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EXTENDED REPORT

Osteoarthritis development is induced by increased dietary cholesterol and can be inhibited by atorvastatin in APOE*3Leiden.CETP mice—a translational model for atherosclerosis

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ABSTRACT

Objective Hypercholesterolaemia, a risk factor for atherosclerosis (ATH), has been suggested to have a role in the development of osteoarthritis (OA). To test this hypothesis, the effect of cholesterol and different cholesterol-lowering treatments on OA was investigated in a mouse model resembling human lipoprotein metabolism.

Methods Female ApolipoproteinE*3Leiden.human Cholesteryl Ester Transfer Protein mice received a western-type diet with 0.1% (w/w) cholesterol (LC), 0.3% (w/w) cholesterol alone (HC) or treated with 3 mg/kg/day atorvastatin or 0.3 mg/kg/day ezetimibe. One group remained on chow (control). After 39 weeks, OA grades of the knees and the extent of ATH were determined. Plasma cholesterol levels were measured throughout the study.

Results LC and HC groups developed significantly more OA at the medial side than the control group in a dose-dependent manner. Atorvastatin but not ezetimibe treatment significantly suppressed OA development. As expected, features of ATH were significantly increased in the LC and HC groups compared with the control group and suppressed by atorvastatin (48%) and ezetimibe (55%) treatment. There were significant correlations between the development of OA on the medial side of the joint and cholesterol exposure (r=0.4) or ATH features (r=0.3).

Conclusions Dietary cholesterol and accordingly increased plasma levels play a role in the development of OA. The correlation found between OA, cholesterol and ATH demonstrates that these variables are connected, but indicates the contribution of other ongoing processes in the development of OA. The suppressive effect on OA development of atorvastatin but not of ezetimibe, which had similar cholesterol exposure levels, corroborates these findings.

Osteoarthritis (OA) is a degenerative joint disease with major implications on the quality of life. Recently, various studies presented a relation between OA and the prevalence of metabolic syndrome (MetS).[1–3] MetS is generally considered a combination of obesity, hypertension, dyslipidemia and impaired glucose tolerance.[4] Puenpatom and Victor[5] demonstrated that prevalence of MetS was over twofold higher in the OA population, and OA was associated with an increased risk of having MetS.[5] These results have led investigators to contemplate common underlying pathologies in OA-related and MetS-related diseases.

Related to MetS is hypercholesterolaemia that can result as a consequence of abnormalities in the cholesterol uptake in the gut and/or improper regulation of plasma cholesterol levels. Interestingly, hypercholesterolaemia was shown to be associated with generalised OA.[6] Furthermore, serum cholesterol and triglycerides are linked with the incidence of bone marrow lesions, which are common in OA patients.[7] Some studies indicate that cholesterol induces low-grade inflammation in humans and mice and that this is dependent on the amount of cholesterol in the diet.[8 9] Low-grade inflammation is also designated to play a role in the development of OA, which suggests that it could be the link between elevated cholesterol levels and OA.[10]

An extended exposure to increased cholesterol levels can lead to the development of atherosclerosis (ATH).[11 12] The question is raised whether ATH and OA are linked by common underlying mechanisms.[13] This suggestion is strengthened by the finding that ATH was associated with OA in several joints in women.[14 15] Furthermore, it has been hypothesised that atheromatous vascular disease is linked to OA and is probably involved more in the progression of OA than in its initiation.[16] It is unclear whether OA and ATH occur as concurrent diseases due to common aetiology or are causally related.[13]

Cholesterol-lowering interventions are used to prevent harmful consequences caused by high cholesterol exposure (eg, ATH).[17] Statins, 3-hydroxy-3-methylglutaryl-coenzyme A (HMG Co-A) reductase inhibitors, are commonly used and have been reported to possess pleiotropic effects independent of their cholesterol-lowering capacity. Statins are also suggested to have important therapeutic value in the context of OA.[18–20] Another drug used to control cholesterol levels, but with a different mode of action to that of statins, is ezetimibe, which inhibits the uptake of cholesterol in the intestine.

Given the emerging evidence that in man cholesterol levels are linked to OA features, we

questioned whether increased intake of cholesterol would be sufficient to induce OA. We used the ApolipoproteinE*3Leiden. Human Cholesteryl Ester Transfer Protein (APOE*3L.CETP) transgenic mouse, which is a well-established model for hyperlipidaemia and ATH and resembles human lipoprotein metabolism in contrary to wild-type mice.[21] Statin and ezetimibe treatments were included to further understand the contribution of cholesterol in the development of OA. The dosages of the treatments were chosen to result in similar plasma cholesterol levels as obtained in mice receiving low cholesterol concentrations in their diet. This enabled us to study the effects of the drug interventions on OA and ATH independent of, but also in addition to, their cholesterol-lowering properties.[22 23]

METHODS

Mice

Female APOE*3Leiden mice, characterised by an ELISA for human APOE, were crossbred with human CETP transgenic mice, which express CETP under control of its natural flanking regions in our animal facility to obtain heterozygous APOE*3L.CETP mice.[24–28] Mice were housed in groups under standard conditions with a 12-hour light–dark cycle and had free access to water and food. Body weight (BW) and food intake were monitored during the study. Experiments were approved by the institutional Animal Care and Use Committee of TNO and were in compliance with European Community specifications regarding the use of laboratory animals.

