HYPOTHESIS

Does endotoxaemia contribute to osteoarthritis in obese patients?

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ABSTRACT

OA (osteoarthritis) is a degenerative condition associated with obesity. A number of metabolic explanations have been proposed to explain the association between obesity and OA in non-weight-bearing joints; however, none of these hypotheses have been demonstrated empirically. In the present Hypothesis article, we recognize that obesity is associated with compromised gut mucosa, translocation of microbiota and raised serum LPS (lipopolysaccharide). The consequent activation of the innate immune response leads to increased serum titres of inflammatory mediators in obese patients, with both local and systemic markers of inflammation associated with onset and progression of OA. Furthermore, a number of workers have shown that articular cartilage repair is impaired by a range of inflammatory mediators, both in vitro and in vivo. We propose that metabolic endotoxaemia, caused by impaired gastric mucosa and low-grade chronic inflammation, may contribute to the onset and progression of OA in obese patients. This may account for the association between obesity and OA at non-weight-bearing joints which cannot be explained by biomechanical factors.

INTRODUCTION

OA (osteoarthritis) is a chronic syndrome of articular cartilage degeneration comprising synovitis (inflammation of the synovial membrane) and the formation of abnormal bone growths (osteoophytes) at the margins of the joints. At the cellular level, there is disequilibrium between matrix synthesis and degradation of the connective tissue cells (chondrocytes) [1,2]. Catabolic activities, such as secretion of degradative proteases, are up-regulated, whereas anabolic activities, including collagen and proteoglycan synthesis, are suppressed [1,2]. Although OA is often described as a non-inflammatory disease, it is now apparent that inflammation is an important contributory factor, although the underlying pathogenesis of OA remains unclear. However, the physiological consequences for patients with OA are severe and include joint pain, stiffness and swelling. At present, OA is the most common form of arthritis and the leading cause of long-term disability in the U.S.A. Although associated with age, gender and joint injury, obesity is a particularly significant risk factor [3]. This has been demonstrated by studies comparing patients awaiting total knee arthroplasty against age-matched controls. Patients with a BMI (body mass index) of <20 were considerably less likely {OR (odds ratio), 0.1 [95 %

Key words: endotoxaemia, endotoxin, lipopolysaccharide, obesity, osteoarthritis.

Abbreviations: AGE, advanced glycation end-product; BMI, body mass index; CI, confidence interval; CRP, C-reactive protein; ECM, extracellular matrix; IL, interleukin; KOA, knee OA; LPS, lipopolysaccharide; MMP, matrix metalloproteinase; NF-κB, nuclear factor κB; NOS, nitric oxide synthase; OA, osteoarthritis; OR, odds ratio; TJ, tight junction; TLR, Toll-like receptor; T2DM, Type 2 diabetes mellitus; TNF, tumour necrosis factor; ZO, zonula occludens.

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One suggests that atherosclerosis in subchondral bone might compromise articular cartilage nutrition. Evidence for this explanation includes the association between OA and cardiovascular mortality, and the value of hypercholesterolaemia in predicting involvement of multiple joints [15]. Although plausible, this hypothesis has not been tested empirically.

Another hypothesis has indicated that adipocytokines, hormones secreted by adipocytes, might contribute to OA, with several lines of evidence to support this theory. Studies have shown that adiponectin, leptin and visfatin are elevated in the infrapatellar fat pads of patients with KOA [16,17], whereas resistin levels are increased in sera and synovial fluid, following traumatic joint injuries [17]. Leptin, adiponectin and visfatin are also known to increase production of MMPs (matrix metalloproteinases) in chondrocytes, which degrade articular cartilage, indicating a catabolic role for these adipocytokines [17,18]. Furthermore, leptin and adiponectin increase IL (interleukin)-6 levels and induce NOS2 (nitric oxide synthase 2), which is central to the synthesis of NO (nitric oxide), a key inflammatory mediator. NO is activated by numerous pro-inflammatory cytokines and chemokines and is known to instigate chondrocyte loss, apoptosis and MMP activation [17]. Patients with severe OA also exhibit higher synovial fluid leptin levels than serum concentrations [18], with higher adiponectin serum levels observed in patients with erosive OA compared with non-erosive OA [17]. Chondrocytes express the leptin functional receptor and high concentrations reduce ECM (extracellular matrix) synthesis in cultured chondrocytes [19]. It has also been observed that advanced osteoarthritic cartilage shows more expression of both leptin and its receptor than minimally osteoarthritic cartilage [20]. However, this model does not account for the evidence suggesting leptin increases chondrocyte synthesis of collagen and proteoglycans [19], whereas studies have also indicated that adiponectin can inhibit MMP-13-mediated induction of IL-1β and may up-regulate the expression of TIMP2 (tissue inhibitor for MMP-2), thus potentially reversing degenerative change [17]. In addition, studies in rodents have shown that artificial leptin administration into rat knees stimulates IGF-1 (insulin-like growth factor-1), TGF-β (transforming growth factor-β) and proteoglycan synthesis [20], which may reduce OA. However, these changes could account for osteophyte formation, which is an important radiographic finding in OA.

