bone plate	BS/BV BS/TV Ct. Th.	n.s.	n.s.	n.s.	BS/BV BS/TV Ct. Th.	BS/BV BS/TV Ct. Th.					BS/BV BS/TV Ct. Th.
Subarticular spongiosa	n.s.	BV/TV Tb.Sp. DA	n.s.	n.s.	n.s.	n.s.	BV/TV Tb.Sp. DA	Tb.Sp DA	n.s.	n.s.	n.s.

Shown are the significant parameters

n.s. not statistically significant

6 months. The data show that an increased loading by valgus overcorrection led to an enlarged specific bone surface (BS/BV) in the subarticular spongiosa compared with unloading by varisation. Interestingly, the subchondral bone plate was 3.9-fold thicker in the central region of the lateral tibial plateau than in the submeniscal periphery, and its thickness in the central region significantly correlated with the thickness of the articular cartilage. Of note, a higher degree of osteoarthritis (OA) correlated with alterations of the subchondral bone plate microstructure. Finally, OA of the submeniscal articular cartilage also correlated with worse matrix staining of the lateral meniscus.

Increased loading of the lateral tibiofemoral compartment resulting from valgus overcorrection led to a 1.33-fold increase in the specific bone surface of the lateral tibial subarticular spongiosa compared with unloading induced by varisation, while the subchondral bone plate remained unaffected by such changes. The bone surface to volume ratio is a measure for the bone surface per given bone volume and is also reflective of the number of bone lining cells that cover a defined volume of bone. The significantly larger bone surface to volume ratio in the subarticular spongiosa of the lateral compartment following overcorrection indicates that the trabecular subchondral bone primarily adapts to the increase in load by enhancing its specific bone surface. Interestingly, it also suggests that the adaptation of the subchondral bone to increased load occurs in the subarticular spongiosa and not in the relatively thick and dense subchondral bone plate in this model. Such adaptation of the subchondral bone to differences in loading was also found in investigations [24] performed after HTO in patients with varus malalignment, demonstrating a reversal of previously pathological density patterns [23].

The present data revealed—independently from the treatment groups—that a higher degree of OA was always accompanied by a lower bone surface/bone volume and specific bone surface/total volume ratio, as well as a higher cortical thickness in the subchondral bone plate. Whether this association of articular cartilage damage with a thickening and a more porous structure of the subchondral bone plate are primarily caused by changes in the articular cartilage or subchondral bone remains unknown. The correlations detected here suggest a linear association between the two individual variables from the subchondral bone, articular cartilage and meniscus. Recently, alterations of the subarticular spongiosa microarchitecture were shown to provoke early osteoarthritis in the femoral heads of adult rabbits [28]. A rabbit medial meniscectomy model highlights the early involvement of the subchondral bone in OA development, when changes of the subchondral bone and bone mineral density (BMD) occur as early as cartilage deterioration begins [1]. Interestingly, the Multicenter OA Study (MOST) identified an association of cartilage loss with attrition of the subchondral bone within the same subregion of a knee [25]. In other clinical studies, a significant association between increase in the loss of lateral tibial cartilage volume was found to be associated with a valgus deformity [4] and that malalignment is associated with the development and progression of radiographic knee OA [3, 36]. Taken as a whole, these data allow to better identify patterns of subchondral bone, articular cartilage and meniscus adaptations and early OA development in the lateral tibiofemoral joint [37] and highlight the important role of the tibial subchondral bone as an active component capable of adapting to the different requirements. These findings also substantiate the close relationship between subchondral bone changes and OA [8, 33].

Table 5 Correlations of the histological scoring (Mankin and OARSI score) with the CT parameters of the subchondral bone plate and the subarticular spongiosa of the lateral tibial plateau