Experimental design and diets

Mice (n=78) were fed standard lab chow (V1534 Ssniff Spezialdiäten GmbH, Soest, Germany) until the start of the study at the age of 10–16 weeks. At t=−4 weeks, 66 mice received a semisynthetic western-type diet (WTD) (AB diets, the Netherlands) supplemented with 0.1% (w/w) cholesterol (low cholesterol, LC group). This resulted in moderately elevated plasma cholesterol levels of mean±SD 10.1±1.1 mmol/l. Twelve mice remained on chow (control group) (plasma cholesterol level of mean±SD 3.5±0.9 mmol/l). After 4 weeks (t=0), mice receiving LC (n=66) were randomised into four groups of 12 animals with on average comparable age, BW, plasma cholesterol and triglyceride levels. Of these four groups, one continued on LC (LC group) and the others switched to a WTD containing 0.3% (w/w) cholesterol (high cholesterol HC group) or HC supplemented with 3 mg/kg/day (0.0036% (w/w) atorvastatin (*Lipitor*, Pfizer, the Netherlands) (HC atorvastatin group) or 0.3 mg/kg/day (0.0003% (w/w) ezetimibe (*Ezetrol*, OPG Pharma, the Netherlands) (HC ezetimibe group). Mice that did not respond to the LC diet were sacrificed immediately (n=18). Atorvastatin and ezetimibe dosages were chosen to reduce plasma cholesterol to the same extent as the levels in the LC group. Mice were sacrificed after 39 weeks (t=39), reaching an age of 53–59 weeks.

Lipid and lipoprotein analysis

Blood samples were collected in EDTA tubes (Sarstedt, Germany) after 4 h of food deprivation by tail incisions at t=−4, 0, 2, 6, 10, 14, 18, 22, 26, 30, 34 and 38 and by heart punction at t=39 weeks. After centrifuging for 10 min at 6000 rpm, plasma was collected. Total plasma cholesterol (Roche Diagnostics, No-1489437) was determined immediately. For lipoprotein profiles, pooled plasma of each group was fractionated using an Äkta FPLC system (Pharmacia, the Netherlands) and analysed for their cholesterol-containing fractions. Thereafter, plasma was stored at −80°C. Total cholesterol exposure (mmol/l * time in weeks) was calculated for each mouse.

Plasma inflammation markers

Alanine aminotransferase (ALT) was determined spectrophotometrically in pooled plasma samples at t=0, 18 and 38 weeks using a reflotron system (Roche diagnostics, USA). Serum Amyloid A (SAA) (Tridelta development, Ireland) (t=0, 18 and 38 weeks), E-selectin and monocyte chemoattractant protein-1 (MCP-1) (both R&D Systems Inc., USA) (t=39 weeks) levels were determined in the individual plasma samples by ELISA according to the manufacturers' instruction. Interleukin 1 (IL-1), IL-6, tumour necrosis factor-alpha (TNF-α) and interferon γ (IFN-γ) levels were determined in plasma samples (t=39 weeks) using multiplex analysis according to the manufacturers' instruction (Millipore, Billerica, USA and Invitrogen, Paisley, UK).

Histological examination of OA and ATH

The knee joints of the left hind limbs were fixed in formalin (Sigma-Aldrich, USA), decalcified in Kristensen solution,[29] dehydrated and embedded in paraffin. Serial coronal 5 μm sections were collected and stained with haematoxylin, fast green and safranine-O and with haematoxylin, phloxine and saffron. Sections were scored by two independent observers, who were blinded for group assignment, at six locations in the joint; femoral condyle and tibial plateau at the lateral and medial side, trochlear groove and the patella (scores 0–6).[30] The whole joint was scored following the guidelines of the Osteoarthritis Research Society International grading system including cartilage structure, proteoglycan depletion and chondrocyte morphology.[31]

Hearts were isolated, formalin-fixed, embedded in paraffin, sectioned and assessed for ATH features (see online supplementary figure S1). ATH lesion areas were calculated and classified into five categories according to the American Heart Association.[32] Types I–III were classified as mild and types IV–V lesions as severe lesions. For each mouse, the percentage of all lesions found in the respective categories was calculated.[25]