A third metabolic explanation postulates a role for insulin resistance. Although associated with an elevated BMI, insulin resistance is independently associated with OA. In a study of overweight patients consisting of BMI- and age-matched cohorts, patients with OA were shown to have higher circulating insulin titres than those without [21], and elevated blood glucose concentrations (>5 mmol/l) were found, predominantly, in overweight patients with OA. A number of mechanisms have been
proposed to explain this association. First, chondrocytes (the cellular component of cartilage) are insulin-sensitive and insulin resistance could impair secretion and maintenance of the cartilaginous matrix. Secondly, AGEs (advanced glycation end-products), a consequence of T2DM (Type 2 diabetes mellitus), induce intracellular signalling cascades in chondrocytes, and animal work suggests that artificially elevated intra-articular AGEs predispose to advanced OA [22].

**METABOLIC ENDOTOXAEMIA AND OA**

**OA and inflammation**

Our hypothesis suggests a role for chronic low-grade inflammation in OA pathophysiology, as the importance of inflammation to OA onset and progression is becoming increasingly recognized. It is widely established that acute-phase proteins are elevated in OA and that CRP (C-reactive protein), a marker of systemic inflammation, correlates positively with severe cartilage degeneration [23]. Serum CRP levels are significantly increased in patients with rapidly destructive hip OA relative to those with slowly progressing disease [24]. Systemic CRP levels are also strongly associated with knee pain and YKL-40, a glycoprotein product of chondrocytes and marker of synovial inflammation [24]. Similarly, CRP is elevated in early OA, even once differences are adjusted for weight, and predicts disease progression [25].

Other pro-inflammatory cytokines have also been implicated in OA, particularly IL-1, IL-6, IL-7 and TNF (tumour necrosis factor)-α. Serum TNF-α, IL-1β, IL-6 and IL-8 are all elevated in patients with temporomandibular joint OA [26]. IL-1 reduces ECM synthesis while simultaneously increasing the production of MMPs and promoting the inflammatory response. Suppression of these processes has been achieved using an IL-1Ra (IL-1 receptor antagonist), which reduces OA progression in a canine model [27]. Pro-inflammatory cytokines appear to act synergistically in mediating cartilage degeneration. For instance, IL-1 and TNF-α together increase the synthesis of IL-6 production which, synergistically with IL-1, promotes collagen degeneration [28]. Evidence suggests the infrapatellar fat pad represents a rich source of local IL-6 production, which could contribute to articular cartilage degeneration in knee OA [29]. Other pro-inflammatory cytokines have also been implicated in OA, for example synovial fluid IL-15 which is elevated in end-stage knee relative to early disease [30].

**Obesity and inflammation**

Obesity is now understood to possess a significant inflammatory component which, although as yet remains unproven, may be important in the development of inflammation in OA. The importance of adiposity in the pathogenesis of OA is highlighted further by a randomized weight-loss trial in which 10% weight loss improved KOA, with the greatest improvements observed with a reduction in fat percentage [31]. Such benefits are not that surprising considering that adipose tissue possesses multiple pro-inflammatory properties; adipocytes express numerous pro-inflammatory chemotactic agents, including MCP-1 (monocyte chemotactic protein-1) and CCR2 (CC chemokine receptor 2). Macrophages accumulate in human abdominal visceral and subcutaneous adipose tissues and their proportion in mouse adipose tissues is positively correlated with total body mass [32].

Fatty acids are pro-inflammatory as they activate key receptors in the innate pathway and up-regulate TLR (Toll-like receptor)-4 signalling in both macrophages and adipocytes [33]. Deletion of TLR-4 reduces inflammatory signalling by these cell lines [34]. TLR-4 expression in human adipose is increased in patients with obesity and diabetes and is likely to be up-regulated during adipocyte differentiation [35].

