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Hepatocyte-specific IKKβ expression aggravates atherosclerosis development in *APOE*3-Leiden* mice

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ABSTRACT

Objective: The liver is the key organ involved in systemic inflammation, but the relation between hepatic inflammation and atherogenesis is poorly understood. Since nuclear factor-κB (NF-κB) is a central regulator of inflammatory processes, we hypothesized that chronically enhanced hepatic NF-κB activation, through hepatocyte-specific expression of IκB kinase-β (IKKβ) (*LIKK*), will aggravate atherosclerosis development in *APOE*3-Leiden* (*E3L*) mice.

Methods and results: *E3L.LIKK* and *E3L* control littermates were fed a Western-type diet for 24 weeks. *E3L.LIKK* mice showed a 2.3-fold increased atherosclerotic lesion area and more advanced atherosclerosis in the aortic root with less segments without atherosclerotic lesions (11% *vs.* 42%), and more segments with mild (63% *vs.* 44%) and severe (26% *vs.* 14%) lesions. Expression of *LIKK* did not affect basal levels of inflammatory parameters, but plasma cytokine levels tended to be higher in *E3L.LIKK* mice after lipopolysaccharide (LPS) administration. *E3L.LIKK* mice showed transiently increased plasma cholesterol levels, confined to (V)LDL. This transient character resulted in a mild (+17%) increased cumulative plasma cholesterol exposure.

Conclusion: We conclude that selective activation of NF-κB in hepatocytes considerably promotes atherosclerosis development which is (at least partly) explained by an increased sensitivity to proinflammatory triggers and transiently increased plasma cholesterol levels.

Abbreviations: apoB, apolipoprotein B; CE, cholesteryl ester; Cpt1a, carnitine palmitoyltransferase 1a; cyclo, cyclophilin; *E3L*, *APOE*3-Leiden*; Fas, fatty acid synthase; FC, free cholesterol; FPLC, fast performance liquid chromatography; Gapdh, glyceraldehyde-3-phosphate dehydrogenase; Hmgcr, HMG-CoA reductase; Hprt, hypoxanthine-guanine phosphoribosyl transferase; HPS, hematoxylin–phloxine–saffron; IFNγ, interferon γ; IκB, inhibitor of κB; IKKβ, IκB kinase-β; IL-1β, interleukin-1β; IL-6, interleukin-6; IL-10, interleukin-10; IL-12p70, interleukin-12p70; *LIKK*, liver-specific IKKβ; MCP-1, monocyte chemoattractant protein-1; MTTP, microsomal triglyceride transfer protein; NF-κB, nuclear factor-κB; PL, phospholipid; RT-PCR, real-time PCR; SAA, serum amyloid A; Srebp-1c, sterol-regulatory element binding protein; TC, total cholesterol; TG, triglyceride; TNFα, tumor necrosis factor α; VLDL, very-low-density lipoprotein.

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1. Introduction

Increased inflammation, in addition to disturbances in lipid metabolism, is the other main contributor to the development of atherosclerosis [1]. Nuclear factor-κB (NF-κB) has been identified as the most important transcription factor in the regulation of inflammatory processes during atherosclerosis development [2]. In unstimulated cells, NF-κB p65/p50 dimer is kept inactive by its inhibitory protein: inhibitor of κB (IκB). A wide range of extracellular stimuli, including cytokines, microbial components, and also free fatty acids, induce activation of the IκB kinase complex, which consists of two kinases (IKKα and -β) and a regulatory subunit, NEMO/IKKγ. This complex mediates the phosphorylation of IκB, resulting in its ubiquitination and degradation, leaving the NF-κB dimer free to translocate to the nucleus and activate its target genes [2].

While general inhibition of the NF-κB pathway by pharmacological agents reduces atherosclerosis development in mice [3,4], the relative contribution of NF-κB may differ at cellular- or tissue-specific level. Suppression of the NF-κB pathway in endothelial cells by ablation of NEMO/IKKγ has been shown to decrease atherosclerosis development [5]. In murine bone marrow transplantation models, inhibition of the NF-κB pathway at distinct levels in hematopoietic cells can have different outcomes, *i.e.* deficiency of the NF-κB p50 subunit resulted in smaller atherosclerotic lesions [6], whereas deletion of IKKβ increased atherosclerosis development [7]. Surprisingly, the role of the NF-κB pathway in hepatocytes on atherosclerosis development has not been investigated thus far.

