


Serum YKL-40 Is Associated with Osteoarthritis and Atherosclerosis in Nonhuman Primates, Thomas C. Register,*† Cathy S. Carlson,* and Michael R. Adams† (1 Department of Pathology, Section on Comparative Medicine, Wake Forest University School of Medicine, Winston-Salem, NC 27157-1040; 2 College of Veterinary Medicine, University of Minnesota, St. Paul, MN 55108; * author for correspondence: fax 336-716-1515)

Osteoarthritis (OA) is the leading cause of chronic disability in the US (1). Because individuals with early stages of the disease are often asymptomatic, a biomarker of early OA may be useful. Adult cynomolgus macaques develop naturally occurring OA that closely resembles the human disease (2–4). Radiographic, histologic, and immunohistochemical analyses show a high frequency of lesions in the knee joints of these animals. Lesions are most severe in the medial tibial plateau and are characterized by fibration, clefting, and loss of articular cartilage with a concomitant, marked thickening of the subjacent subchondral bone (4).

YKL-40 (also known as human cartilage glycoprotein-39 or 38-kDa heparin-binding glycoprotein), a major secretory protein of human chondrocytes and synovial cells (5), is increased in the serum and synovial fluid of individuals with joint or cartilage disease (5–9). YKL-40 mRNA expression is also increased in the cartilage of patients with rheumatoid arthritis (10). YKL-40, first identified as a major product secreted by osteosarcoma MG63 cells (11), is also produced by fibrotic liver cells and breast cancer cells and not by skin or lung fibroblasts [for a review, see Ref. (8)]. YKL-40 expression is increased during the late stages of differentiation in the transition of monocytes to activated macrophages (12, 13), and YKL-40 mRNA is present in macrophages in human atherosclerotic lesions (14).

The N-terminal amino acid sequence of YKL-40 begins with Tyr-Lys-Leu (YKL), and the protein migrates at 40 kDa on sodium dodecyl sulfate polyacrylamide gel electrophoresis; hence the YKL-40 designation. YKL-40 binds heparin (15, 16) and chitin and has structural similarity to chitinolytic enzymes, although it lacks chitinase activity (10, 15). Thus, YKL-40 may function as a lectin or glycohydrolase of unknown specificity. YKL-40 expression patterns suggest roles in tissue remodeling or in immune function.

The purpose of the present study was to determine the associations of YKL-40 in serum and synovial fluid with naturally occurring OA and diet-induced atherosclerosis in cynomolgus monkeys. The subjects were subsets of animals obtained from previously described studies [study 1, Adams et al. (17), n = 13; study 2, Williams et al. (18), n = 1] or from a breeding colony (n = 2) at Wake Forest University School of Medicine. Study 1 and 2 animals were ovarioctomized and fed a moderately atherogenic diet (40% of calories as fat and 0.28 mg cholesterol/kcal) for 34 months. Before necropsy, the hips, knees, and feet of the animals were radiographed and sera collected and stored at −70 °C for later analysis. At necropsy, the monkeys were anesthetized deeply with pentobarbital (30 mg/kg intravenous), and the cardiovascular system was flushed with normal saline. The heart of each animal was excised after ligation of the vena cava, and pulmonary arteries and perfusion were fixed via the aorta with neutral (pH 7.4) phosphate-buffered paraformaldehyde (40 g/L) and sucrose (50 g/L) with EDTA (1 mmol/L) at a pressure of 100 mmHg. The heart was then immersed in the fixative above. Synovial fluid was obtained from the knee joint by injection of 3 mL of sterile saline solution into the joint followed by aspiration, after which the knee joints were collected. Synovial fluid saline aspirates were stored at −70 °C until analysis. All procedures involving animals were conducted in compliance with state and federal laws and standards of the US Department of Health and Human Services and were approved by the Wake Forest University Animal Care and Use Committee.

OA severity in individual animals was ranked as none,
mild to moderate, or severe on the basis of gross, radiographic, and histologic assessment of the knee joints as described previously (3). Radiographs of the hips, knees, and feet obtained before necropsy showed OA lesions only in the knee and not in the feet or hips. Lateral and antero-posterior radiographs of the intact knee joints were examined, and each knee joint was opened and examined grossly. Midcoronal sections of tibia from knee joints were examined histologically. No gross or radiographic lesions were observed in knee joints classified histologically as normal or as mild OA. No histologic lesions were present in joints assessed as being without OA, and a mild degree of articular cartilage fibrillation involving the medial tibial plateau was present in joints assessed as having mild OA, whereas in moderate and severe OA, there was a reduction in joint space radiographically, evidence of articular cartilage fibrillation grossly, and moderate to severe cartilage fibrillation involving the medial tibial plateau histologically. Coronary artery atherosclerosis was determined as described previously (17). Atherosclerotic plaque size reported here is the mean intimal area of 15 sections of left circumflex, left anterior descending, and right coronary arteries.