Statistical analysis

Data are presented as mean±SD. Statistical differences were assessed using the non-parametrical Kruskal–Wallis test followed by Mann–Whitney U test (OA scores, ATH lesion area, total cholesterol exposure and MCP-1 and E-selectin levels). BW, cholesterol and SAA levels were tested with repeated-measure analysis followed by Dunnett T3 post hoc test. OA scores, ATH lesion area and cholesterol exposure correlations were calculated with the Spearman correlation test. A partial correlation was performed for OA scores and ATH lesion areas after correction for cholesterol exposure. Inter-observer correlation for OA scores was measured with Spearman correlation test. SPSS V.20.0 for Windows (SPSS, USA) was used for statistical analyses. For all the analyses, p less than 0.05 was considered significant.

RESULTS

Mice characteristics

Every 2 weeks, BW of all mice was measured and revealed no significant differences between groups during the course of the study (figure 1A).

To assess the effects of cholesterol intake, plasma cholesterol levels were determined every 4 weeks (mmol/l). The LC and HC groups had significantly higher cholesterol levels over time than the control group (both $p<0.001$) (figure 1B). This was

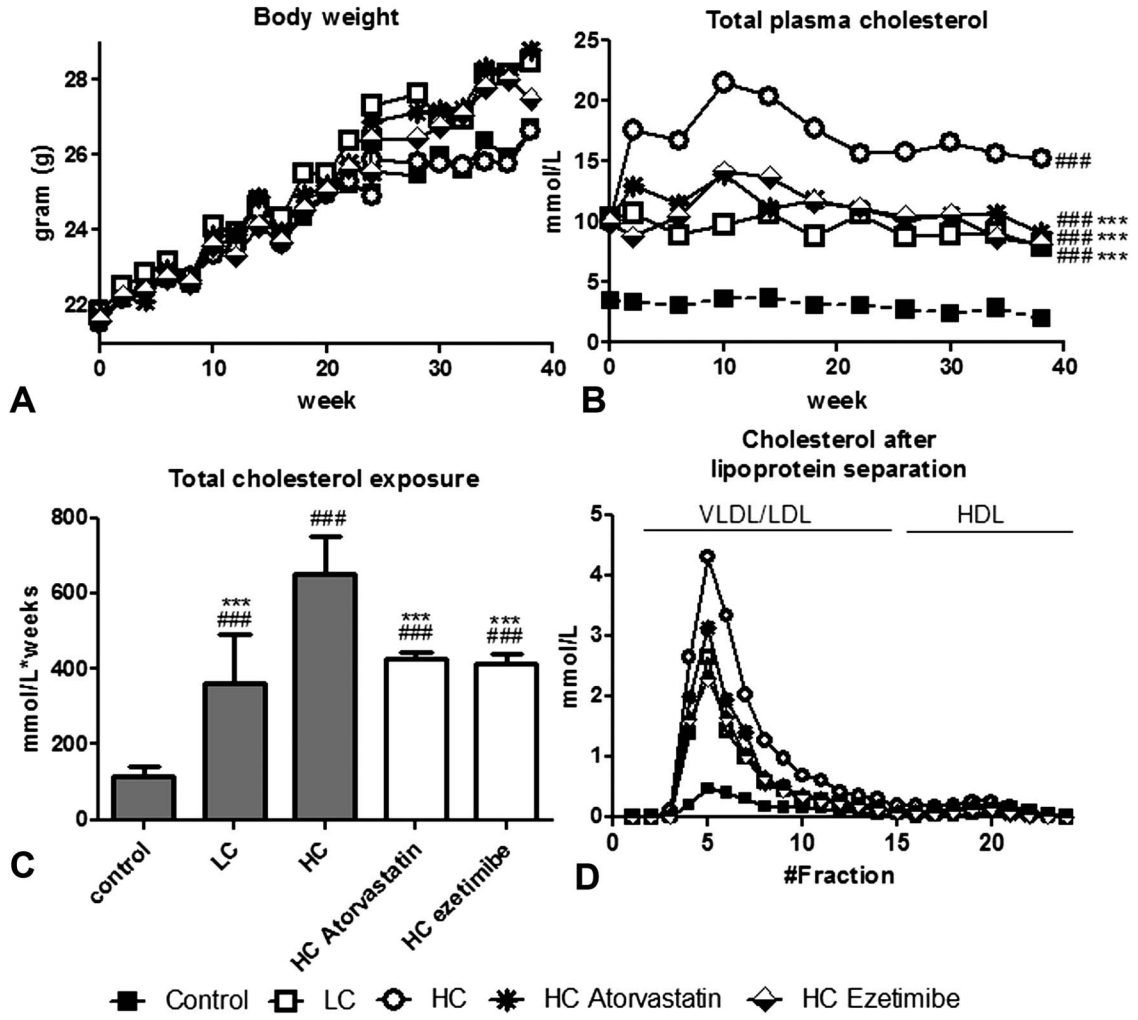

Figure 1 Characteristics of APOE*3Leiden.CETP mice on a control, low-cholesterol and high cholesterol diet (A) Body weight (g), (B) total plasma cholesterol levels (mmol/l), (C) total cholesterol exposure (mmol/l × weeks) in the complete study, (D) representative cholesterol distribution after lipoprotein separation (mmol/l) at t=34 weeks. Fractions 4–15 contain the ApoB containing lipoproteins (very/low-density lipoprotein) and fractions 16–24 contain the HDL particles. Control (n=12), low cholesterol (LC) (n=10), high cholesterol (HC) (n=12) or HC group treated with atorvastatin (n=12) or ezetimibe (n=11) (HC atorvastatin and HC ezetimibe), ###p<0.001 versus control; ***p<0.001 versus HC.

confirmed by the total cholesterol exposure (mmol/l×time in weeks) (figure 1C); the LC group had 3.1 and the HC group had 5.6 times more cholesterol exposure than the control group (both p<0.001).

Elevated cholesterol levels due to HC were significantly reduced by atorvastatin and ezetimibe treatment (both p<0.001) to similar levels as the LC group (figure 1B). Atorvastatin reduced the total cholesterol exposure levels by 35% and ezetimibe by 38% (both p<0.001), to a level comparable as in the LC group (figure 1C). In general, lipoprotein profiling revealed that the reduction in total cholesterol by the atorvastatin and ezetimibe treatments was mainly confined to the (very–)low-density lipoprotein fractions, which is demonstrated by representative data obtained at t=34 (figure 1D).

Inflammation markers