The liver plays a central role in both lipid metabolism [8] and inflammation [9]. Disturbances in lipid metabolism and increased inflammation are the two main risk factors for atherogenesis [1]. Hepatocytes form the largest population of cells in the liver and execute most of its important functions. During inflammation, acute phase proteins are mainly synthesized by the hepatocytes [10]. Interestingly, hepatocyte-specific deficiency of gp130, a receptor component of IL-6 signaling which signals independent of the NF-κB pathway, decreases atherosclerosis in *apoe*$^{-/-}$ mice [11], suggesting that reduced hepatic inflammation is associated with less atherosclerosis development.

Despite ample evidence implicating the involvement of NF-κB in atherogenesis, the hepatocyte-specific role of NF-κB in atherosclerosis has not been investigated directly. Therefore, in this study we aimed to investigate whether chronic activation of hepatocyte-specific NF-κB aggravates atherosclerosis development. We used transgenic mice with hepatocyte-specific expression of human IKKβ (liver-specific IKKβ or *LIKK* mice), resulting in an increase of active NF-κB [12], crossbred with atherosclerosis-prone *APOE*3-Leiden* (*E3L*) mice. *E3L* mice exhibit a human-like lipoprotein distribution on a cholesterol-rich diet due to transgenic expression of a human mutant of the *APOE3* gene, and are therefore susceptible to atherosclerosis development [13]. Collectively, our results show that hepatocyte-specific NF-κB activation markedly aggravates atherosclerosis development in *E3L* mice.

2. Methods

Brief descriptions of the most important procedures of this study are provided in this section. An expanded description is available in the supplemental data (available online at http://atherosclerosis-journal.com).

2.1. Animals

Transgenic *LIKK* mice expressing constitutively active human IKKβ in hepatocytes under the control of an albumin promoter [12] were crossbred with *E3L* mice [13] to generate heterozygous *E3L.LIKK* and control *E3L* littermates, as described before [14]. Ten-12 weeks old female mice were fed a Western-type diet for 24 weeks. Blood was drawn every 4 weeks after a 4-h fast.

2.2. Plasma analysis

Plasma levels of serum amyloid A (SAA), inflammatory cytokines, total cholesterol (TC), triglycerides (TG) and phospholipids (PL) levels were determined.

2.3. Lipopolysaccharide stimulation

Mice were injected i.v. with *Salmonella minnesota* Re595 lipopolysaccharide (LPS) (50 mg/kg body weight). Blood was collected 90 min after injection and plasma was assayed for cytokines.

2.4. Atherosclerosis quantification

The extent of atherosclerosis was assessed in the aortic root area. After staining with hematoxylin–phloxine–saffron (HPS), atherosclerotic lesion severity and area were determined.

3. Results

3.1. LIKK causes low-grade inflammation

The overall appearance of *E3L* and *E3L.LIKK* mice during the study was similar. To assess whether expression of *LIKK* affects body weight gain, we measured food intake and body weight weekly. Both were not different between *E3L.LIKK* and *E3L* control mice (Supplemental Fig. 1A and B). The liver- and spleen weight and histological morphology of the liver were also comparable between *E3L.LIKK* and *E3L* mice (data not shown). To gain more insight in the effects of *LIKK* on inflammation, we determined whether *LIKK* expression increased the inflammatory state of the liver and systemic inflammatory markers in *E3L.LIKK* mice on a Western-type diet. We confirmed previous findings [14] showing that the enhanced expression of hepatocyte-specific human IKKβ (Supplemental Fig. 2A) resulted in a 1.4-fold increased hepatic NF-κB activation, as shown by an increase in the phosphorylated p65 subunit (pNF-κB^{Ser536}) (Supplemental Fig. 2B). IKKβ kinase phosphorylates subunit p65 of NF-κB at the position Ser536, which activates the transcriptional activity of NF-κB [15]. The transgenic expression of human IKKβ mRNA was present only in *E3L.LIKK* mice and did not alter murine IKKβ mRNA expression (Supplemental Fig. 2C and D). The enhanced hepatic NF-κB activation in *E3L.LIKK* mice did not result in increased IL-6 expression in whole liver, but did result in a tendency towards increased IL-1β expression ($P = 0.085$) and a significant increase in MCP-1 expression (Supplemental Table 2).

To evaluate whether the increased hepatocyte-specific NF-κB activation in *E3L.LIKK* mice enhanced the systemic inflammatory state, we determined the plasma inflammation marker SAA and plasma cytokines under basal conditions. *LIKK* expression did not affect SAA before (3.1 ± 0.17 μg/mL *vs.* 3.4 ± 0.15 μg/mL) and after 8 weeks (4.4 ± 0.28 μg/mL *vs.* 4.2 ± 0.31 μg/mL) and 24 weeks (4.9 ± 0.51 μg/mL *vs.* 5.4 ± 0.78 μg/mL) of Western-type diet feeding (Fig. 1), and neither the determined plasma cytokine levels (Supplemental Fig. 3A–F). SAA levels increased with Western-type diet feeding in both *E3L* and *E3L.LIKK* mice, but this difference only reached statistical significance in *E3L.LIKK* mice (Fig. 1).