CRP is another pro-inflammatory factor positively correlated with BMI in otherwise healthy individuals, and its elevation in obesity may be a consequence of IL-6 production. IL-6 is secreted by subcutaneous adipose tissue and increases hepatic CRP production. In addition, serum CRP is positively correlated with adipose tissue expression of IL-6, and IL-6 is necessary for human CRP gene expression in transgenic mice [36]. Adipocytes also co-ordinate inflammation through the secretion of leptin and adiponectin. Other inflammatory components increased in obesity include endothelial adhesion molecules, for example E-selectin and ICAM-1 (intercellular adhesion molecule-1), which are increased in overweight children and in subjects with T2DM [37].

**Intestinal permeability and endotoxin**

Both obesity and OA are aetiologically linked with low-grade chronic inflammation. A number of findings have suggested that low-grade chronic inflammation can result from the absorption of endotoxin across the intestinal tract [35,38,39]. Emerging evidence also suggests that endotoxin absorption is positively correlated with obesity [40–42], with recent studies demonstrating a significant and prolonged elevation in circulating endotoxin levels in obese and T2DM subjects following post-prandial high-fat intake [43]. Further to this, findings by Griffin et al. [44] have described the OA-inducing effects of a high-fat diet in mouse models, paralleled with increased levels of inflammatory cytokines. As such, endotoxin may be a contributing factor to the onset and progression of OA in obese patients. A potential model for this interaction is represented by Figure 1.
The intestinal mucosa provides a selectively permeable barrier between the circulation and intestinal lumen contents. Paracellular transport through the intact epithelial cell layer occurs through apical junctional complexes, composed of TJs (tight junctions) and adherens junctions. This structure is illustrated by Figure 2. TJs are composed of intracellular [ZO (zonula occludens) and catenin family members] and transmembrane (claudins, occludins and junctional adhesion molecules) proteins. They regulate barrier permeability in response to physiological and pathological stimuli, including polyunsaturated fatty acids and pro-inflammatory cytokines, particularly IFN (interferon)-γ and TNFα [45]. The intestinal mucosa permits limited paracellular transport of bacterial LPS (lipopolysaccharide), and TJ dysfunction increases intestinal permeability to such toxic luminal contents [46].

In addition, evidence from murine models suggests that obesity is associated with endotoxin absorption [38]. Although obesity results in enhanced nutrient absorption, this is probably a consequence of increased absorptive mucosal surface. However, two strains of leptin-deficient mice (ob/ob and db/db) exhibiting hyperphagia and obesity were found to have increased intestinal permeability and portal endotoxaemia. Confocal microscopic analysis revealed disrupted occludin and ZO-1 proteins in the ileum of these mice [38]. Further evidence comes from modulation of murine microbiota with Bifidobacteria spp., which reduced plasma LPS and inflammatory markers as well as improving TJ integrity [40]. Emerging evidence in human subjects supports an association between obesity and subacute endotoxaemia. Endotoxin levels are increased in sedentary males relative to those undergoing regular high-intensity exercise [47], and correlate with BMI and waist circumference in apparently healthy Chinese subjects (P<0.01) [42].

A number of mechanisms could explain the observation of endotoxaemia in obese mice. First, diet may impair intestinal barrier function through effects on intestinal flora or motility; the intestines of mice fed on a high-fat diet are colonized by a greater proportion of LPS-containing bacteria [40]. Introduction of dietary fibre reduces the proportion of Gram-negative bacteria in the gut lumen and plasma endotoxaemia [48]. Secondly, the ecology of murine gut microbiota is altered by obesity [41], an effect potentially mediated by insulin resistance as reduced intestinal motility and bacterial overgrowth are observed in both hyperglycaemic and hyperinsulinaemic states [49]. Hyperglycaemia increases gut mucosal permeability in LPS-treated rats independently of the plasma insulin concentration. However, insulin can also act directly on the intestine to increase gut absorption. One large cohort study demonstrated increased endotoxin activity in diabetics compared with non-diabetic individuals [50] and serum LBP (LPS-binding protein) was found to correlate with indicators of insulin resistance [including insulin, HbA1c (glycated haemoglobin) and HOMA (homeostatic model assessment)-1] in apparently healthy Chinese subjects (P<0.05). The role of insulin resistance in OA has been discussed previously [1,15,16], but endotoxaemia could provide a further mechanism to explain this association.