The acute phase protein SAA, the liver damage marker ALT, the adhesion molecule E-selectin and the pro-inflammatory chemokine MCP-1 were measured as functional markers for inflammation. SAA levels were higher in the LC and HC compared with the control group during the course of the study (non-significant). The LC and HC groups showed a tendency for higher ALT levels, but significance could not be tested since this parameter could only be measured in pooled plasma. At end point, MCP-1 levels in the LC and HC groups were significantly higher than in the control group (p<0.001). E-selectin levels were significantly higher in the HC group than in the control group (p<0.001).

After atorvastatin and ezetimibe treatment, mice had lower SAA and ALT levels compared with the untreated HC group (not significant). Both treatments resulted in significantly reduced E-selectin levels (p<0.01). MCP-1 levels were not affected by the treatments (table 1).

IL-1, IL-6, TNF-α and IFN-γ levels in the plasma samples at endpoint were below the lowest level of detection in the multiplex assays.

Osteoarthritis development

The knee joints were analysed to assess the effect of increased dietary cholesterol on OA development (inter-observer rate R=0.8, p<0.001). Representative histological pictures are presented in figure 2A. Mice developed significantly more OA features at the medial side of the joint in the LC (p=0.03) and HC (p=0.01) groups than in the control group (figure 2B). No effects of increased cholesterol intake were seen on the lateral side or on the trochlear groove. When giving an integrated score

Table 1 Functional plasma markers for inflammation

	SAA (μg/ml)			ALT (μg/ml)			E-selectin (ng/ml)	
t=0	t=18	t=38	t=0	t=18	t=38	t=39	t=39	MCP-1 (pg/ml)
Control	4.6 (4.6)	2.3 (0.9)	7.0 (4.5)	59.7	56.9	51.3	40.6 (9.4)	26.1 (7.7)
LC	3.4 (0.8)	3.1 (1.0)	7.4 (4.2)	43.9	68.3	169	41.8 (6.8)***	45.5 (12.2)*
HC	3.75 (0.61)	5.9 (2.1)	9.1 (5.0)	50.2	109	192	52.9 (7.3)*	51.4 (11.0)*
HC atorvastatin	3.9 (3.2)	3.3 (1.2)	6.4 (1.8)	48.9	64.5	164	42.9 (8.2)***	51.4 (17.4)*
HC ezetimibe	3.5 (3.0)	3.0 (0.9)	8.6 (5.4)	40.3	52.2	174	43.9 (12.0)***	46.2 (15.2)*

Serum amyloid A (SAA) and alanine aminotransferase (ALT) levels at t=0, 18 and 38 weeks and E-selectin and monocyte chemoattractant protein-1 (MCP-1) levels at t=39 weeks. Data are indicated as mean (SD). SAA, E-selectin and MCP-1 levels were determined in individual plasma samples. ALT levels were measured in pooled plasma samples. Control (n=12), low cholesterol (LC) (n=10), high cholesterol (HC) (n=12) or HC were treated either with atorvastatin (n=12) or ezetimibe (n=11) (HC atorvastatin and HC ezetimibe) groups.
*p<0.001 versus control.
***p<0.01 versus HC.

of the entire knee joint (including all components, ie, total OA grade), effects of increased cholesterol intake on OA development were also observed, although not significant (table 2).

