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Aspartic acid racemization reveals a high turnover state in knee compared with hip osteoarthritic cartilage

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SUMMARY

Objective: We investigated tissue turnover in healthy and osteoarthritic cartilage. We challenge long held views that osteoarthritis (OA) is dominated by a similar turnover process in all joints and present evidence that hip and knee cartilage respond very differently to OA.
Methods: D- and L-Aspartate (Asp) were quantified for whole cartilage, collagen and non-collagenous components of cartilage obtained at the time of joint replacement. We computed the Asp racemization ratio (Asp-RR = D/D + L Asp), reflecting the proportion of old to total protein, for each component.
Results: Compared with hip OA, knee OA collagen fibrils ($P < 0.0001$), collagen ($P = 0.007$), and non-collagenous proteins ($P = 0.0003$) had significantly lower age-adjusted mean Asp-RRs consistent with elevated protein synthesis in knee OA. Knee OA collagen had a mean hydroxyproline/proline (H/P) ratio of 1.2 consistent with the presence of type III collagen whereas hip OA collagen had a mean H/P ratio of 0.99 consistent with type II collagen. Based on Asp-RR, the relative age was significantly different in knee and hip OA ($P < 0.0005$); on average OA knees were estimated to be 30yrs 'younger', and OA hips 10yrs 'older' than non-OA.
Conclusions: The metabolic response to OA was strikingly different by joint site. Knee OA cartilage evinced an anabolic response that appeared to be absent in hip OA cartilage. These results challenge the long held view that OA cartilage is capable of only minimal repair and that collagen loss is irreversible.

Introduction

Due to the presence of an asymmetrical carbon atom, all amino acids (AAs) except glycine exist in nature in two non-superimposable, mirror image L- and D-racemic forms. Although racemic forms share almost identical chemical properties, differing only in their optical properties, only L-AAs are incorporated into proteins. This stereochemical selection is important for the correct folding of proteins. However, racemized AAs (D-AAs) spontaneously form post-translationally and build up slowly over time in long-lived extracellular matrix proteins. Racemized AAs have been detected in a wide array of proteins and tissues including tooth dentin, bone, cartilage, skin, intervertebral disc, brain and eye lens proteins[1–7]. The rate of racemization is influenced by environmental factors such as temperature, pH and ionic strength as well as the AA R-group[8]. Racemization is a spontaneous clock-like physical process used to estimate the age of a wide range of biological materials including silk[9], shells[10] and teeth[11]; it has the added advantage over carbon dating of requiring only very small amounts of sample.

Although AA racemization is slow, Asp racemization occurs on a biologically relevant timescale and is the most readily and reliably detected racemized AA. Asp racemization occurs spontaneously through a succinimide intermediate [Fig. 1(A)]. Acid hydrolysis, required to release AAs, causes spontaneous deamidation of Asn to Asp; therefore, racemized Asp represents a combination of racemized Asn and Asp. Rates of D-Asp racemization for tooth dentin (tissue devoid of turnover), are estimated to be $7.7–8.3 \times 10^{-4}$/yr[6].

We investigated racemized AAs as a method of estimating protein turnover in joint tissues[7]. Given uniform turnover throughout a tissue, the ratio of racemized AAs to total AAs is unaffected by catabolism but decreased by anabolism. When

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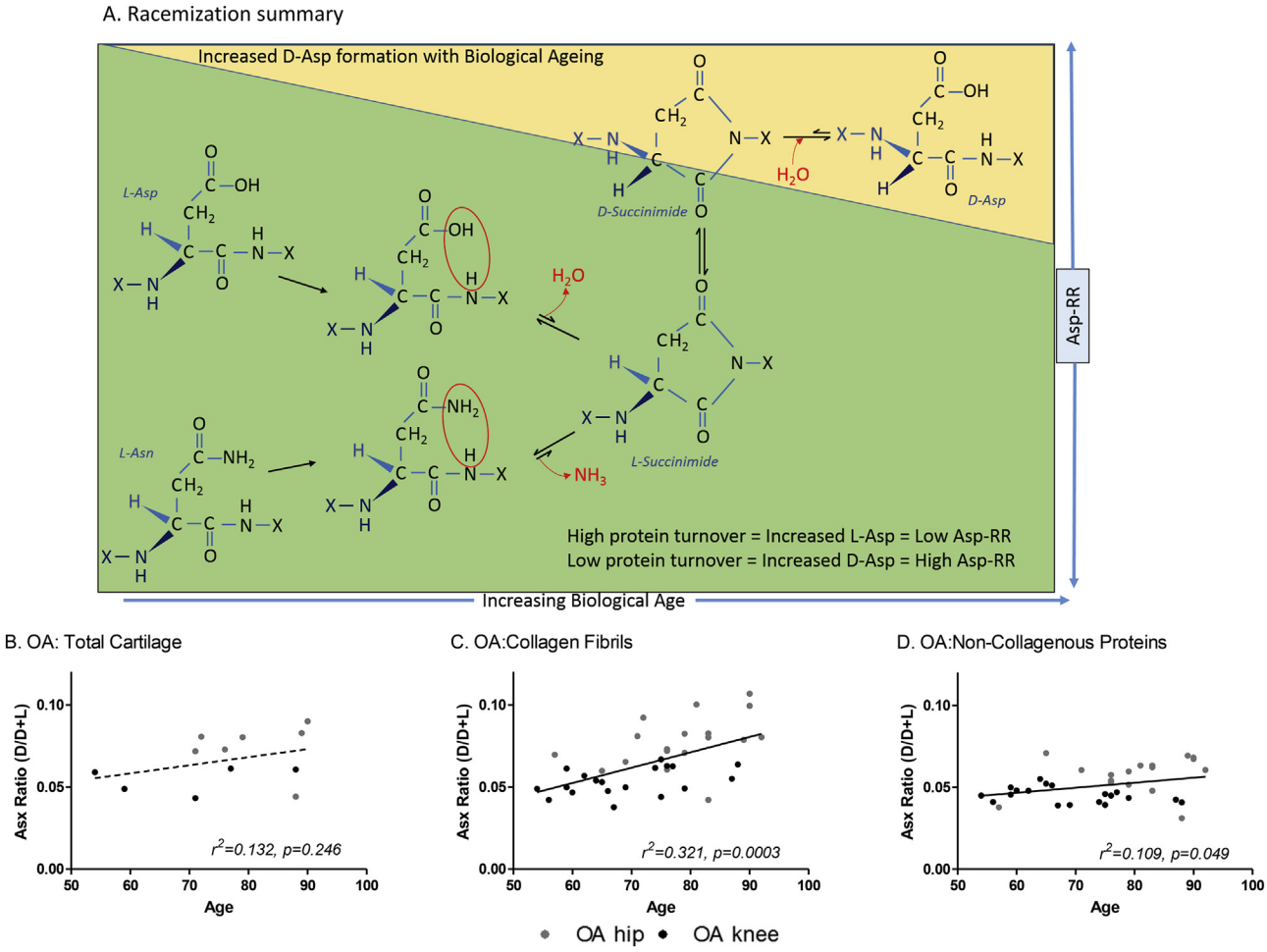

