High systemic levels of low-density lipoprotein cholesterol: fuel to the flames in inflammatory osteoarthritis?

Wouter de Munter1, Peter M. van der Kraan1, Wim B. van den Berg1 and Peter L. E. M. van Lent1

Abstract
There is increasing evidence that low-density lipoprotein (LDL) cholesterol plays a role in the pathology of OA. Specifically, oxidized LDL (oxLDL), which has been shown to play an essential role during development of atherosclerosis, could be involved in processes such as synovial inflammation, cartilage destruction and bone deformations. OxLDL can activate synovial cells such as macrophages, endothelial cells and synovial fibroblasts, resulting in release of growth factors, MMP and pro-inflammatory cytokines. In this review article, we discuss the role of LDL and oxLDL in OA joint pathology and share our viewpoint of possible mechanisms by which these proteins could influence the development and progression of OA. The proposed theory could provide insight into the aetiopathology of OA and give rise to new potential treatments.

Key words: osteoarthritis, cholesterol, low density lipoproteins, oxidized low density lipoproteins, metabolic syndrome, synovium, inflammation, macrophages, joint pathology, statins.

Introduction
Already at the beginning of the last century, OA was seen as a disease in which metabolic processes play a role. In 1937, Matthew B. Ray (senior physician of the British Red Cross Clinic for Rheumatism) described OA patients as ‘well nourished individuals of a fairly robust type and frequently of a plethoric habit of body’ [1]. Fifty years earlier, John Kent Spender of the Royal Mineral Water Hospital in Bath, UK, described the first stage of OA as the early synovial stage, hinting towards a theory in which synovial pathology precedes other OA pathology, such as cartilage damage and osteophyte formation [2]. During the 20th century, however, OA was typically seen as a wear-and-tear disease, and hardly any research was performed concerning synovial involvement and systemic factors. Nevertheless, over the past two decades, the paradigm of OA has been shifting from a cartilage-specific disease towards that of an organ disease, in which low-grade inflammation and systemic pathology do play an important role. Several recent reviews discuss the role of (systemic) inflammation in OA [3-5]. In this article, we postulate a role specifically for low-density lipoprotein (LDL)-cholesterol in the pathology of inflammatory OA.

Methods
Literature investigating the role of the metabolic syndrome, and specifically LDL-cholesterol, on innate immunity was recapitulated and integrated with OA literature to form a literature-based hypothesis (Fig. 1). In essence, we included literature based on (the combination of) the search terms shown in Table 1 using the PubMed
search engine. Non-English literature was excluded from our search. The main focus of this hypothesis article will be the effects of LDL and oxidized LDL (oxLDL) on OA joint pathology, possible mechanisms by which these proteins influence OA, and potential treatments.

Risk factors for primary OA

OA is recognized as a disease that affects the whole joint, including subchondral bone, ligaments, menisci, peri-articular muscle, infrapatellar fat pad, synovial tissue and articular cartilage [6, 7]. Articular cartilage mainly consists

TABLE 1 Basic search terms for literature

<table>
<thead>
<tr>
<th>Disease</th>
<th>Determinant</th>
<th>Comparator</th>
<th>Outcome</th>
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<tr>
<td>Atherosclerosis</td>
<td>Cholesterol</td>
<td>Macrophages</td>
<td>Inflammation</td>
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<td>Osteoarthritis</td>
<td>LDL</td>
<td>Chondrocytes</td>
<td>Infiltration</td>
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<td>Metabolic syndrome</td>
<td>Oxidized LDL</td>
<td>Fibroblasts</td>
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<td>Endothelial cells</td>
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<td>Monocytes</td>
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<td>Synovium</td>
<td>Damage</td>
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<td>Cartilage</td>
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<td>Bone</td>
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<td>Growth factors</td>
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<td>Pain</td>
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LDL: low-density lipoprotein.
of proteoglycans and collagen type II fibres, in which chondrocytes are embedded. Homeostasis of articular cartilage is constantly balanced by complex interplay between anabolic and catabolic processes. Failure to maintain this balance (by either abnormal loading or abnormal functioning of tissue) lies at the base of OA development and is characterized by release of cartilage fragments, cytokines and catabolic enzymes that could activate synovial cells and further enhance cartilage degradation [7, 8]. OA is, however, not confined only to the joint. Serum levels of proteins such as collagen type II-associated products, COMP, MMPs, adipokines and many more have been widely studied and shown to be increased in OA patients compared with healthy controls (extensively reviewed by Lotz et al. [9] and Hunter et al.[10]). The Dutch Cohort Hip and Cohort Knee (CHECK) study consists of early OA patients (aged between 45 and 65 years) who, on entry, had pain or stiffness of the knee or hip. They had not yet consulted their physician for these symptoms, or the first appointment occurred within 6 months before cohort entry. We recently found that, even in these early patients, high plasma levels of alarmins S100A8/100A9 (>200 ng/ml) increased odds of disease progression (odds ratio 4.0) 2-5 years later, whereas high ESR (up to 31 mm/h) and high levels of CRP (up to 14 ng/l) were not predictive [11]. S100A8 (myeloid-related protein-8) and S100A9 (myeloid-related protein-14) proteins are produced mainly by granulocytes, monocytes and activated macrophages [12–14]. In addition to their function as inflammatory markers [15], these proteins have also been shown to be involved in cartilage damage and synovial activation during OA [16, 17].