To investigate whether cholesterol-lowering drugs can diminish the development of OA, treatment with atorvastatin or ezetimibe was included. Atorvastatin suppressed the development of OA at the medial side significantly compared to the HC group (p=0.046), whereas ezetimibe did not have this effect (figure 2A). No significant effects of these treatments were seen on the lateral side, the trochlear groove or the total OA grade (table 2).

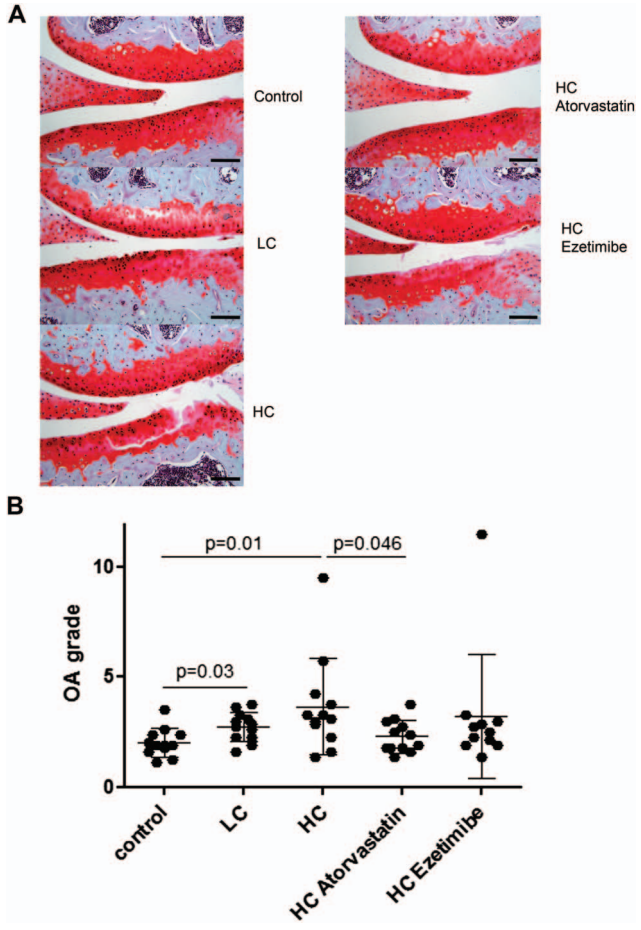

Figure 2 Effect of dietary cholesterol on osteoarthritis (OA) development in the knee joints of APOE*3Leiden.CETP mice. (A) Representative pictures of haematoxylin, fast green and safranine-O-stained medial knee joints. Scale bar indicates 100+m. (B) Sum total of the OA grades at the medial femur and tibia of the knee joint. Each dot is an individual mouse. Line indicates the mean±SD. Control (n=12), low cholesterol (LC) (n=10), high cholesterol (HC) (n=12) or HC group treated with atorvastatin (n=12) or ezetimibe (n=11) (HC atorvastatin and HC ezetimibe) group.

Atherosclerosis development

The origin of the aorta in the hearts was analysed for ATH features. Regular ATH studies in APOE*3L.CETP mice lasted on average 19–21 weeks;[21 24 25] however, in this study the exposure to high cholesterol was almost twice as long. After 39 weeks, the LC and HC groups developed severe ATH in the aortic root (figure 3A). The total lesion area per cross-section was 33.5 times higher in the LC group and 91.8 times higher in the HC group than the control group (both p<0.001) (figure 3B). The LC and HC groups had significantly more severe lesions than the control group (both p<0.001) (figure 3C).

Atorvastatin and ezetimibe treatment led to significantly reduced lesion areas compared with the untreated HC group (48% and 55%, respectively; both p<0.001) (figure 3B). Mice receiving atorvastatin treatment had significantly less severe (types V–IV) lesions (p<0.05) compared with the untreated HC group, which was not the case for ezetimibe.

