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No detectable remodelling in adult human menisci: an analysis based on the C^{14} bomb pulse

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► Additional material is published online only. To view please visit the journal online (http://dx.doi.org/10.1136/bjsports-2019-101360).

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Accepted 21 February 2020
Published Online First 14 May 2020

To cite: Våben C, Heinemeier KM, Schjerling P, *et al*. *Br J Sports Med* 2020;**54**:1433–1437.

ABSTRACT

Objectives Bone and other human tissues remodel through life, for example, as a response to increasing load, and this prevents permanent destruction of the tissue. Non-traumatic meniscal rupture is a common musculoskeletal disease, but it is unknown if it is caused by inability of the menisci to remodel. The aim of this study was to determine whether meniscal collagen is remodelling throughout life.

Methods The life-long turnover of the human meniscal collagens was explored by the ^{14}C bomb pulse method. ^{14}C levels were determined in menisci from 18 patients with osteoarthritis and 7 patients with healthy knees.

Results There was a negligible turnover of the meniscal collagen in adults. This low turnover was observed in menisci from patients with knee osteoarthritis and in healthy menisci.

Conclusion This study provides evidence that essentially no remodelling occurs in the adult human meniscal collagen structure and explains the clinical degeneration that is often seen in menisci of middle-aged and elderly persons. It suggests that strengthening of the collagen structure of menisci, as response to physical activity, may occur during childhood, while it is not possible in the adult population.

INTRODUCTION

The human menisci are essential for weight distribution in the knee joint and preservation of the joint cartilage, and the architectural structure of the meniscus is basis for its weight-absorbing properties. Injury and degeneration of menisci are the most common symptomatic conditions of the knee and represent a significant economic burden for society and the individual patients.[1]

Several human tissues, like bone, possess the ability to remodel and strengthen as a response to increasing load. This strategy of remodelling prevents destruction of the tissue. Even though deterioration of the meniscal tissue (often termed non-traumatic meniscal rupture) is the most common musculoskeletal disease,[1] it is unknown if it is caused by inability of the menisci to remodel and thus prevent tissue deterioration.

The menisci have a very complex structure. They are semilunar fibrocartilages[2] with a skeleton of collagen. Seventy per cent of the dry weight is collagen and 90% of this is collagen type I. Furthermore, there are smaller amounts of collagen type II, III, V and VI.[2] The ring structure of collagen I, which attaches to the tibia at the anterior and posterior meniscal horns, is essential for the capacity of the menisci to transfer vertical load from femur to tibia. Glycosaminoglycans (GAGs) make up 2.5% of the meniscus dry weight[2] and are responsible for creating osmotic pressure inside the collagen meshwork of the menisci and thus for load distribution. Once the ring structure is weakened, this capacity is reduced, and a higher proportion of load is transferred directly between the femoral and tibial cartilages.

The content of GAGs and water is subject to constant adaption. For instance, these contents change temporarily during running as evaluated by MRI,[3] and they are also higher with knee osteoarthritis (OA) and with increasing age.[2 4 5] There are conflicting results regarding changes in the content of collagen in menisci with degenerative conditions.[2 4 6 7]

Remodelling is the active, cellular process of existing collagen being replaced by newly formed collagen. This involves degradation of the collagen structure, which is being replaced, and cellular production and deposition of new collagen fibrils in the extracellular environment. It is unknown if there is any ability of the meniscus to remodel the collagen meshwork during adult life and adapt to changing conditions, such as increasing body weight, or changes in physical activity. It is also unknown during which period of life any potential capacity to remodel the menisci is highest. This knowledge is relevant in relation to recommendations for physical activities in childhood, in adolescence and in the adult population.

The main aim of this study was to investigate whether the meniscal collagen matrix is remodelled throughout life. Based on a hypothesis that vascularisation may impact positively on the potential for collagen remodelling, we analysed the inner, non-vascularised parts of the meniscus and the capsular, vascularised parts from the medial and lateral menisci. Furthermore, menisci from healthy as well as osteoarthritic knees were analysed.