Although most of the above-mentioned factors are believed to be the result of, rather than causative for, OA-related joint pathology, it suggests that OA is not merely a local disease. The fact that basic inflammatory markers, such as ESR and CRP, do not always reflect OA pathology might preclude the disease as a common inflammatory disease but does not exclude a systemic component.

Moreover, there are systemic processes that have been shown to increase the risk of primary human OA. Two of these undeniably important risk factors are ageing and the metabolic syndrome. Ageing could induce OA simply via wear and tear, which means that long-term or excessive loading locally increases vulnerability of the joint and, in combination with systemic factors that increase susceptibility, such as weight and female sex, cause cartilage damage [18]. Also, age could induce OA via chondrocyte senescence. Shortening of telomerase length and decrease of mitotic activity in chondrocytes limits the ability of these cells to form new cartilage [19], although this is an unlikely cause of OA in a non-dividing tissue, such as adult cartilage. A more recent theory is that, with age, cartilage balance shifts from homeostasis towards terminal differentiation [20–23]. Finally, ageing also induces low-grade systemic inflammation that could influence OA. The word inflamm-ageing is often used these days, describing a process in which continuous antigen load acts as stressor and eventually results in low-grade systemic inflammation [24]. This low-grade inflammation is coupled to increased levels of pro-inflammatory factors, such as S100A8 and S100A9, that are also important during OA processes [25].

A second important risk factor that is described for primary OA is the metabolic syndrome [26]. The metabolic syndrome, also known as insulin resistance syndrome or metabolic syndrome X, affects ~22% of the United States population, with a slightly higher prevalence in female adults compared with males [27]. The metabolic syndrome includes a variety of conditions that, in combination, cause cardiovascular disease and diabetes. These conditions are high blood pressure, decreased high-density lipoprotein (HDL) levels, increased LDL and triglyceride levels, raised blood glucose levels and abdominal obesity [28]. Obesity has been extensively associated with OA, often leading to the theory that OA is induced via a biomechanical link [29]; however, the association of obesity with development of OA in non-weight-bearing joints [30–32] suggests a systemic mechanism, rather than plain overloading. This, taken together with studies that report no direct relation between obesity and OA [33, 34], supports the recent theory in which the metabolic syndrome, rather than obesity itself, is seen as a risk factor for OA [35, 36]. Obviously, the metabolic syndrome is often accompanied by obesity and, in reality, both mechanisms (overloading and a systemic component) will contribute to initiation and aggravation of OA.

**LDLs in OA**

A study by Gierman et al. [37] showed a relationship between metabolic stress and spontaneous development of OA in mice. A high-fat diet increased cartilage damage independently of body weight, and this effect could be abolished by lowering cholesterol levels, suggesting a cholesterol-mediated mechanism for cartilage damage [37]. In a later study, this group also showed that a cholesterol-rich diet in a murine model for hyperlipidaemia and atherosclerosis led to increased spontaneous cartilage damage, further underlining an important role for total cholesterol [38]. In both studies, the authors claim that metabolic stress-induced inflammation is more likely to be at the base of cholesterol-induced cartilage damage than mechanical overload, because anti-inflammatory intervention suppressed the development of OA. The evidence that atherosclerosis and OA are both associated with the metabolic syndrome and that the cell types that are important in the development of atherosclerosis (such as macrophages and endothelial cells) [39] also play an important role in OA pathology [40, 41] suggests that LDL-cholesterol and LDL modifications (such as oxLDL) play a role in OA pathology.