Correlations

To explore how and to what extent OA was related to cholesterol exposure and ATH lesion area, linear regression analysis was performed. OA grades at the medial side of the knee joint in the control, LC and HC groups were significantly associated with total cholesterol exposure (R=0.43; p<0.01) (figure 4A). Cholesterol exposure levels of each mouse in the control, LC and HC groups were strongly and significantly correlated with the corresponding lesion area (R=0.91; p<0.001) (figure 4B), and therefore, it was consistent such that the lesion area and OA grades were also significantly correlated (R=0.34; p=0.03) (figure 4C). To evaluate the contribution of ATH features in addition to cholesterol exposure, partial correlation correcting for cholesterol exposure was performed and revealed no significant correlation between ATH features and OA grades (R=0.09, p=0.6).

DISCUSSION

There is currently mounting evidence that the MetS and OA are associated with one another.[2 3] Hypercholesterolaemia is related to MetS and a risk factor for ATH. Associations between OA and ATH suggest similarities in their underlying pathologies.[11 15 33 34] In this study, using the APOE*3L.CETP mouse

Table 2 Osteoarthritis (OA) grades

		Control	LC	HC	HC atorvastatin	HC ezetimibe
Medial	Femoral condyle	0.73 (0.37)	1.20 (0.49)*	1.63 (1.23)*	0.84 (0.34)***	1.36 (1.57)
	Tibia plateau	1.29 (0.42)	1.51 (0.30)	2.04 (1.02)*	1.46 (0.54)	1.85 (1.26)
	Total	2.02 (0.64)	2.71 (0.65)*	3.67 (2.17)*	2.30 (0.73)**	3.22 (2.80)
Lateral	Femoral condyle	1.72 (0.26)	1.68 (0.35)	1.33 (0.61)	1.45 (0.47)	1.49 (0.67)
	Tibia plateau	1.61 (0.55)	1.59 (0.67)	1.81 (0.81)	1.83 (0.94)	1.70 (0.53)
	Total	3.33 (0.76)	3.26 (0.94)	3.15 (0.97)	3.28 (1.27)	3.19 (1.03)
Patella	Patella	0.83 (0.71)	1.20 (1.69)	1.26 (1.42)	1.09 (0.83)	1.09 (1.01)
	Trochlear groove	1.01 (0.61)	1.11 (1.14)	1.23 (0.88)	0.98 (0.56)	1.24 (0.89)
Total OA grade		5.85 (1.57)	7.00 (2.96)	7.35 (3.46)	5.68 (2.03)	7.11 (3.71)

OA grades were scored at the medial, lateral side and patella in control (n=12), low cholesterol (LC) (n=10), high cholesterol (HC) (n=12) or HC were treated either with atorvastatin (n=12) or ezetimibe (n=11) (HC atorvastatin and HC ezetimibe) group. Data are indicated as mean (SD).
*p<0.05 versus control.
**p<0.05.
***p<0.1 versus HC.

model, we demonstrate that hypercholesterolaemia indeed plays a role in the development of OA since an increased intake of cholesterol led to more severe OA features in the knee joints. Furthermore, a significant correlation between cholesterol exposure and OA features on the medial side of the knee joint indicates that high levels of cholesterol in the blood contribute to the OA process. However, correlation coefficients suggest that other processes, evoked by the intake of cholesterol, are also involved in the pathogenesis of OA. The suppressive effect on OA development by atorvastatin but not by ezetimibe, which had similar effects on cholesterol exposure levels, corroborates these findings.

Impaired lipid metabolism has been suggested as an important factor in OA development. We used the APOE*3L.CETP mice as translational model for human lipoprotein metabolism and development of ATH. To our knowledge, this is the first study in which the effect of solely cholesterol, without interference of other variables such as BW, on OA development has been investigated. Since elevated cholesterol intake led to more advanced OA features, these data suggest a role for cholesterol in OA development. Compared with other spontaneous models, such as high-fat diet (HFD)-induced OA, the development of OA is mild, although it is difficult being conclusive due to differences in strains, gender and lengths of the studies.[10 35–37] The differences were only seen at the medial side of the joint, which is known to be most susceptible in mice models, and therefore, in line with our expectations.[38]

Whether the development of OA after cholesterol intake is due to a direct effect of cholesterol (local effects), other mechanisms induced by cholesterol (systemic effects), or a consequence of ATH is uncertain. With respect to local effects, several mechanisms of altered lipid mechanisms on OA development are suggested. For example, in human OA chondrocytes, the expression of genes regulating cholesterol efflux is impaired, resulting in a toxic accumulation of lipid droplets in the chondrocyte.[39 40] In addition, an association of OA pathogenesis with SREBP-2, a gene that plays a key role in cholesterol homoeostasis, was demonstrated.[41] These findings suggest a direct role for cholesterol in triggering degenerative processes in the cartilage by altered cholesterol homoeostasis in the chondrocytes. However, in our *in vivo* mouse model, only a moderate correlation between plasma cholesterol exposure and OA development was observed, which suggests that cholesterol is not solely responsible for the development of OA. Altered underlying systemic processes such as inflammation that are the result of increased cholesterol load may therefore be more relevant. This is substantiated by the fact that SAA, ALT, E-selectin and MCP-1 levels in the mice were increased when fed a cholesterol diet. Kleemann *et al*[8] performed a gene expression analysis, which indicated four key inflammatory pathways consisting of IFNγ, TNFα, IL-1 and platelet -derived growth factor to play a central role in the evolution of cholesterol-induced inflammation in the liver.[8] These pathways are also critical for lesion development in the vessel wall and known to be important in the development of OA.[42] Circulating levels of cytokines thought to be important in OA, for example, TNFα. IFNγ, IL-6 and IL-1, were not detectable in this study, which is consistent with previous findings in our lab with these transgenic mice on a cholesterol diet (unpublished data).