We used the radiocarbon bomb pulse dating method for the analyses. This method exploits the doubling of atmospheric ^{14}C in the 1960s due to the testing of nuclear bombs (figure 1). The consequence of the ^{14}C increase is that high levels of this isotope were introduced in all organic tissue being formed at that time.[8] By analysing the ^{14}C content of the meniscal collagen from patients born before, during and after the bomb pulse peak, it is possible to establish the pattern of collagen formation and remodelling in the meniscus during life. This method has previously been used in different tissues,[9–13] showing no turnover in the eye lens crystalline, hyaline cartilage collagen of the knee and

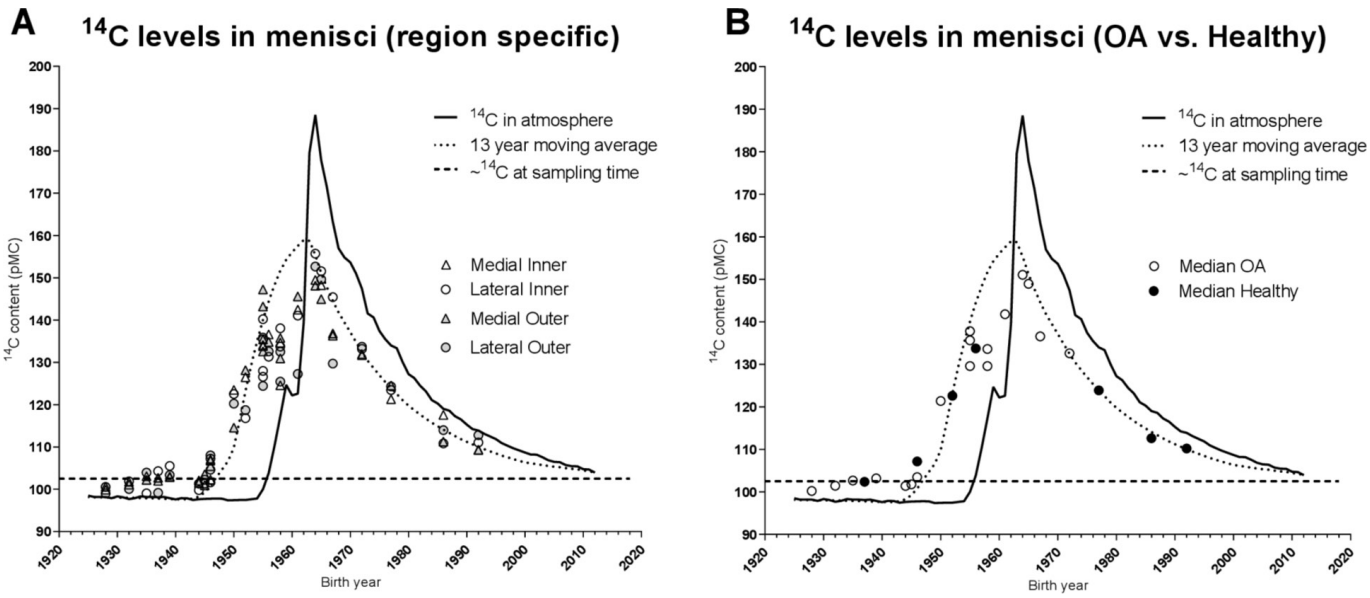

Figure 1 ^{14}C content in menisci: ^{14}C content in collagen purified from menisci in relation to birth year of donors. Solid black line shows the ^{14}C level in per cent modern carbon (pMC) in the atmosphere based on Kueppers *et al* up to 2001[25] and Levin *et al* from 2002.[26] The dotted line shows the 13-year moving average of the atmospheric ^{14}C level, with each point representing the average atmospheric ^{14}C level of the next 13 years. The horizontal dashed line represents the ^{14}C level in the atmosphere at time of tissue sampling. (A) ^{14}C levels in regional areas of menisci. (B) Median ^{14}C content of four meniscal regions from the same donors of OA and healthy menisci. Vertically aligned symbols represent data from the same individual (except for the data points in 1946 (two donors), 1953 (three donors) and 1958 (two donors). OA, osteoarthritis.

healthy Achilles tendon during adulthood[9 10 12] and a high turnover in fat and skeletal muscle.[10 13]

Secondary aims were to compare the contents of water, GAGs and collagen in menisci from OA and healthy knees, in medial and lateral menisci and the in the inner and outer parts of the menisci.