One of the features of the metabolic syndrome is increased levels of LDL [42]. In the course of metabolic syndrome, high levels of non-esterified fatty acids, together with hyperinsulinaemia and low adiponecetin levels, provide substrate for increased production of very low density lipoprotein (VLDL) in the liver. VLDL,
with its associated apolipoprotein B, is converted to LDL particles, which form the main transport vehicle of cholesterol from the liver to all other cells in the body. Normally, LDL particles reverse cholesterol transport; however, the metabolic syndrome is characterized by low levels of HDL, thus resulting in increased LDL levels [43]. LDL is internalized by cells via the LDL receptor (LDLR), where it releases its cholesterol after lysosomal hydrolysis. Cholesterol is an essential component of the plasma membrane, serving a barrier function, modulating fluidity and forming lipid rafts [44]. After taking up LDL, LDLR production by the cell is decreased, blocking further uptake of LDL and efficiently regulating intracellular cholesterol levels [45, 46]. As a result of this regulated LDL uptake by cells and low levels of HDL, the serum levels of LDL remain high in patients with the metabolic syndrome. Although LDL has been extensively studied in the pathology of cardiovascular diseases [47, 48], it has gained relatively little attention in clinical OA research. Comparative analysis of serological parameters has demonstrated that OA patients have significantly higher serum LDL levels compared with age-, sex- and BMI-matched healthy controls [49, 50].

Also in mice there is evidence that LDL is related to OA pathology. Recently, we observed that increased LDL levels (by LDLR deficiency or a cholesterol-rich diet), in a model for inflammatory OA, increased pathology. Mice with enhanced LDL levels showed increased synovial S100A8 production and TGF-β1 signalling, suggesting synovial activation. Furthermore, we observed increased ectopic bone formation, both at the bone margins and in the collateral ligaments, due to increased levels of LDL [51]. Although we proposed in that study a specific role for oxLDL, the main focus was the indirect effects of high LDL levels on OA processes. In vivo experiments specifically directed to oxLDL or oxLDLRs in the joint could provide conclusive evidence of oxLDL involvement.

Oxidation of LDL and its effects on cellular level

A variety of factors have been proposed to induce LDL oxidation [e.g. sphingomyelinase, reactive oxygen species (ROS), secretory phospholipase-2, other lipases and MPO]. Although the precise mechanism by which LDL is oxidized in vivo is unknown, ROS is generally seen as the main suspect [52–54]. OxLDL can be taken up by endothelial cells, fibroblasts and macrophages, among others, via scavenger receptors (SR) such as SR-A [55], CD36 (SR-B) [56] and lectin-type oxLDL receptor 1 (LOX-1; SR-E) [57, 58]. As these receptors lack the self-regulating mechanism of LDLR, high levels of oxLDL could have far-reaching effects on the cells that take up these molecules. Different cell types have been described to respond upon oxLDL uptake. Fibroblast stimulation by oxLDL is mainly mediated via LOX-1, inducing production of MMP-1 and MMP-3 [59]. Also, endothelial cells can be activated by oxLDL via LOX-1, resulting in increased expression of leucocyte adhesion molecules and enhanced secretion of chemoattractants such as chemokine (C-C motive) ligand 2 [CCL2, also referred to as monocyte chemotactic protein 1 (MCP-1)] [60]. Mostly described, however, is oxLDL uptake by macrophages via SR-A and CD36. A review by Maiolino et al. [61] thoroughly discusses the role of oxLDL uptake by macrophages and the different effects on inflammation. Uptake of oxLDL by macrophages causes production of pro-inflammatory mediators such as IL-6, IL-8, MCP-1 and S100A8/9 [51, 62], matrix-degrading proteinases MMP-1 and MMP-3 [63] and growth factors such as TGF-β-1 [51]. Very recently, Hossain et al. [64] also reported that LOX-1, which is normally expressed in relatively low levels on macrophages, potentially plays an important role in oxLDL uptake by macrophages. Toll-like receptor 4-stimulation strongly up-regulates surface expression of LOX-1 in macrophages, resulting in elevated oxLDL uptake.

In addition to synovial cells, it has also been described that chondrocytes exhibit LOX-1 receptors [65, 66]. Stimulation of chondrocytes by oxLDL results in intracellular ROS production [67], mediates intracellular MMP-3 accumulation and secretion [68] and up-regulates mRNA levels of VEGF and mRNA and protein levels of MCP-1 [69, 70] (Fig. 1, mechanism A). Furthermore, Kishimoto et al. [71] show that bovine chondrocytes, cultured with oxLDL, increase expression of runt-related transcription factor 2, resulting in hypertrophic, type X collagen-producing chondrocytes, thereby suggesting a possible molecular mechanism by which oxLDL could lead to terminal chondrocyte differentiation and cartilage destruction. Nevertheless, studies investigating the effect of oxLDL directly on chondrocytes are scarce and are confined to in vitro experiments.