Since we did not observe a clear increased systemic proinflammatory state under basal conditions, we challenged the mice with LPS to boost the inflammatory response. Interestingly, after injection of LPS, proinflammatory cytokines (*e.g.* IL-1β, IFNγ) showed

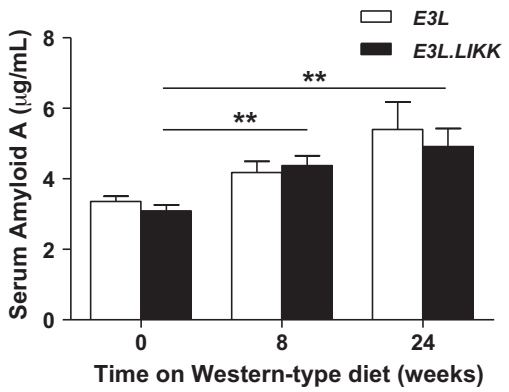

Fig. 1. *LIKK* does not increase plasma SAA levels. SAA levels were determined in plasma from *E3L.LIKK* (black bars) and *E3L* (white bars) mice fed a Western-type diet for 0, 8 and 24 weeks. Values are means ± SEM; $n = 15$/group; $^{**}P < 0.01$.

a tendency towards increased plasma levels in *E3L.LIKK* mice as compared to *E3L* mice (Fig. 2A–F). The anti-inflammatory IL-10:IL-1β ratio was significantly lower in *E3L.LIKK* mice (Fig. 2G). Overall, these data indicate that *E3L.LIKK* mice are more sensitive to proinflammatory triggers compared to their *E3L* littermates.

To study whether this chronic low-grade inflammation in *E3L.LIKK* mice also resulted in increased inflammatory cell counts in liver and plasma, we determined the hepatic mRNA expression of various cell-type markers of inflammatory cells present in the liver, which are likely to influence atherogenesis [16], and the number of circulating monocytes. Hepatic mRNA expression of CD68 (Kupffer cells), CD3 ((NK)T cells), and Vα14 (NKT cells) were not different between the genotypes (Supplemental Table 2), neither were the total number of circulating monocytes, the proinflammatory Ly6C-hi monocyte subset, the intermediate Ly6C-med monocyte subset and the less inflammatory Ly6C-lo monocyte subset (Supplemental Fig. 4A–D). Together, the above findings indicate that the enhanced hepatocyte-specific NF-κB activation in *E3L.LIKK* mice results in