MATERIALS AND METHODS

Meniscus samples

Eighty-seven human knee menisci (44 medial and 43 lateral) were donated from patients with OA undergoing total knee arthroplasty at Department of Orthopedic Surgery, Bispebjerg and Frederiksberg Hospital, Denmark, between 2012 and 2016. The birth years of these patient were evenly spread from 1928 to 1972. 14 human knee menisci (7 medial and 7 lateral) were donated from patients with tumour undergoing bone resection and reconstruction with a knee tumour prosthesis at the Department of Orthopedic Surgery, Rigshospitalet, Denmark, during the same period. The cancer did not affect the menisci and the tibial plateau, and the knee joints were without any degenerative changes; therefore, we regard these menisci as healthy. The birth years of these patients were from 1937 to 1992. Thus, both healthy and OA menisci were obtained from donors born before, during and after the peak of the bomb pulse.

The menisci were visually assessed, and if it was not possible to obtain samples from the inner and outer part of the meniscus as well as from the medial and lateral meniscus, the patient was excluded from the study. In most excluded cases, the meniscal tissue was too degenerated to obtain samples from the inner part of the meniscus. Of the 101 menisci that were assessed, 18 pairs of medial and lateral menisci from patients with OA and 7 pairs of medial and lateral healthy menisci were available for analysis.

All meniscus samples were dissected in to inner (white zone) pieces and outer (red zone) pieces. All samples went through a collagen purification process and ^{14}C analysis. Secondary to this, GAG and hydroxyproline contents were measured. Details of laboratory processes and statistical analyses are described in online supplementary methods.

RESULTS

^{14}C content/isotope analysis

Figure 1A shows the content of ^{14}C in all samples. The level of ^{14}C in the samples of meniscal collagen indicates a very slow collagen turnover, since the pattern of the bomb pulse peak is retained in the collagen. If a continuous turnover of the meniscal collagen had taken place, the ^{14}C content of the all samples would have corresponded to the atmospheric ^{14}C at the time of sampling (shown by the dashed horizontal line in figure 1A). However, if we imagine that the menisci had no collagen added or renewed after birth, the samples would have been placed on the bomb pulse curve. What we observe is that the trend of the data points mirrors the bomb pulse but shifted to a ^{14}C level 5–10 years after birth, indicating that an incorporation of ^{14}C after birth and also showing that this incorporation does not continue in adult life. To model the timing of the formation and turnover of the meniscal collagen matrix, similarly to what has previously been done for human tendon tissue,[10] a 13-year moving average of the bomb pulse curve has been added to figure 1. Each point on this curve represents the average atmospheric ^{14}C level of the next 13 years. The 13-year moving average fits well with the levels of ^{14}C in the meniscus samples, suggesting that a gradual incorporation of ^{14}C in the collagen matrix has occurred during the first 13 years of life on average. There are samples that do not fit this curve perfectly. This is to be expected, since the timing of the formation of the menisci is not the same in each individual, and even small individual variations in the meniscal development pattern will impact the ^{14}C level. Especially in the persons that were growing during 1960s and 70s, where the atmospheric levels rose and fell dramatically, the variation in ^{14}C is greater. Similarly, in the donors born between 1930 and 1940, it is seen