Oxidized LDL as a potential inducer of OA pathology

Given that the same cells that have been described as being affected by oxLDL in cardiovascular diseases, such as macrophages, endothelial cells and fibroblasts, are dominant cells within synovium, we postulate that oxLDL can play an important role in OA pathology. A substantial population of OA patients develops synovitis, in which cells such as synovial macrophages are suggested to play a key role [3, 40, 72–74]. OxLDL could therefore be a strong modulator of OA pathology. Figure 1 depicts a putative pathway showing how oxLDL influences OA pathology via activation of synovial cells. Increased levels of oxLDL activate synovial cells such as macrophages, fibroblasts and endothelial cells via SRs (Fig. 1, mechanism B). These cells are than able to produce inflammatory and chemotactic factors, stimulating monocyte influx and resulting in aggravated inflammation (Fig. 1, mechanism C) [59, 60, 62, 63]. In mice, we have already shown that increased accumulation of modified LDL in synovium is associated with increased production of S100A8 and S100A9 [51]. Additionally, oxLDL could stimulate synovial fibroblasts to produce catabolic enzymes, as described by Ishikawa et al. [59] and...
stimulate lining macrophages to produce growth factors such as TGF-β [51] (Fig. 1, mechanism D). These processes could then aggravate synovial inflammation, cartilage destruction and ectopic bone formation, eventually increasing oxidative stress and resulting in a vicious cycle of increased OA pathology.

### Treatment

Much is still unknown about the exact role of oxLDL in inflammatory OA; nevertheless, involvement of oxLDL in OA pathology stands to reason. This creates new opportunities for possible therapies. To date, no drug has been consistently found to have modifying effects on the structural progression of OA. While cytokine targeting has been shown to be an effective treatment in RA, there is little evidence that this is also the case in OA. Studies focusing on biologics in OA, such as anti-IL-1 [75–82] and anti-TNF [83, 84], show no convincing therapeutic effects. Hence, current therapies (predominantly involving analgesics and NSAIDs) focus merely on symptomatic relief [85]. More insight into OA progression, and the possible role of (ox)LDL, may provide new leads for effective therapies.

Considering a role for LDL-cholesterol in the development of OA, the use of statins would be one of the most obvious therapies. Statins lower cholesterol biosynthesis and thus systemic levels of LDL. In mice, the statin atorvastatin (lowering cholesterol biosynthesis) could diminish the severity of OA in cholesterol-induced joint pathology, whereas the total-cholesterol-lowering non-statin ezetimibe (limited to inhibiting cholesterol uptake in the intestine) could not [38]. Given that it has been described that statins, in addition to specifically lower serum LDL levels, also reduce development of atherosclerosis by modulating oxLDL-mediated pathology [86, 87], the theory arises that specifically LDL-cholesterol and LDL modifications (such as oxLDL) play a role in OA pathology. Interestingly, another study by the same group showed no effect on cartilage destruction by the statin simvastatin in the spontaneous OA model in SRT/Ort mice [88]. It is important to mention, however, is that these specific STR/Ort mice display abnormal lipidaemic symptoms that could interfere with statin treatment [89].