	Mankin score										Little score									
	Structure		Chondrocytes		Safranin O staining		Tidemark		Total		Structure		Chondrocyte density		Cell cloning		Matrix staining		Tidemark		Total	
	ρ	P	ρ	P	ρ	P	ρ	P	ρ	P	ρ	P	ρ	P	ρ	P	ρ	P	ρ	P	ρ	P
Subchondral bone plate																						
BV/TV (%)	0.354	0.029	0.14	n.s.	−0.031	n.s.	0.153	n.s.	0.233	n.s.	0.328	0.045	0.14	n.s.	0.194	n.s.	−0.022	n.s.	0.1	n.s.	0.225	n.s.
BS/BV (1/mm)	−0.474	0.003	−0.027	n.s.	−0.216	n.s.	−0.123	n.s.	−0.467	0.003	−0.435	0.006	−0.027	n.s.	−0.13	n.s.	−0.284	n.s.	−0.179	n.s.	−0.452	0.004
BS/TV (1/mm)	−0.472	0.003	−0.022	n.s.	−0.226	n.s.	−0.122	n.s.	−0.473	0.003	−0.434	0.006	−0.022	n.s.	−0.125	n.s.	−0.298	n.s.	−0.176	n.s.	−0.455	0.004
Ct.Th (mm)	0.379	0.019	0.144	n.s.	0.274	n.s.	0.033	n.s.	0.442	0.005	0.4	0.013	0.144	n.s.	0.205	n.s.	0.301	n.s.	0.153	n.s.	0.447	0.005
Subarticular spongiosa																						
BV/TV (%)	0.038	n.s.	−0.433	0.009	0.183	n.s.	0.041	n.s.	0.141	n.s.	0.017	n.s.	−0.433	0.009	−0.255	n.s.	0.192	n.s.	−0.101	n.s.	0.018	n.s.
BS/BV (1/mm)	0.042	n.s.	0.31	n.s.	−0.208	n.s.	0.031	n.s.	−0.105	n.s.	0.045	n.s.	0.31	n.s.	0.19	n.s.	−0.225	n.s.	0.02	n.s.	−0.045	n.s.
BS/TV (1/mm)	0.082	n.s.	0.12	n.s.	−0.152	n.s.	0.083	n.s.	−0.041	n.s.	0.071	n.s.	0.12	n.s.	0.061	n.s.	−0.167	n.s.	−0.049	n.s.	−0.05	n.s.
Tb.Pf (1/mm)	0.243	n.s.	0.249	n.s.	0.065	n.s.	−0.068	n.s.	0.197	n.s.	0.334	n.s.	0.249	n.s.	−0.013	n.s.	0.116	n.s.	0.117	n.s.	0.284	n.s.
SMI	0.207	n.s.	0.268	n.s.	0.05	n.s.	0.047	n.s.	0.195	n.s.	0.274	n.s.	0.268	n.s.	0.093	n.s.	0.074	n.s.	0.168	n.s.	0.266	n.s.
Tb.Th (mm)	−0.026	n.s.	−0.187	n.s.	0.132	n.s.	−0.044	n.s.	0.065	n.s.	−0.003	n.s.	−0.187	n.s.	−0.126	n.s.	0.148	n.s.	−0.009	n.s.	0.046	n.s.
Tb.N (1/mm)	0.033	n.s.	0.022	n.s.	−0.11	n.s.	0.137	n.s.	−0.035	n.s.	−0.011	n.s.	0.022	n.s.	0.056	n.s.	−0.135	n.s.	−0.055	n.s.	−0.087	n.s.
Tb.Sp (mm)	−0.048	n.s.	0.679	<0.0001	0.073	n.s.	−0.057	n.s.	0.074	n.s.	0.069	n.s.	0.679	< 0.0001	0.373	0.027	0.084	n.s.	0.33	n.s.	0.281	n.s.
DA	0.101	n.s.	0.364	0.032	−0.095	n.s.	−0.033	n.s.	0.017	n.s.	0.01	n.s.	0.364	0.032	0.389	0.021	−0.192	n.s.	−0.065	n.s.	−0.055	n.s.
FD	−0.106	n.s.	0.245	n.s.	−0.135	n.s.	−0.057	n.s.	−0.162	n.s.	−0.14	n.s.	0.245	n.s.	0.155	n.s.	−0.151	n.s.	0.02	n.s.	−0.117	n.s.

Shown are the Spearman ρ coefficient and the P values

n.s. not statistically significant

Table 6 Correlations of the histological scoring (Mankin and Little score) of the lateral tibial plateau with the histological scoring of the lateral meniscus

	Lateral tibial plateau																					
	Mankin score										Little score											
	Structure		Chondrocytes		Safranin O staining		Tidemark		Total		Structure		Chondrocyte density		Cell cloning		Matrix staining		Tidemark		Total	
	ρ	P	ρ	P	ρ	P	ρ	P	ρ	P	ρ	P	ρ	P	ρ	P	ρ	P	ρ	P	ρ	P
Lateral meniscus																						
Femoral surface	0.180	n.s.	−0.275	n.s.	−0.066	n.s.	−0.064	n.s.	−0.082	n.s.	0.180	n.s.	−0.275	n.s.	−0.303	n.s.	−0.066	n.s.	−0.108	n.s.	−0.161	n.s.
Surface tibia	0.147	n.s.	−0.251	n.s.	−0.263	n.s.	0.002	n.s.	−0.149	n.s.	0.147	n.s.	−0.251	n.s.	−0.010	n.s.	−0.192	n.s.	0.128	n.s.	−0.006	n.s.
Surface inner border	0.042	n.s.	−0.364	n.s.	−0.262	n.s.	0.311	n.s.	−0.203	n.s.	0.042	n.s.	−0.364	n.s.	−0.080	n.s.	−0.208	n.s.	0.016	n.s.	−0.058	n.s.
Cellularity	0.121	n.s.	−0.265	n.s.	−0.121	n.s.	0.320	n.s.	−0.009	n.s.	0.121	n.s.	−0.265	n.s.	0.100	n.s.	−0.078	n.s.	0.014	n.s.	−0.049	n.s.
Collagen organisation	−0.209	n.s.	0.076	n.s.	−0.075	n.s.	0.111	n.s.	−0.103	n.s.	−0.209	n.s.	0.076	n.s.	0.239	n.s.	−0.060	n.s.	0.090	n.s.	0.044	n.s.
Matrix staining	−0.401	n.s.	−0.333	n.s.	−0.439	n.s.	−0.139	n.s.	**−0.560**	0.011	−0.401	n.s.	−0.333	n.s.	−0.072	n.s.	−0.395	n.s.	−0.236	n.s.	−0.356	n.s.
Total score	0.110	n.s.	−0.381	n.s.	−0.314	n.s.	0.208	n.s.	−0.202	n.s.	0.110	n.s.	−0.381	n.s.	−0.101	n.s.	−0.238	n.s.	0.047	n.s.	−0.105	n.s.
Type I collagen immunoreactivity	0.291	n.s.	0.045	n.s.	−0.076	n.s.	0.066	n.s.	0.116	n.s.	0.291	n.s.	0.045	n.s.	−0.185	n.s.	−0.129	n.s.	−0.098	n.s.	−0.120	n.s.