Fig. 1. Correlation of Asp racemization ratio with subject age for OA whole cartilage and extracted matrix components. (A) Process of Asp racemization through a succinimide intermediate. Asp racemization ratio (Asp-RR = D-Asp/D + L-Asp) was determined for OA cartilage including: (B) whole cartilage (full thickness macroscopically normal cartilage), (C) collagen fibrils (cartilage extensively extracted with Gu-HCl to remove soluble cartilage proteins leaving mainly collagen) and, (D) non-collagen cartilage components (proteins extractable from cartilage by Gu-HCl, mainly aggrecan). Briefly, Hydrolysates were buffered with 0.4M boric acid (pH9.0) and derivatized with o-phthaldialdehyde and *N*-tert-tiarybutyloxycarbonyl-L-cysteine (Sigma–Aldrich, USA) in methanol. Resulting fluorescent derivatives were separated by reverse-phase HPLC. A total of 100 μl of derivatized sample was injected onto a Chromolith RP-18e 100 × 4.6 mm column (VWR International LLC, USA) at a 1 ml/min flow rate with fluorescent detection (excitation 344 nm, emission 443 nm) using the following gradient: 30 min 9.5–15.5% buffer B (100% acetonitrile); 5 min 60% buffer B column wash. Mobile phase consisted of 0.2M acetic acid adjusted to pH6.0 with NaOH. The Asp racemization ratio (Asp-RR) was defined as D/D + L Asp.

catabolism and anabolism are at steady state, this ratio reflects protein/matrix turnover. For instance, under slow turnover conditions, the ratio will rise with age; under fast turnover conditions, the ratio will fall with age; when turnover matches the racemization rate, the ratio will remain unchanged with age. D-Asp increases with age at an estimated rate of 2.58×10^{-4}/yr for type II collagen of normal cartilage indicative of a slowly turning over component[12]. AA racemization reveals differential protein turnover in osteoarthritic articular and meniscal cartilages[6,7,13]. In disease states, such as osteoarthritis (OA), where the matrix is no longer in a steady state, lower quantities of racemized AAs relative to normal tissue can be considered to reflect high turnover in attempted repair of the cartilage with new protein synthesis.

Cartilage matrix is enriched for two main molecular components, namely type II collagen and aggrecan, comprising 60% and 40% by dry weight, respectively[14]. Turnover and synthesis of matrix molecules in normal cartilage are slow but in equilibrium. Based on analysis of human femoral head cartilage, the predicted half-life is 25yrs for aggrecan[15] and at least 120yrs for collagen[12,16]. Upon cytokine stimulation, proteoglycan is rapidly lost[17–19] but rapidly replaced[18]. In contrast, collagen is much less readily broken down but generally believed to be minimally replaced, so is considered to represent an irreversible step in cartilage degradation[20]. Our previous data suggested that for Cartilage Oligomeric Matrix Protein (COMP), turnover was higher in knee OA than hip OA cartilage[21]. The goal of this study was to understand the reparative responses in OA through analyses of D-Asp in soluble (primarily aggrecan) and insoluble (primarily collagen) matrix components from knee and hip OA cartilage.

Methods

Samples and demographics

Articular cartilage specimens were obtained under Duke Institutional Review Board approval as anonymized waste surgical specimens from knee and hip arthroplasties performed at Duke University Medical Center to alleviate symptoms of OA. Non-arthritic control cartilages were obtained either from the National Disease Research Interchange (Philadelphia, PA), or trauma