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Perturbations in the HDL metabolic pathway predispose to the development of osteoarthritis in mice following long-term exposure to western-type diet

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SUMMARY

Objective: Recent data suggest that obesity and related metabolic aberrations are associated with osteoarthritis (OA) development, a phenomenon that is attributed at least in part to the consumption of lipid-rich diets. To date, the molecular mechanisms that govern the lipid-OA connection remain largely unknown. Given the important role of high-density lipoprotein (HDL) in plasma and tissue lipid metabolism, the main purpose of the present study was to investigate the role of HDL metabolism in the pathobiology of OA.

Methods: We used apolipoprotein A-I (apoA-I)$^{-/-}$ mice that lack classical apoA-I containing HDL, LCAT$^{-/-}$ mice that have only immature HDL and relatively reduced HDL-cholesterol levels and control C57BL/6 mice. Mice were placed on chow or western-type (WTD) and monitored for 24 weeks. Knee joints were removed and articular cartilage was isolated for further analyses.

Results: The LCAT$^{-/-}$ mice were significantly more sensitive to the development of diet-induced obesity compared to the C57BL/6 and apoA-I$^{-/-}$ mice. Morphological, biochemical and molecular analyses revealed that the LCAT$^{-/-}$ obese mice developed OA, while the C57BL/6 mice that were fed WTD did not. Notably, apoA-I$^{-/-}$ mice that received WTD also developed OA although their body-weight gain was similar to their wild-type counterparts. Interestingly, bone marrow from LCAT$^{-/-}$ and apoA-I$^{-/-}$ mice contained significantly increased number of adipocytes, compared to the other groups.

Conclusions: Our findings suggest that perturbations in HDL metabolism predispose to OA following chronic insult with WTD and raise the challenging possibility that HDL has a causative relation to OA in patients with metabolic syndrome.

Introduction

Osteoarthritis (OA) is a very common degenerative joint disease that accounts for a considerable proportion of disability in adults[1]. The atiology of OA is multifactorial, inasmuch as increased mechanical stimulation, trauma, metabolic factors and genetic susceptibility contribute to its pathogenesis. Definitely, one of the most common conditions that lead to OA is obesity. Excess body-weight is associated with elevated mechanical stimulation of the weigh-bearing joints that leads to cartilage destruction and ultimately OA development[2]. Notably, however, even though a connection between obesity and OA is well-established, the contribution of excessive body-weight to OA development remains vague. Indeed, several epidemiological studies have demonstrated that aside from the large weight-bearing joints like knee and hip, the small non-bearing articulations of the hand of obese individuals also develop OA, a finding that cannot be attributed to increased body-weight[3,4]. Along the same lines, another recent study on obese patients showed an association between obesity and hand OA that was independent of other metabolic parameters raising the possibility that altered lipid metabolism has a cardinal role in OA pathogenesis[5]. This hypothesis is further supported by a considerable volume of reports having shown that OA is tightly associated with cardiovascular-related conditions, such as hypertension, hypercholesterolemia, abdominal obesity, dyslipidemia, type 2 diabetes and metabolic syndrome, which are coupled to distorted lipid

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metabolism[6]. Recent epidemiological studies have demonstrated that high-density lipoprotein cholesterol (HDL-C) is decreased in the serum of OA patients compared to non-OA individuals, clearly suggesting a potential relationship between HDL and OA aetiopathogenesis[7,8].

HDL, a key-component of the lipoprotein transport system possesses a number of functions associated with maintenance of plasma and tissue lipid metabolism and homeostasis. The biogenesis of mature HDL requires apolipoprotein A-I (apoA-I) and the functions of lipid transporter ABCA1 and plasma enzyme lecithin:cholesterol acyltransferase (LCAT). LCAT is a critical component of the lipoprotein metabolic system. It is produced and secreted mainly by the liver and catalyzes the esterification of free cholesterol of lipoproteins by transferring a fatty-acyl group from the C-2 position of lecithin to the 3-hydroxyl group of cholesterol[9]. In plasma, LCAT is activated by apoA-I. Following initial lipidation of apoA-I by ABCA1, apoA-I forms discoidal particles that are then converted to mature, fully active, spherical HDL by the action of LCAT[10]. It has been recently reported that LCAT is a key enzyme in the metabolism of triglyceride-rich lipoproteins[11], while apoA-I or LCAT deficiency sensitizes mice to hepatic lipid deposition associated with non-alcoholic fatty liver disease[12,13].

In metabolic syndrome, obesity is associated with dyslipidemia consisting of increased triglyceride and reduced HDL-cholesterol levels. Based on the recently uncovered link between obesity and OA in patients with metabolic syndrome[7,8], in the present study we sought to investigate the potential link between HDL metabolic pathway and the pathogenesis of OA.

