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Metabolic enrichment of omega-3 polyunsaturated fatty acids does not reduce the onset of idiopathic knee osteoarthritis in mice

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SUMMARY

Objective: We evaluated the effect of a reduction in the systemic ratio of n-6:n-3 polyunsaturated fatty acids (PUFAs) on changes in inflammation, glucose metabolism, and the idiopathic development of knee osteoarthritis (OA) in mice. We hypothesized that a lower ratio of n-6:n-3 PUFAs would protect against OA markers in cartilage and synovium, but not bone.

Design: Male and female *fat-1* transgenic mice (Fat-1), which convert dietary n-6 to n-3 PUFAs endogenously, and their wild-type (WT) littermates were fed an n-6 PUFA enriched diet for 9–14 months. The effect of gender and genotype on serum PUFAs, interleukin (IL)-6, tumor necrosis factor (TNF)-α, and glucose tolerance was tested by 2-factor analysis of variance (ANOVA). Cortical and trabecular subchondral bone changes were documented by micro-focal computed tomography (CT), and knee OA was assessed by semi-quantitative histomorphometry grading.

Results: The n-6:n-3 ratio was reduced 12-fold and 7-fold in male and female Fat-1 mice, respectively, compared to WT littermates. IL-6 and TNF-α levels were reduced modestly in Fat-1 mice. However, these systemic changes did not reduce osteophyte development, synovial hyperplasia, or cartilage degeneration. Also the *fat-1* transgene did not alter subchondral cortical or trabecular bone morphology or bone mineral density.

Conclusions: Reducing the systemic n-6:n-3 ratio does not slow idiopathic changes in cartilage, synovium, or bone associated with early-stage knee OA in mice. The anti-inflammatory and anti-catabolic effects of n-3 PUFAs previously reported for cartilage may be more evident at later stages of disease or in post-traumatic and other inflammatory models of OA.

Introduction

Inflammation mediates osteoarthritis (OA) pathogenesis through a mosaic-like pattern of classical immune cell mediated cytokine signaling and activation of molecular inflammatory pathways in native cells of intra-articular joint tissues[1,2]. While these inflammatory responses are most evident in post-traumatic knee OA[3,4], they are also observed in primary knee OA, suggesting that age-dependent changes in inflammatory pathways contribute to an increase in OA risk[5,6].

Recent studies suggest that chronic dietary factors can exacerbate or inhibit joint inflammation and thus may be important

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mediators of aging-associated knee OA. Obesity is a well established risk factor for knee OA, and several recent studies indicate that altered joint biomechanics alone are insufficient to increase OA risk with obesity[7–10]. While most studies have focused on adipokines as systemic mediators of obesity-associated OA, lipids are also potent regulators of inflammation[11]. In particular, the ratio of omega-6 (n-6) to omega-3 (n-3) polyunsaturated fatty acids (PUFAs) is considered one of the most important dietary mediators of inflammation[12]. Arachidonic acid (AA), a major n-6 PUFA, promotes inflammation by being converted into pro-inflammatory eicosanoids, such as prostaglandins, thromboxanes, and leukotrines. In contrast, n-3 PUFAs such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) inhibit inflammation and accelerate the resolution of inflammation. The anti-inflammatory effects of n-3 PUFAs occur through multiple mechanisms, including inhibition of the AA conversion into pro-inflammatory eicosanoids, synthesis of anti-inflammatory agents such as protectins and resolvins, and down-regulation of pro-inflammatory gene expression through n-3 receptor G-protein coupled receptor (GPR) 120[13,14]. Thus, variation in the dietary ratio of n-6:n-3 PUFAs, which is elevated in modern Western diets[15] and attributed to the increase in risk of numerous chronic diseases[16], may also contribute to differences in OA risk.

Previous studies support a role for n-6 and n-3 PUFAs in modifying OA severity. In middle-aged individuals without clinical knee OA, dietary intake of n-6 PUFAs was positively associated with the future prevalence, but not the incidence, of subchondral bone marrow lesions[17,18]. In individuals who have or are at high risk for knee OA, fasting plasma AA was positively associated with synovitis, whereas patella-femoral cartilage loss was negatively associated with DHA[19]. Animal and cell studies also indicate that n-3 PUFAs protect against OA. Feeding an n-3 enriched diet to OA-prone Dunkin–Hartley Guinea pigs reduced markers of OA without altering OA markers in a non-prone strain[20]. In addition, mice expressing the *fat-1* transgene were moderately protected from developing knee OA following transection of the medial meniscus, medial collateral ligament, and anterior crutiate ligament[21]. This transgene induces endogenous conversion of n-6 to n-3 PUFAs by encoding a desaturase enzyme absent in mammals that adds a double bond into the omega-3 position of an unsaturated fatty acid. The result is a systemic reduction in the n-6:n-3 ratio[22]. The protective effects of the *fat-1* transgene was attributed to a reduction in inflammation, decreased protein expression of matrix metalloproteinase-13 and A Disintegrin and Metalloproteinase with Thrombospondin Motifs (ADAMTS)-5, and enhanced autophagy[21]. In bovine cartilage explant and cell culture models, EPA and DHA inhibited the expression of pro-inflammatory and pro-catabolic genes and reduced glycosaminoglycan catabolism induced by exposure to interleukin (IL)-1[23,24]. Yet not all aspects of n-3 PUFAs necessarily protect against OA. EPA and DHA levels are positively associated with bone strength and bone density[25,26], and a low ratio of n-6:n-3 fatty acids protected against ovariectomy-induced bone loss in mice[27]. These pro-anabolic effects of n-3 PUFAs on bone strength and mass may promote OA by stimulating osteophyte development or subchondral bone thickening.