After these studies, YKL-40 was measured in coded samples at Metra by a sandwich ELISA with a monoclonal anti-YKL-40 capture antibody, an alkaline phosphatase-conjugated polyclonal anti-YKL-40 antibody for detection, and \( p \)-nitrophenylphosphate as substrate (Metra YKL-40 assay; Quidel Corporation). The reference intervals are 25–93 µg/L for women and 24–125 µg/L for men, and the minimum detection limit is 20 µg/L (8). Interassay CVs of 2.8% and 3.7% were found at YKL-40 concentrations of 79 and 208 µg/L, and intraassay CVs of 3.5–7.0% were found at YKL-40 concentrations of 36, 86, and 177 µg/L (8). Paired serum and synovial fluid samples were available from 13 animals, in which the OA severity ranged from absent to severe; synovial fluid alone was available from an additional 3 animals with moderate to severe OA.

Data were log transformed if necessary to normalize variance and analyzed by analysis of variance. Data presented are mean ± SE of the untransformed data. Correlations were carried out on untransformed data to examine relationships between individual variables.

YKL-40 was measured in 13 serum and 16 synovial fluid samples from female cynomolgus macaques. Serum YKL-40 was 166-2801 µg/L (Table 1). In the six animals with no detectable OA in the knee joints, serum YKL-40 was <466 µg/L, but it was >466 µg/L in the seven animals with mild to severe OA in the knee. Mean YKL-40 was higher in animals with any severity of OA than in subjects without OA in both serum (1136 ± 331 vs 329 ± 51 µg/L; \( F[1,14] = 9.62; P < 0.01 \)) and synovial fluid (70 ± 24 vs 15 ± 3 µg/L; \( F[1,11] = 10.90; P < 0.01 \)). Serum and synovial fluid concentrations of YKL-40 were highly correlated (\( r = 0.68; P < 0.01 \)). We cannot exclude a contribution of non-knee OA to serum YKL-40 because we did not analyze all joints in detail. However, the hips, knees, and feet were evaluated radiographically, and lesions were observed only in the knees, consistent with previous findings in the macaque.

Among animals with no detectable OA of the knee, the extent of coronary artery atherosclerosis (CAA) was significantly correlated with serum YKL-40 (Fig. 1). No significant relationship between YKL-40 and CAA was observed among the seven animals with OA (\( r = -0.37; P \), not significant).

YKL-40 (human cartilage glycoprotein-39) mRNA is markedly up-regulated in macrophages of developing human atherosclerotic lesions (14). We observed a strong correlation (\( r = 0.85; P < 0.05 \)) between serum YKL-40 and CAA in the six animals with no detectable OA. The origin of the serum YKL-40 in these animals is not known, but it may arise from processes occurring in the atherosclerotic arteries of these animals. The relationship between serum YKL-40 and CAA may represent an important new avenue for investigation, and additional studies should be performed to investigate the possible utility of serum YKL-40 as a marker for vascular disease.

### Table 1. Serum YKL-40 concentrations in animals with and without OA.

<table>
<thead>
<tr>
<th>OA status</th>
<th>YKL-40, µg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>167</td>
</tr>
<tr>
<td>None</td>
<td>219</td>
</tr>
<tr>
<td>None</td>
<td>282</td>
</tr>
<tr>
<td>None</td>
<td>391</td>
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<tr>
<td>None</td>
<td>447</td>
</tr>
<tr>
<td>None</td>
<td>466</td>
</tr>
<tr>
<td>Moderate</td>
<td>466</td>
</tr>
<tr>
<td>Severe</td>
<td>489</td>
</tr>
<tr>
<td>Moderate</td>
<td>564</td>
</tr>
<tr>
<td>Moderate</td>
<td>659</td>
</tr>
<tr>
<td>Moderate</td>
<td>1198</td>
</tr>
<tr>
<td>Severe</td>
<td>1779</td>
</tr>
<tr>
<td>Moderate</td>
<td>2801</td>
</tr>
</tbody>
</table>

![Fig. 1. Correlation between serum YKL-40 and CAA in control animals with no detectable OA.](image)

\( R^2 = 0.74; r = 0.85; P < 0.05 \).
The processes occurring during atherogenesis and the progression of OA exhibit several similarities. Remodeling of the extracellular matrix is a prominent component of OA [for a review, see Ref. (19)], and atherosclerosis (20–24). Degradation and loss of large chondroitin sulfate proteoglycan aggregates has been observed in atherogenesis (20, 25) and in OA (26), and proteoglycan biosynthesis and accumulation of unique proteoglycan epitopes are markedly altered in both diseases (2, 23, 24). These alterations in matrix metabolism and carbohydrate composition might precede or coincide with alterations in YKL-40, which have been associated with remodeling processes (7). The exact function of YKL-40 is not known. To date, no endogenous chitin-like structures have been identified in humans, although a mammalian homolog to a bacterial chitin synthase has been identified that may play a role in the synthesis of hyaluronic acid (27, 28). Interestingly, exogenous chitins and chitosans have been used to stimulate hyaluronic acid synthesis and promote wound healing (29). These findings suggest that YKL-40 function is related to interactions with hyaluronate or other oligo- or polysaccharides in the extracellular matrix. Elucidation of the exact carbohydrate moieties YKL-40 interacts with will provide important information about its role in tissue remodeling and connective tissue metabolism.

In summary, YKL-40 appears to be a serum and synovial fluid marker of OA, as well as a possible marker of vascular and other diseases in which inflammation and/or tissue remodeling occur. The cymolugus macaque represents a particularly useful animal model for the study of YKL-40 because it is closely similar to humans, and because the YKL-40 ELISA assay is specific for human and nonhuman primates and cannot be used in rodent or rabbit models.

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References