Finally, obesity is a disorder of chronic low-grade inflammation and inflammation has been implicated in impaired intestinal permeability. Patients with Crohn’s disease have increased absorption of PEG400 [poly(ethylene glycol) 400] and lactulose when compared with healthy controls [51]. Pro-inflammatory cytokines in obese patients may disrupt TJs, impairing the intestinal barrier to gut microbiota. TNF-α modifies permeability by acting on TJs and reduces the expression of the P-glycoprotein MDR (multidrug resistance)-1 [52]. It alters the lipid composition and fatty acyl structure of phospholipids in microdomains at TJs and increases translocation of Escherichia coli through a monolayer of glutamine-starved epithelial cells in vitro [53]. In addition, treatment of Crohn’s disease with the anti-TNF infliximab restores the intestinal barrier [54], although
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Evidence suggests a complex relationship between metabolic factors, inflammation and intestinal permeability. For example, TNF-α causes insulin resistance by increasing serine phosphorylation of IRS (insulin receptor substrate) and hyperglycaemia-enhanced intestinal permeability is mediated by this cytokine [55]. Dietary fatty acids activate TLR-4 in adipocytes and macrophages and deletion of this receptor impairs inflammatory signalling [33,34]. Furthermore, TLR-4 gene transcription is enhanced during adipocyte differentiation. Metabolic endotoxaemia may also initiate obesity and insulin resistance [40], suggesting a positive-feedback loop between intestinal permeability and these two metabolic states.

Transport of luminal contents across the intestinal mucosa initiates the innate pathway through binding of TLRs to bacterial antigen, for example LPS, a component of the Gram-negative cell membrane. Activation of TLRs results in transduction of NF-κB to the nucleus and subsequent transcription of the inflammatory mediators IL-1, IL-6 and TNF-α. LPS has been shown to up-regulate TLR-2 expression with the induction of both IL-6 and TNF-α in human adipocytes [35].

Many studies of gut barrier function have shown an impairment that results in plasma endotoxaemia [40,46]. Circulating endotoxin may, in turn, compound injury at the intestinal barrier by promoting mucosal immunodeficiency [46]. Murine studies [38,40] have shown that continuous infusion of endotoxin increased gut permeability, as did feeding on a high-fat diet. Although this mechanism is not well understood, LPS has a particular affinity for chylomicrons, i.e. lipoproteins responsible for transporting fatty acids across the intestinal wall. This affinity has been implicated in the post-prandial inflammatory response [56] and may account for the translocation of LPS across the intestinal wall. Endotoxaemia could therefore partially explain the chronic low-grade inflammation associated with obesity.

Metabolic endotoxaemia has been associated with clinical effects at distant locations around the body, including T2DM [35], non-alcoholic steatohepatitis [38] and cardiovascular disease [57]. In light of increasing evidence for the role of insulin resistance, impaired gut permeability, endotoxaemia and low-grade chronic inflammation, we propose that this mechanism could promote articular cartilage degeneration, thus increasing the risk of OA onset and progression among obese patients.

**TESTING THE HYPOTHESIS**

Our hypothesis relies on a number of discreet steps: each of which has been demonstrated experimentally and/or in population studies. First, obesity is associated with insulin resistance. Secondly, insulin resistance is associated with intestinal mucosa dysfunction [34,37]. Thirdly, intestinal mucosa dysfunction causes the translocation of LPS into the circulation, endotoxaemia...
and increased serum titres of pro-inflammatory cytokines [35,38,43]. Finally, pro-inflammatory cytokines have a detrimental effect on articular cartilage and thus may cause, precipitate or promote OA in obese patients.

However, the hypothesis has not yet been tested in its entirety. It has been established that obesity is associated with metabolic endotoxaemia and chronic low-grade inflammation [38,40–42,47]. *In vitro* studies have demonstrated that inflammatory mediators disrupt articular cartilage [28,29], whereas exposure of human chondrocytes to endotoxin induces NOS [58]. Furthermore, the activation of macrophages in the synovial fluid, via TLR-4, which is also activated by endotoxin, offers potential mechanisms via which endotoxinaemia may induce OA. However, it remains to be shown that circulating endotoxin accentuates the local inflammatory response in OA and/or contributes to cartilage degeneration in diseased joints. As such, the effects of circulating endotoxin may be most readily demonstrated using animal work. For example, metabolic endotoxaemia can be artificially induced in mice either by modifying intestinal permeability [48] or directly through the injection of endotoxin [59]. The articular cartilage of treated mice could be compared with that of controls to demonstrate or refute the contribution of circulating endotoxin.

Although human studies may be limited by confounding factors (for example BMI), the hypothesis could be tested by determining whether endotoxaemia correlates with OA in non-weight-bearing joints. A retrospective or prospective study design could control for confounding factors, for example BMI and insulin resistance.

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