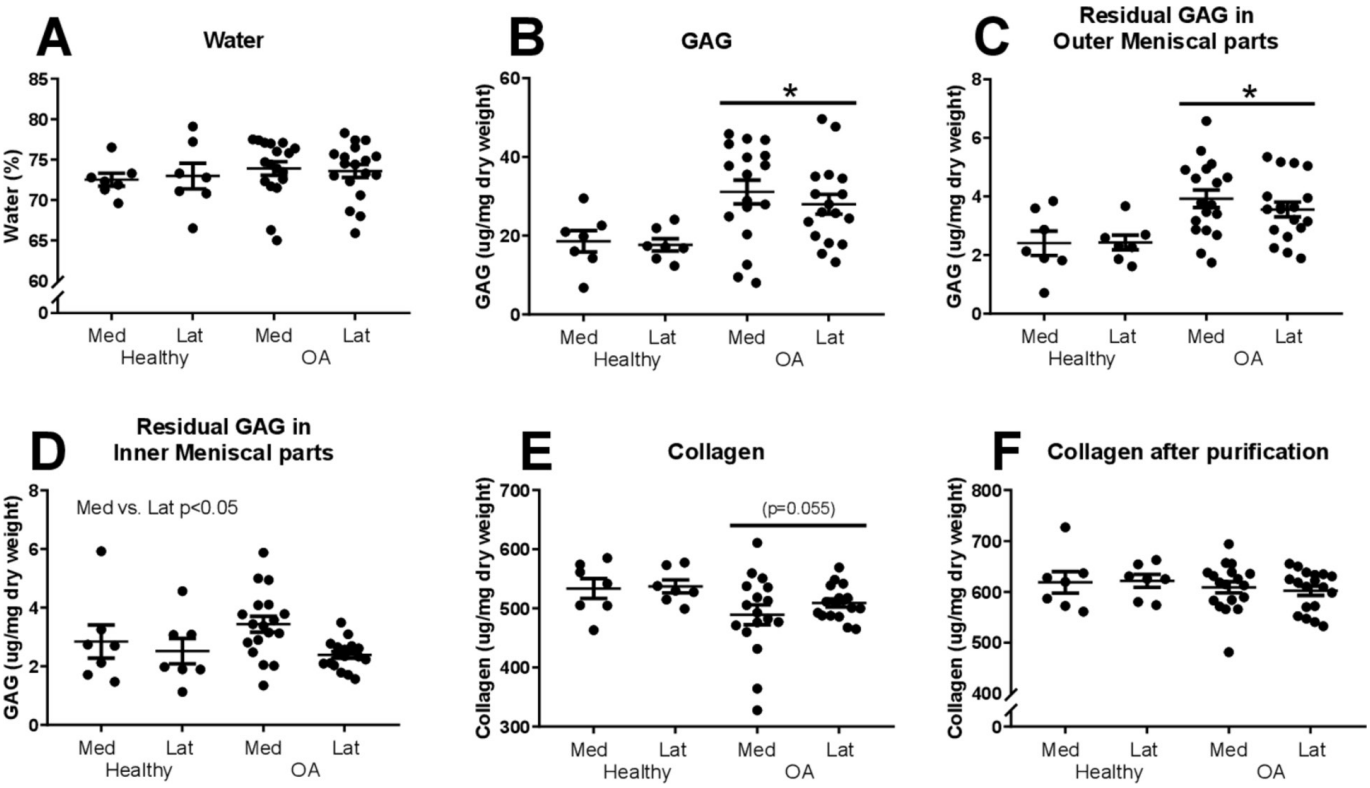

Figure 2 Water, GAG and collagen content (shown as mean and SEM): (A) water content (%) (mean of inner and outer part) of medial (Med) and lateral (Lat) healthy and OA menisci; (B) GAG content in outer part of raw menisci; (C) GAG content in purified outer meniscal tissue; (D) GAG content in purified inner meniscal tissue; (E) collagen content in outer part of raw menisci; (F) collagen content (mean of inner and outer part) in purified meniscal tissue. *Significant differences between OA and healthy menisci ($p<0.05$); significant difference ($p<0.05$) between medial (Med) and lateral (Lat) menisci is indicated on the graphs. Error bars indicate SEM. GAG, glycosaminoglycan; OA, osteoarthritis.

that some of their meniscal samples have had a slight incorporation the elevated ^{14}C from the bomb pulse after the age of 13 year, indicating that those parts of their menisci were completed at a slightly later age than 13 years. This fits well with the individual variation in growth. Thus, the 13-year moving average should be seen as a working model, and we do not conclude that the meniscal collagen is fully formed in all individuals at exactly the age of 13 years. In spite of these limitations of the model, the ^{14}C data clearly show that the meniscal collagen matrix is formed before adulthood and that essentially no further remodelling takes place later in life.