Our goal was to determine how a life-long reduction in the ratio of n-6:n-3 PUFA levels affects the development of idiopathic knee OA in mice. We hypothesized that a low ratio of n-6:n-3 PUFAs protects against OA markers in cartilage and synovium, but not bone. We tested this hypothesis using a transgenic mouse model containing the *fat-1* transgene from *Caenorhabditis elegans*[22]. By feeding mice an n-6 PUFA-enriched diet, Fat-1 mice reduce the n-6:n-3 ratio in the serum and tissues approximately 20-fold[22]. Here we examined changes in serum lipids and inflammatory markers in 9–14 month-old male and female C57BL/6 mice with and without the *fat-1* transgene. We determined the effect of *fat-1* transgene expression on idiopathic knee OA changes in cartilage, bone, and synovium. We also conducted regression analyses to evaluate whether *fat-1* expression altered associations between cartilage and bone or synovium and bone OA outcome measures.

Materials and methods

Animals

We obtained *fat-1* transgenic breeder mice from Dr JX Kang[22]. Animals were bred while housed in the University of Oklahoma Health Sciences Center (OUHSC) vivarium on a 12-h light/dark cycle under temperature-controlled conditions (20–22°C). Animals were allowed *ad libitum* access to water and a modified AIN-76A purified rodent diet supplemented with 10% Safflower oil by weight (#180465; Dyets, Inc., Bethlehem, PA) as previously described[28]. The diet contains 59% kcal carbohydrate, 18% kcal protein, and 23% kcal fat, with an n-6:n-3 ratio of 274[28]. Wild-type (WT) (C57BL/6) females were mated with male hemizygous *fat-1*$^{+/0}$ transgenic mice maintained on a C57BL/6 background. Male and female littermates carrying either one copy of the *fat-1* gene (hereafter referred to as Fat-1) or no copy of the *fat-1* gene (WT) were studied. Genotyping was conducted as previously described[28]. Our initial cohort of animals (cohort 1) included the following number and ages of animals: female WT mice ($N = 10$; 59.6 ± 1.7 wks of age [58–62 wks], mean ± SD [range]), female Fat-1 mice ($N = 10$; 59.5 ± 1.6 wks of age [58–62 wks]), male WT mice ($N = 10$; 55.4 ± 4.4 wks of age [52–61 wks]), and male Fat-1 mice ($N = 10$; 57.7 ± 3.8 wks of age [51–61 wks]). Additional male mice were included (cohort 2) to increase the sample size for lipid, cytokine, and histologic analyses: male WT mice ($N = 8$; 43.6 ± 4.3 wks of age [36–48 wks]) and male Fat-1 mice ($N = 7$; 43.1 ± 3.9 wks of age [40–48 wks]). Although cohort 2 mice were younger than cohort 1, no significant age-dependent differences were observed so data were pooled for both cohorts. Following euthanasia, the hind limbs were dissected and stored in phosphate buffered saline at −80°C. All experimental procedures were conducted in accordance with a protocol approved by the OUHSC Animal Care and Use Committee.

Serum analyses

Blood was collected from anesthetized mice just before the mice were killed. The blood was allowed to clot for 60 min at room temperature and then centrifuged for 15 min at 3500 revolutions per min, and the serum was aliquoted for immediate storage at −80°C until analysis. Serum n-6 and n-3 PUFA analyses were performed as previously described[28]. Briefly, lipid classes of serum lipid extracts were resolved, derivatized to form fatty acid methyl esters, and analyzed using gas–liquid chromatography in an Agilent 6890N gas chromatograph with flame ionization detector (GC-FID) (Agilent Technologies, Wilmington, DE). Authentic standards (NU-CHEK PREP, Elysian, MN) were used to calculate relative mole percentages for the following fatty acids: 20:4n6, 18:3n3, 20:5n3, 22:5n3, and 22:6n3. Serum concentrations of IL-6 and TNFα were investigated as prognostic markers of the effect of *fat-1* transgene expression on systemic inflammation. They were measured using quantitative enzyme linked immunosorbent assays (ELISA) designed specifically for mice (eBioscience, San Diego, CA). All samples were analyzed as recommended by the protocols provided by the manufacturer. Glucose tolerance tests were conducted as previously described[29].

Micro-computed tomography (CT) analyses

Right knee joints of mice from Cohort 1 were thawed, fixed in 10% neutral buffered formalin, and scanned using a micro-CT