Alterations due to ATH may provide an alternative explanation for the development of OA in this model. For example, effects of a reduced blood flow in the small vessels in the subchondral bone may modify the cartilage homoeostasis and cause OA changes.[7 13 14 16] This notion is supported by the analysis of human data that revealed a significant association between intima-media thickness and knee OA after adjustment, for example, cholesterol levels.[14] Moreover, hand OA was linearly associated with ATH features after adjustment for potential confounders.[15] In our model, the lack of correlation between OA and ATH, when corrected for cholesterol exposure, debilitate ATH as main driving force for OA. This is further substantiated by the fact that ezetimibe, in contrast to atorvastatin, did not lead to significant suppressive effects on OA development, whereas these drugs had comparable effects on plasma cholesterol levels and ATH characteristics. Atorvastatin is known to have several pleiotropic effects, in contrary to ezetimibe, including anti-inflammatory immunomodulation, plaque stabilisation, decreased activation of the blood coagulation cascade and inhibition of platelet aggregation, which may be responsible for the different effect of atorvastatin compared with ezetimibe.[43] In a previous study, we have shown that rosuvastatin suppressed the development of HFD-induced OA through its anti-inflammatory capacities.[10] Moreover, it has been shown that intraarticular administration of mevastatin-reduced inflammatory cell infiltration and matrix-degrading enzyme expression in a rabbit model of experimental OA indicated local effects of atorvastatin.[19 20] Our data suggest that statins may be an important candidate for the management of OA, and additional studies are warranted.

In conclusion, elevated levels of cholesterol play a role in OA as mice receiving cholesterol in their diet develop OA features