Although there was a variation in the ^{14}C content between the different regions of the menisci within individuals, no systematic difference was seen among the lateral and medial menisci or among the inner part and the outer part of the meniscus. For example, it is seen for the person born in 1935 that the lateral inner tissue had lowest ^{14}C content and the lateral outer had the highest content. Oppositely for the person born in 1937, the lateral inner tissue had the highest ^{14}C content and the lateral outer had the lowest. The lack of a systematic difference between the regions suggests that the meniscal collagen deposition and turnover during life is not dependent on the tissue region.

Because no systematic difference was seen between the different regions of the two menisci, the median ^{14}C content of all four regions (medial inner, medial outer, lateral inner and lateral outer) from each person was plotted, comparing healthy and OA patients (figure 1B). There was no apparent difference in the pattern of ^{14}C contents between menisci from OA and healthy patients. If the menisci from patients with OA had undergone a greater turnover during adult life than the healthy menisci, they would generally have been placed lower on the graph due to incorporation of modern, low levels of ^{14}C.

Water content

The water content in all menisci was 73%±4% (mean±SD). There was 2% more water in the inner part compared with the outer part of the menisci ($p=0.009$). However, we considered this difference negligible and used the mean of the two parts for further comparison (figure 2A). We found no significant differences in water content between menisci from patients with OA and patients with healthy knee joints and between medial and lateral menisci when comparing these means (figure 2A).

GAG content

The overall content of GAGs in dry raw and purified samples was 25.5 µg/mg ±10.8 µg/mg and 3.2 µg/mg ± 1.2 µg/mg, respectively, meaning that about 90% of GAGs had been removed during the purification process. Measurement of the overall GAG content was therefore made on dry, raw samples. Unfortunately, the menisci samples obtained during surgery were of varying size, and especially the inner meniscal samples were small due to the decrease in meniscal thickness in the inner part. Since the primary goal of the study was to look at the collagen, the priority was made to get large samples for the collagen purification process leaving smaller parts left for further raw analysis. This resulted in 43% of the inner meniscal samples being too small for the GAG analysis on raw tissue. Therefore, comparisons of overall GAG content could only be made for the outer

(capsular) part of the meniscus, while the GAG content in purified tissue was made for inner as well as outer meniscus.

The raw meniscal samples from patients with OA had significantly ($p<0.05$) higher content of GAGs (mean±SEM 29.54 µg/mg ±1.97 µg/mg) compared with healthy menisci (18.12 µg/mg ±3.07 µg/mg) (figure 2B). The relative differences between the OA and healthy menisci was preserved after purification (figure 2C). For the inner parts, the GAG level in purified samples was similar to what was found in the outer pieces, indicating a similar purification efficiency (figure 2D). However, there was a significant difference between the medial and lateral pieces, suggesting that within the inner part the medial meniscus had higher GAG content than the lateral meniscus (figure 2D).

Hydroxyproline/collagen measurements

There was no difference in the collagen contents measured on purified samples between outer and inner pieces, OA and healthy menisci and medial and lateral menisci (figure 2F).

DISCUSSION

It was not possible in this study to demonstrate any turnover of collagen in menisci during adult life, and even in patients with OA, the meniscal collagen had not been replaced. This is true regardless of the meniscal region as there were no systematic differences between the inner and outer zone of the menisci nor between the medial and lateral menisci. The data support the view that remodelling of the collagen structures within intact human menisci does not occur during adult life.