Methods

Animal studies

We used male 10–12-week-old LCAT-deficient ($LCAT^{-/-}$) mice (Dr. Silvia Santamarina-Fojo laboratory[14]) as well as apoA-I-deficient ($apoA\text{-}I^{-/-}$) and C57BL/6 mice (Jackson Labs, Bar Harbor, Maine, USA) that were caged individually and allowed unrestricted access to food and water. The age and starting body-weights of the tested animals were similar. Mice were fed the standard western-type diet (WTD) and standard chow diet (CD) (both form Mucedola SRL, Italy) for the indicated period. The standard WTD (42% energy from fat) is composed of 17.3% protein, 48.5% carbohydrate, 21.2% fat, 0.2% (0.15% added, 0.05% from fat source), and contains 4.5 kcal/g. The standard CD (10% energy from fat) is composed of 29.0% protein, 60.4% carbohydrates and 10.6% fat. Body-weight was measured at the indicated time-points after diet initiation[12]. At the end of each experiment, mice were sacrificed and plasma and knee joints were collected for biochemical, histological and molecular analyses. Carcasses were stored at −80°C. All animal studies were performed according to the EU guidelines for the Protection and Welfare of Animals. The work was approved by Committee of the Laboratory Animal Center of The University of Patras Medical School and the Veterinary Authority of the Prefecture of Western Greece.

Fractionation of plasma lipoproteins by density gradient ultracentrifugation

In order to determine plasma cholesterol and triglyceride levels in various plasma lipoproteins, 0.5 ml of pools of plasma from six $LCAT^{-/-}$, six apoA-I and six C57BL/6 mice were fractionated by density gradient ultracentrifugation as previously described[12,15]. The cholesterol and triglyceride content of different density fractions were determined as described above.

Determination of daily food consumption

Food intake was assessed by determining the difference in food weight during a 7-day period to ensure reliable measurements, as described previously[16,17]. Results were then expressed as average food consumption per mouse per strain over the 7-day period ± standard error of the mean.

Histological analysis of tissue samples

At the end of each experiment, mice were sacrificed. Hyaline cartilage from femora and tibiae was isolated under direct inspection with a Nikon SMZ660 stereomicroscope, finely diced and stored at −80°C or fixed in 10% formalin for molecular/biochemical and histological analyses, respectively. Fixed sections were decalcified with Ethylenediaminetetraacetic acid (EDTA), embedded in paraffin and sectioned at 4 μm for histological evaluation. Hematoxyline and eosin (H&E) stain was applied for the assessment of articular cartilage and bone marrow micromorphology. Five serial sections were obtained from one knee joint of each animal. In order to assess cartilaginous tissue structural integrity matrix proteoglycans were visualized with the use of the Safranin O/Fast green histochemical stain according to the manufacturer's instructions (S8884, Sigma) and observed with Olympus BX41 bright-field microscope. OA changes were graded semi-quantitatively in a blindfolded manner by one bone-and-soft-tissue pathologist (DJP) and one investigator (I-ET) on a scale from 0 to 6 according to the scoring system criteria recommended by the Osteoarthritis Research Society International (OARSI)[18]. The severity of the OA changes is expressed as maximal scores for the entire joint[18]. For the calculation of the number of bone marrow adipocytes five different areas per section were photographed using a Nikon Eclipse 80i microscope with a Nikon DXM 1200C digital camera (original magnification 10×). The digital images were imported into Photoshop CS4 and grid was added. For each area the number of lipid vacuoles that were intersected by the grid was determined and calculated blindly by DJP and I-ET. For each animal the average score was used for statistical analysis.

Micro-computer tomography (μCT) analysis

Fixed knee joints were mounted on the CT specimen tube for scanning at a resolution of 12 μm, with a slice increment of 10 μm (Scanco vivaCT 40 in vivo μCT scanner, Scanco Medical AG, Switzerland). Reconstructed 3D μCT scans were evaluated for the detection of knee cartilage architectural alterations.

Protein extraction

Knee cartilage derived from C57BL/6, $apoA\text{-}I^{-/-}$ and $LCAT^{-/-}$ mice, fed with WTD or CD was used for the determination of matrix metalloproteases (MMPs) expression. As mentioned previously, each specimen was finely diced and the macromolecules contained were extracted for 24 h at 4°C in the dark with 4 M GdnHCl–0.05 M sodium acetate, using 10 vols of extraction buffer per gram of tissue[19]. A protease inhibitor cocktail was included containing 5 mM benzamidine hydrochloride, 0.4 mM phenylmethylsulfonyl fluoride, 10 mM *N*-ethylmaleimide, 0.1 M ε-amino-n-caproic acid and 0.01 M Na_2EDTA. Each one of the extracts was stored at −20°C until use.

Zymographic examination of MMP-2 and MMP-9

The presence and gelatinolytic activity of MMP-2 and -9 was estimated using zymographic techniques as previously described[20]. Gels were scanned on a digital scanner (Hewlett Packard, ScanJet 6100 C/T), using the Adobe Photoshop 7 and analyzed with the Image