**Table 2: Evidence for involvement of low-density lipoprotein in OA (in order of appearance in main text)**

<table>
<thead>
<tr>
<th>Reference</th>
<th>Evidence</th>
<th>Species</th>
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<tr>
<td>Gierman et al. [37]</td>
<td>Lowering cholesterol levels decreases cartilage damage during spontaneous OA</td>
<td>Mouse</td>
</tr>
<tr>
<td>Gierman et al. [38]</td>
<td>A cholesterol-rich diet increases spontaneous cartilage damage</td>
<td>Mouse</td>
</tr>
<tr>
<td>Mishra et al. [49]; Oliviero et al. [50]</td>
<td>OA patients have higher serum LDL levels than healthy controls</td>
<td>Human</td>
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<tr>
<td>de Munter et al. [51]</td>
<td>High LDL levels result in increased synovial TGF-β signaling, S100A8/9 production and ectopic bone formation during experimental OA</td>
<td>Mouse</td>
</tr>
<tr>
<td>Ishikawa et al. [59]</td>
<td>In vitro fibroblast stimulation with oxLDL increases MMP-1 and MMP-2 production</td>
<td>Human</td>
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<tr>
<td>Sawamura et al. [60]</td>
<td>In vitro endothelial cell stimulation with oxLDL increases leucocyte adhesion molecules and enhances secretion of CCL2</td>
<td>Human</td>
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<tr>
<td>van Tits et al. [62]</td>
<td>In vitro macrophage stimulation with oxLDL causes production of IL-6, IL-8, CCL2, S100A8/9, MMP-1, MMP-3 and TGF-β</td>
<td>Human</td>
</tr>
<tr>
<td>Galis et al. [63]</td>
<td>In vitro macrophage stimulation with oxLDL causes production of MMP-1 and MMP-3</td>
<td>Rabbit</td>
</tr>
<tr>
<td>de Munter et al. [51]</td>
<td>In vitro macrophage stimulation with oxLDL causes production of S100A8/9 and TGF-β</td>
<td>Mouse</td>
</tr>
<tr>
<td>Nishimura et al. [67]</td>
<td>In vitro chondrocyte stimulation with oxLDL induces intracellular ROS production</td>
<td>Bovine</td>
</tr>
<tr>
<td>Kakinuma et al. [68]</td>
<td>Ex vivo chondrocyte stimulation with oxLDL induces MMP-3 accumulation and secretion</td>
<td>Human</td>
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<tr>
<td>Kanata et al. [69]</td>
<td>In vitro chondrocyte stimulation with oxLDL induces VEGF expression</td>
<td>Bovine</td>
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<tr>
<td>Akagi et al. [70]</td>
<td>In vitro chondrocyte stimulation with oxLDL induces CCL-2 production</td>
<td>Human</td>
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<tr>
<td>Kishimoto et al. [71]</td>
<td>In vitro chondrocyte stimulation with oxLDL induces Runx2 expression and type X collagen production</td>
<td>Bovine</td>
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<tr>
<td>Gierman et al. [38]</td>
<td>Atorvastatin diminishes OA severity, ezetimibe (non-statin) does not</td>
<td>Mouse</td>
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<tr>
<td>Wei et al. [88]</td>
<td>Simvastatin does not affect cartilage damage</td>
<td>Mouse</td>
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<tr>
<td>Akasaki et al. [90]</td>
<td>Mevastatin reduces cartilage damage in experimental OA</td>
<td>Rabbit</td>
</tr>
<tr>
<td>Aktas et al. [91]</td>
<td>Simvastatin diminishes inflammation in experimental knee OA</td>
<td>Rat</td>
</tr>
<tr>
<td>Clockaerts et al. [93]; Kadam et al. [94]; Valdes et al. [95]</td>
<td>Statins are beneficial for OA patients</td>
<td>Human</td>
</tr>
<tr>
<td>Beattie et al. [96]; Riddle et al. [97]</td>
<td>Statins do not affect disease outcome in OA patients</td>
<td>Human</td>
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CCL: C-C motive ligand; LDL: low-density lipoprotein; oxLDL: oxidized low-density lipoprotein; ROS: reactive oxygen species; Runx2: runt-related transcription factor 2.
other in vivo animal studies show beneficial effects of statins on cartilage degradation in animal OA models [90, 91]; however, evidence of beneficial effects in human OA is scarce [92]. A Dutch prospective population-based cohort study [93] and two studies from the UK [94, 95] show positive effects of statins, whereas two studies from the USA do not show beneficial effects [96, 97]. The controversies regarding statin use as a treatment for OA could be explained by the assumption that, besides high LDL-cholesterol levels, specific oxidative triggers also play a role in OA pathology; emphasizing that high LDL levels alone are not enough to induce/aggravate OA without oxidation.

Perhaps combining statin treatment with antioxidants could result in decreased OA morbidity; however, even in atherosclerosis, where oxLDL involvement is apparent, a beneficial role for antioxidants has not been proved [98, 99].

Specifically blocking the mechanism by which oxLDL could lead to joint pathology might be a better option. For example, locally blocking SRs, which are the main receptors for oxLDL, could stop uptake of oxLDL by synovial cells and prevent progression of OA. It would be worthwhile to investigate the role of SR inhibition in OA pathology.

Conclusion

In this viewpoint article, we discuss the increasing in vitro and animal model-based evidence that LDL-cholesterol plays a role in OA pathology. Specifically, oxidized LDL, which has been shown to play an essential role in development of atherosclerosis, could be involved in processes such as synovial inflammation, cartilage destruction and ectopic bone formation (Table 2). Lowering local levels of oxLDL could be an effective treatment strategy in early OA. In vivo studies focusing on the direct role of oxLDL during OA and local blocking of oxLDL-mediated processes could give more insight into the role of oxLDL and could provide new leads for effective treatment of patients with OA.

Funding: No specific funding was received from any funding bodies in the public, commercial or not-for-profit sectors to carry out the work described in this manuscript.

Disclosure statement: The authors have declared no conflicts of interest.

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