If the intact meniscus had the ability to remodel collagen, it would be expected that renewal or growth of collagen content was seen in particular in the outer parts of the menisci that are vascularised.[14] This was found not to be the case, and there was no sign of remodelling of the meniscal collagen after the menisci had fully developed when growth was completed. This is in line with the clinical experience that meniscal lesions, in which the collagen structure has been interrupted (typically flap or radial lesions), cannot heal, even though they are sutured. Healing of longitudinal lesions, in which the circular collagen fibres are intact and the tear is parallel to these fibres, seem from animal studies to be provided by extrinsic repair tissue with an unstructured collagen architecture and not by intrinsic repair of the natural collagen structure.[15 16]

The consequence of this understanding is that meniscal collagen most likely cannot be strengthened by introduction of exercises in adults. Whereas bone and muscle react to increasing load by formation of stronger tissue,[17 18] the inability of menisci to remodel later in life indicates a diminished ability to adapt to changes in external mechanical loading on the tissue. Interestingly, a similar pattern has been demonstrated for hyaline cartilage in the knee.[9] Therefore, the positive effect that has been reported of knee exercises on subjective pain in patients with knee pain[19] cannot be explained by strengthening of the collagen structure of the load transforming tissue (menisci and cartilage) of the knee itself. However, load can lead to a temporary increase the production of GAGs,[20 21] and in intact menisci and hyaline cartilages, it increases the hydrostatic/osmotic pressure and the ability to absorb load.[22]

While this study shows that meniscal collagen content cannot increase in adult life, it is possible that the meniscal collagen structure can strengthen as response to load during the development in childhood and the teenage years. This would add to the already known benefits of active lifestyle in childhood and adolescence (reviewed in ref 23), but it is speculative.

Clearly, we cannot from this study conclude regarding potential meniscus collagen remodelling in relation to severe degenerative tissue changes, as we were not able to use tissue from OA knees with severely damaged menisci where the amount of meniscus tissue was too low for analysis. However, it can be concluded that in OA knees with intact meniscus there was no sign of elevated collagen turnover.

Secondary aims in the present study were to study the composition of the meniscus, that is, the contents of water, collagen and GAGs. It was surprising that the water per cent was similar in all samples, as a higher water content has previously been described in menisci from OA knees.[2 4 6] However, it is not known to which degree the menisci analysed in previous studies from patients with OA were degraded. We excluded the most degraded menisci in our study, and differences in the degree of degeneration might explain the different figures for water content. The menisci were removed during arthroplasty operations, and the indication for this operation varies in different countries; as in some traditions, the prosthesis is introduced early in the cause of degenerative disease, while in others, it is a salvage procedure. Meniscal tissue obtained this way may therefore not be comparable between countries.

The GAG content was around 50% higher in the outer part of OA patient menisci than in the outer part of healthy menisci. This corresponds well with earlier studies showing that GAG content is higher in the menisci of patients with OA.[2 4] As the GAG content is subject to fluctuations as response to load,[3] the increased GAG levels in menisci from patients with OA may be a response to a constant, increased load on the menisci due to reduced load absorbing capacity of the hyaline cartilage in OA.

The collagen content in the analysed menisci was lower than what has been described earlier.[2] However, when the collagen content was calculated from hydroxyproline content, multiplication factors between 7 and 10 have been used,[24] and this can be the reason for the differences. Also, collagen content correlates negatively with degree of meniscal degeneration,[2] and our patients were older with an increased risk of degeneration in the meniscal tissue and thereby lower collagen content.

Limitations

Regarding the collagen purification, we were successful in removing 90% of the GAGs from the menisci (with use of the purification procedure). However, the collagen fraction is not pure collagen, but likely contains other matrix proteins and cell debris. The ^{14}C data show that this composite material has extremely limited turnover in adults, suggesting that other parts of the matrix, in addition to the collagen matrix, are also relatively inert.