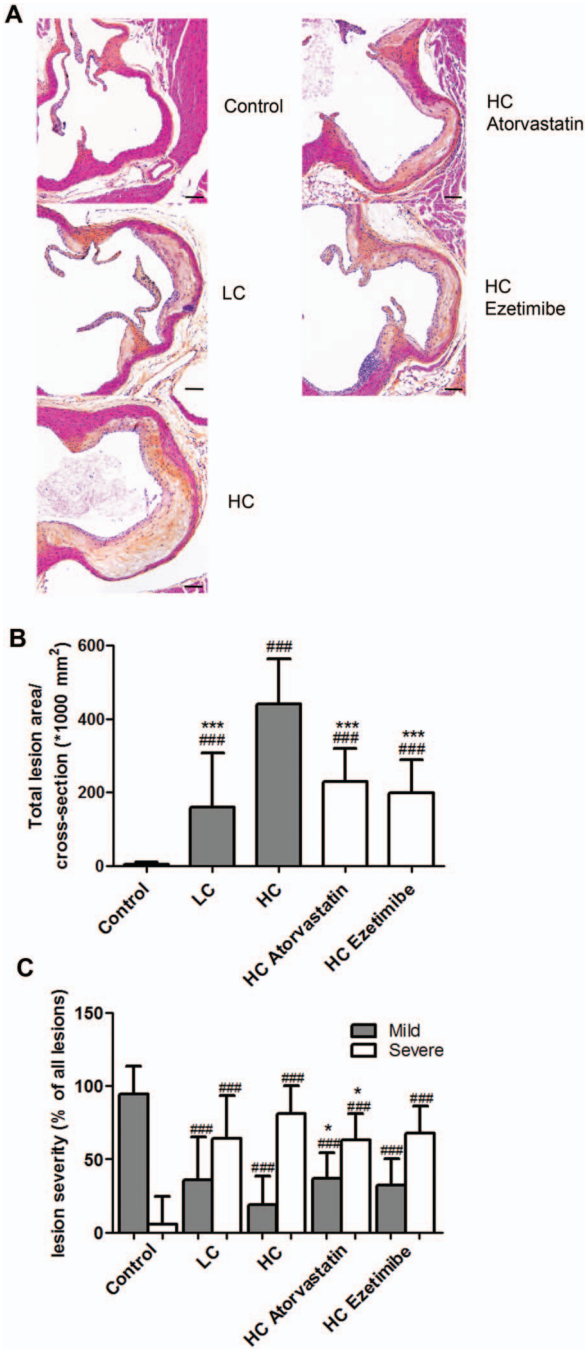

Figure 3 Effect of dietary cholesterol on atherosclerotic development in the aortic root area of APOE*3Leiden.CETP mice. (A) Representative pictures of atherosclerotic lesions in the root stained with haematoxylin, phloxine and saffron (HPS). Scale bar indicates 100 + m. (B) Total lesion area per cross section * 1000 m^2. (C) Lesion severity as a percentage of all lesions (mild: types I–III and severe: types IV–V). Control (n=12), low cholesterol (LC) (n=10), high cholesterol (HC) (n=12) or HC group treated with atorvastatin (n=12) or ezetimibe (n=11) (HC atorvastatin and HC ezetimibe), ###p<0.001, #p<0.05 versus control; ***p<0.001, *p<0.05 versus HC.

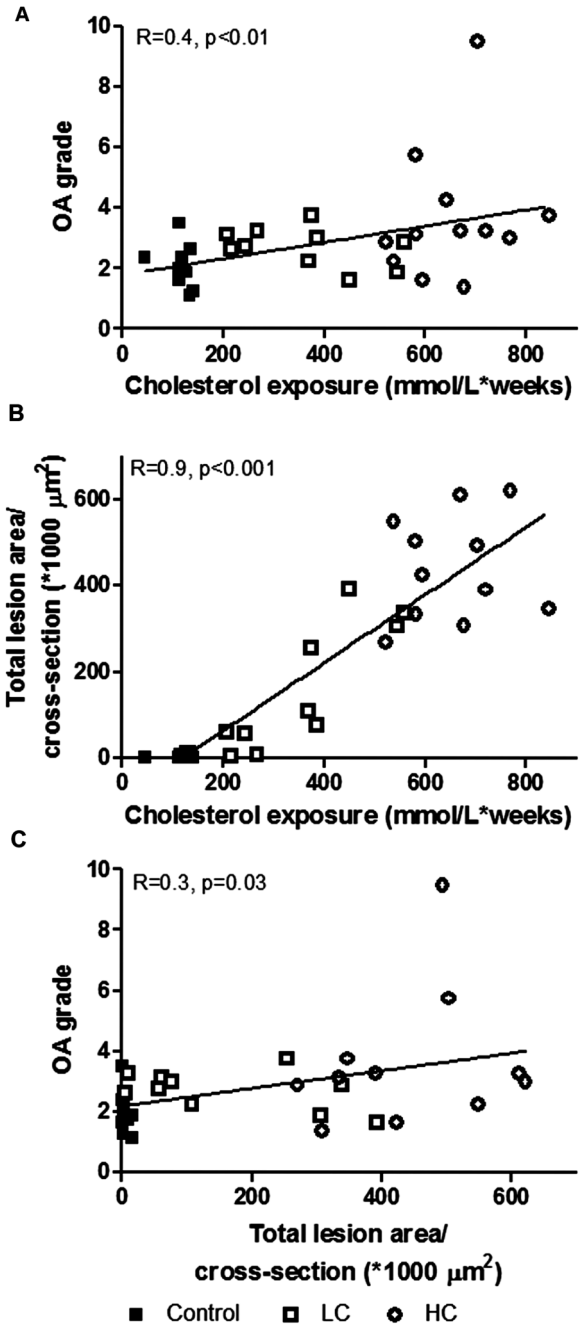

Figure 4 Overview of correlations in the control, low cholesterol (LC) and untreated high cholesterol (HC) groups (A) OA grade (sum total of the OA grades at the medial femur and tibia of the knee joint) and cholesterol exposure (mmol/l * weeks), (B) Cholesterol exposure (mmol/l * weeks) and atherosclerosis (ATH) development (total lesion area/cross-section (*1000 m^2), (C) OA grade (sum total of the OA grades at the medial femur and tibia of the knee joint) and ATH development (total lesion area/ cross-section (*1000 m^2).

on the medial side of the knee joint. The moderate correlation between OA and cholesterol exposure suggests that processes independent of cholesterol exposure also contribute to the development of OA features. This is further supported by the differences in the effect of atorvastatin and ezetimibe on OA. Both drugs reduced cholesterol levels to the same extent, but only atorvastatin significantly diminished OA severity, which may be attributed to its anti-inflammatory properties. This study supports the notion that components related to MetS, such as hypercholesterolaemia, could significantly contribute to the development, progression and severity of OA; however, further research is needed to elucidate the underlying molecular mechanisms.

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Notes