Shown are the Spearman ρ coefficient and the P values

n.s. not statistically significant

Intriguingly, this study also identified specific topographical relationships between variables of the submeniscal and central regions of the lateral tibiofemoral compartment. First, the subchondral bone plate in the central region of a normal lateral tibial plateau in sheep is 3.9-fold thicker than the subchondral bone plate in the submeniscal periphery. Moreover, in the central region of the lateral tibial plateau, the thickness of the subchondral bone plate significantly correlated with the thickness of the articular cartilage. These findings are well in line with the previous finding of the topographical differences in the articular cartilage thickness [38].

In the lateral submeniscal region, the significantly thinner subchondral bone and cartilage attest to the important role of the lateral meniscus as a shock-absorber and load-transmitter [12, 26, 39]. Conversely, in the central region—where such a protective buffer is absent—the cartilage adapts by an expanded thickness. Of note, OA changes in the submeniscal region correlated with a worse matrix staining of the lateral meniscus after 6 months in vivo. In a Framingham study, meniscal damage was associated with increased local subchondral BMD in the same compartment [14]. These important findings highlight the vital role of the meniscus as a weight-absorbing buffer and its protective function [26] for both the articular cartilage and the subchondral bone plate [5, 12, 26, 39]. Moreover, damage of the meniscus tissue is highly associated with OA [2, 9].

Limitations of the present study include the use of a clinical CT (to analyse the entire ovine lateral tibial subchondral bone) compared to a micro CT (more appropriate to analyse small subregions of the ovine tibial subchondral bone [29]) and the general constraints of the quadruped sheep as a preclinical animal model. Strengths of the study are the separate assessment of the subchondral bone plate and subarticular spongiosa and of the topographically different regions of the tibial plateau, and the determination of correlations between key parameters of the subchondral bone, the articular cartilage and the meniscus.

From a clinical perspective, the combined follow-up data from this and previous studies [20, 38] suggest that open wedge valgus high tibial osteotomy applying a plate fixator [15] is a safe and well-established procedure to manage medial osteoarthritis of the knee with varus malalignment in the short term [6]. Extended follow-up using this experimental model will reveal its long-term outcome.

Conclusion

Opening wedge HTO resulting in both standard and overcorrection is a safe procedure for the lateral tibial subchondral bone at least in short-term follow-up. Cartilage

OA changes are associated with alterations of the subchondral bone plate microstructure. Specific topographical relationships exist in the central region between the articular cartilage and subchondral bone plate thickness, and in the submeniscal periphery between and the articular cartilage and lateral meniscus, underscoring the importance of their interactions.

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Conflict of interest None.

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7 Publikationen

7.1 Originalarbeiten

Ziegler R, Goebel L, Seidel R, Cucchiarini M, Pape D, Madry H. Effect of open wedge high tibial osteotomy on the lateral tibiofemoral compartment in sheep. Part III: analysis of the microstructure of the subchondral bone and correlations with the articular cartilage and meniscus. Knee Surg Sports Traumatol Arthrosc. 2015; 23(9):2704-14

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7.2 Übersichtsarbeiten

Madry H, **Ziegler R**, Pape D, Cucchiarini M. Strukturelle Veränderungen im lateralen femorotibialen Kompartiment nach Tibiakopfosteotomie. Orthopäde 2014; 43(11):958-65

7.3 Vorträge

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7.4 Posterpräsentationen

Goebel L, Pape D, **Sicks R**, Kohn D, Cucchiarini M, Madry H. Einfluss der Tibiakopfosteotomie bei bestehendem medialen femoralen Knorpeldefekt auf den Innenmeniskus im Schafmodell. Deutsche Gesellschaft für Orthopädie und Orthopädische Chirurgie (Berlin), 98. Tagung, 2012

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