As discussed previously for cartilage tissue,[9] it is possible that a small fraction of the insoluble collagen matrix has a more dynamic nature. This is possible, since a small fraction with higher turnover would not dilute the ^{14}C levels enough to abolish the remnants of the bomb pulse ^{14}C that are clearly present in the meniscal samples. We cannot, based on the current data, determine if a potential small fraction with continuous turnover originates from the collagen matrix or from other tissue fractions or impurities.

Finally, it should be mentioned that any soluble collagen in the menisci will be removed during the purification of the tissue during trypsin digestion. This soluble collagen may contain newly synthesised collagen that is not incorporated/cross-linked into the stable collagen network. However, this collagen fraction is unlikely to contribute to the mechanical stability to the collagen matrix, and the data clearly show that no significant remodelling of the stable collagen matrix occurs in adults.

CONCLUSION

Taken together, the present study provides evidence that there is no remodelling capacity of human menisci in adults. This implies that there is no possibility for any major or permanent adaptations of the menisci in response to exercise in adult patients with knee symptoms. However, during growth when the meniscal structure is formed, it might be possible to increase the collagen contents and thereby the lifelong load absorbing capacity of the menisci by regular loading, obtained by an active lifestyle during childhood and early adolescence.

What are the findings?

- This study shows that essentially no remodelling of the collagen structure of human menisci occurs in adult life.
- This understanding explains the clinical observations of increasing degradation of meniscal tissue and clinical symptoms during adulthood and the inability of exercise in adults to strengthen meniscal tissue.
- It introduces the hypothesis that lifetime maximal strength and load absorbing capacity of meniscal tissue may be provided through a physically active lifestyle at the time when the menisci are developed during childhood and early adolescence.

Contributors KMH, MK and MRK planned the study. MRK provided samples from patients with osteoarthritis, and MMP provided samples from patients with healthy menisci. CV and KMH prepared samples for isotope analyses and performed all other analyses. JO performed the isotope analyses. CV, KMH and PS structured the results, PS performed the statistical analyses and all authors participated in interpretation of the results and writing of the manuscript.

Funding The study was funded by The Danish Rheumatism Association and The Augustinus Foundation.

Competing interests None declared.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Not required.

Ethics approval The study was approved by the Ethical Committee of the Capital Region of Denmark (H-4-2012-131), and all persons gave informed written consent to donate their tissue.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement All data relevant to the study are included in the article or uploaded as supplementary information. All data associated with this study are available in the main text or the supplementary materials.

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REFERENCES

1 Clayton RAE, Court-Brown CM. The epidemiology of musculoskeletal tendinous and ligamentous injuries. *Injury* 2008;39:1338–44.
2 Herwig J, Egner E, Buddecke E. Chemical changes of human knee joint menisci in various stages of degeneration. *Ann Rheum Dis* 1984;43:635–40.
3 Subburaj K, Kumar D, Souza RB, *et al*. The acute effect of running on knee articular cartilage and meniscus magnetic resonance relaxation times in young healthy adults. *Am J Sports Med* 2012;40:2134–41.
4 Roller BL, Monibi FA, Stoker AM, *et al*. Characterization of knee meniscal pathology: correlation of gross, histologic, biochemical, molecular, and radiographic measures of disease. *J Knee Surg* 2015;28:175–82.
5 Di Giancamillo A, Deponti D, Addis A, *et al*. Meniscus maturation in the swine model: changes occurring along with anterior to posterior and medial to lateral aspect during growth. *J Cell Mol Med* 2014;18:1964–74.
6 Mine T, Ihara K, Kawamura H, *et al*. Collagen expression in various degenerative meniscal changes: an immunohistological study. *J Orthop Surg* 2013;21:216–20.
7 Melrose J, Smith S, Cake M, *et al*. Comparative spatial and temporal localisation of perlecan, aggrecan and type I, II and IV collagen in the ovine meniscus: an ageing study. *Histochem Cell Biol* 2005;124:225–35.
8 Sarachine Falso MJ, Buchholz BA. Bomb pulse biology. *Nucl Instrum Methods Phys Res B* 2013;294:666–70.
9 Heinemeier KM, Schjerling P, Heinemeier J, *et al*. Radiocarbon dating reveals minimal collagen turnover in both healthy and osteoarthritic human cartilage. *Sci Transl Med* 2016;8:346ra90.
10 Heinemeier KM, Schjerling P, Heinemeier J, *et al*. Lack of tissue renewal in human adult Achilles tendon is revealed by nuclear bomb (14)C. *Faseb J* 2013;27:2074–9.
11 Bergmann O, Bhardwaj RD, Bernard S, *et al*. Evidence for cardiomyocyte renewal in humans. *Science* 2009;324:98–102.
12 Lynnerup N, Kjeldsen H, Heegaard S, *et al*. Radiocarbon dating of the human eye lens crystallines reveal proteins without carbon turnover throughout life. *PLoS One* 2008;3:e1529.
13 Arner P, Bernard S, Salehpour M, *et al*. Dynamics of human adipose lipid turnover in health and metabolic disease. *Nature* 2011;478:110–3.
14 Shybut T, Strauss EJ. Surgical management of meniscal tears. *Bull NYU Hosp Jt Dis* 2011;69:56–62.
15 Nakagawa Y, Muneta T, Kondo S, *et al*. Synovial mesenchymal stem cells promote healing after meniscal repair in microminipigs. *Osteoarthritis Cartilage* 2015;23:1007–17.
16 Duygulu F, Demirel M, Atalan G, *et al*. Effects of intra-articular administration of autologous bone marrow aspirate on healing of full-thickness meniscal tear: an experimental study on sheep. *Acta Orthop Traumatol Turc* 2012;46:61–7.
17 Maycas M, Esbrit P, Gortázar AR. Molecular mechanisms in bone mechanotransduction. *Histol Histopathol* 2017;32:751–60.
18 Blaauw B, Schiaffino S, Reggiani C. Mechanisms modulating skeletal muscle phenotype. *Compr Physiol* 2013;3:1645–87.
19 Henriksen M, Klokker L, Graven-Nielsen T, *et al*. Association of exercise therapy and reduction of pain sensitivity in patients with knee osteoarthritis: a randomized controlled trial. *Arthritis Care Res* 2014;66:1836–43.
20 Parkkinen JJ, Ikonen J, Lammi MJ, *et al*. Effects of cyclic hydrostatic pressure on proteoglycan synthesis in cultured chondrocytes and articular cartilage explants. *Arch Biochem Biophys* 1993;300:458–65.
21 Roos EM, Dahlberg L. Positive effects of moderate exercise on glycosaminoglycan content in knee cartilage: a four-month, randomized, controlled trial in patients at risk of osteoarthritis. *Arthritis Rheum* 2005;52:3507–14.
22 Lu XL, Sun DDN, Guo XE, *et al*. Indentation determined mechanoelectrochemical properties and fixed charge density of articular cartilage. *Ann Biomed Eng* 2004;32:370–9.
23 Loprinzi PD, Cardinal BJ, Loprinzi KL, *et al*. Benefits and environmental determinants of physical activity in children and adolescents. *Obes Facts* 2012;5:597–610.
24 Hoemann CD. Molecular and biochemical assays of cartilage components. *Methods Mol Med* 2004;101:127–56.
25 Kueppers LM, Southon J, Baer P, *et al*. Dead wood biomass and turnover time, measured by radiocarbon, along a subalpine elevation gradient. *Oecologia* 2004;141:641–51.
26 Levin I, Kromer B, Hammer S. Atmospheric Δ14CO2 trend in Western European background air from 2000 to 2012. *Tellus B: Chemical and Physical Meteorology* 2013;65:20092.
27 Olsen J, Tikhomirov D, Grosen C, *et al*. Radiocarbon analysis on the new AARAMS 1MV Tandetron. *Radiocarbon* 2017;59:905–13.
28 Edwards CA, O'Brien WD. Modified assay for determination of hydroxyproline in a tissue hydrolyzate. *Clinica Chimica Acta* 1980;104:161–7.