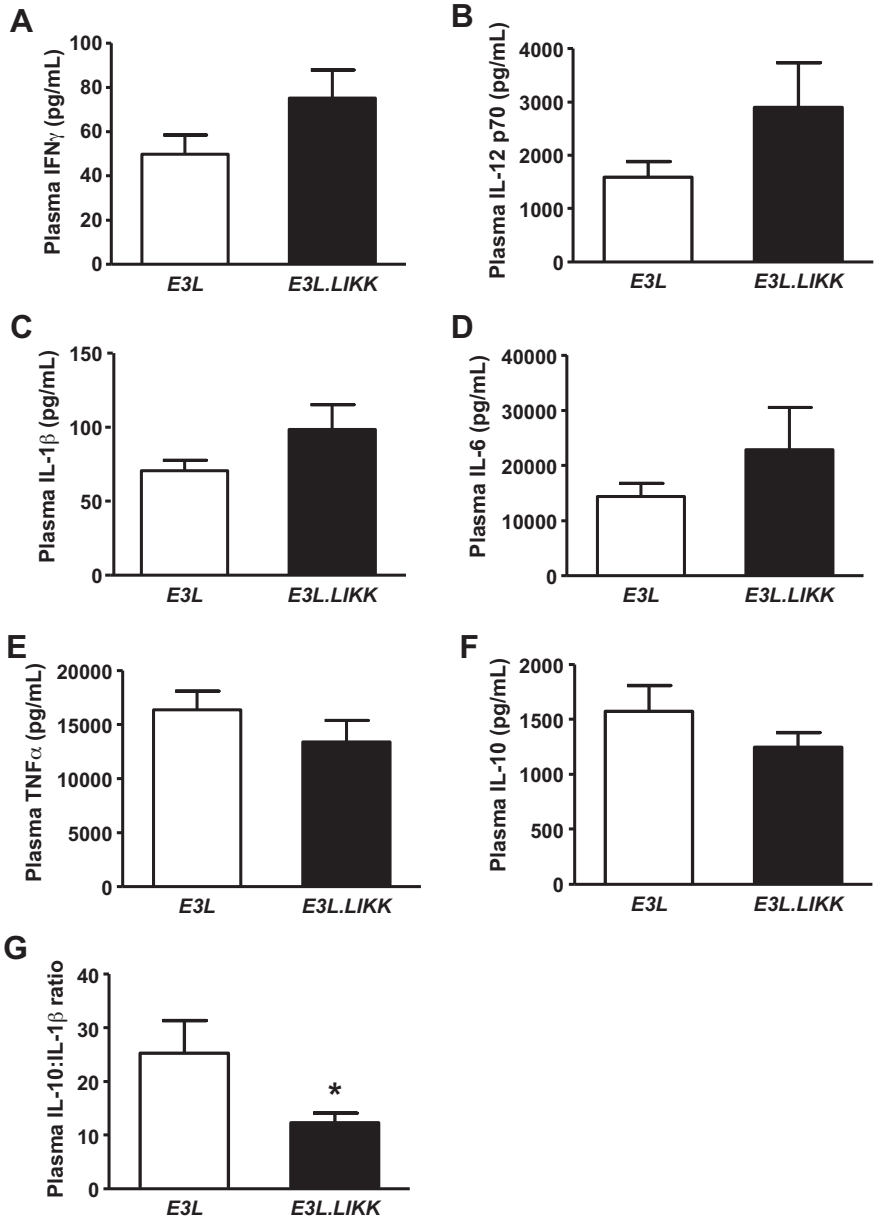

Fig. 2. *LIKK* tends to enhance plasma cytokines after LPS stimulation. LPS was injected intravenously in *E3L.LIKK* (black bars) and *E3L* (white bars) mice. Cytokine levels were measured 90 min after LPS injection (A–F). The IL-10:IL-1β ratio was calculated (G). Values are means ± SEM; $n = 7$/group; $^{*}P < 0.05$.

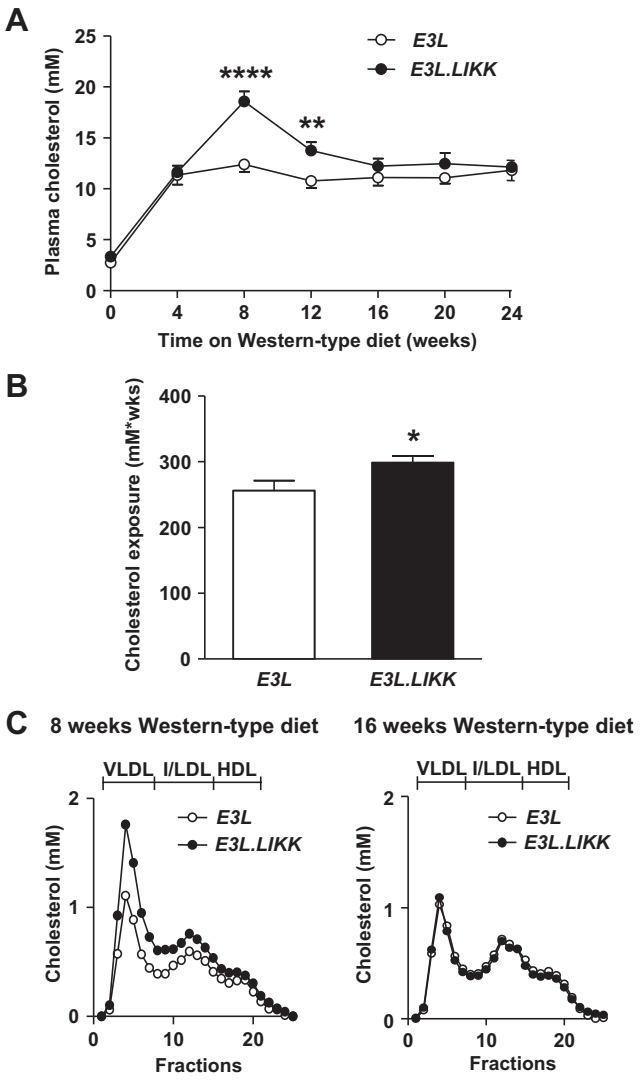

Fig. 3. *LIKK* transiently increases (V)LDL. Plasma cholesterol levels of *E3L.LIKK* (black symbols) and *E3L* (white symbols) mice fed a Western-type diet were assessed (A), and cumulative total cholesterol exposure was calculated (B). Lipoprotein profiles were determined at 8 (*left*) and 16 (*right*) weeks (C). Values are means ± SEM; $n = 15$/group; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{****}P < 0.0001$.

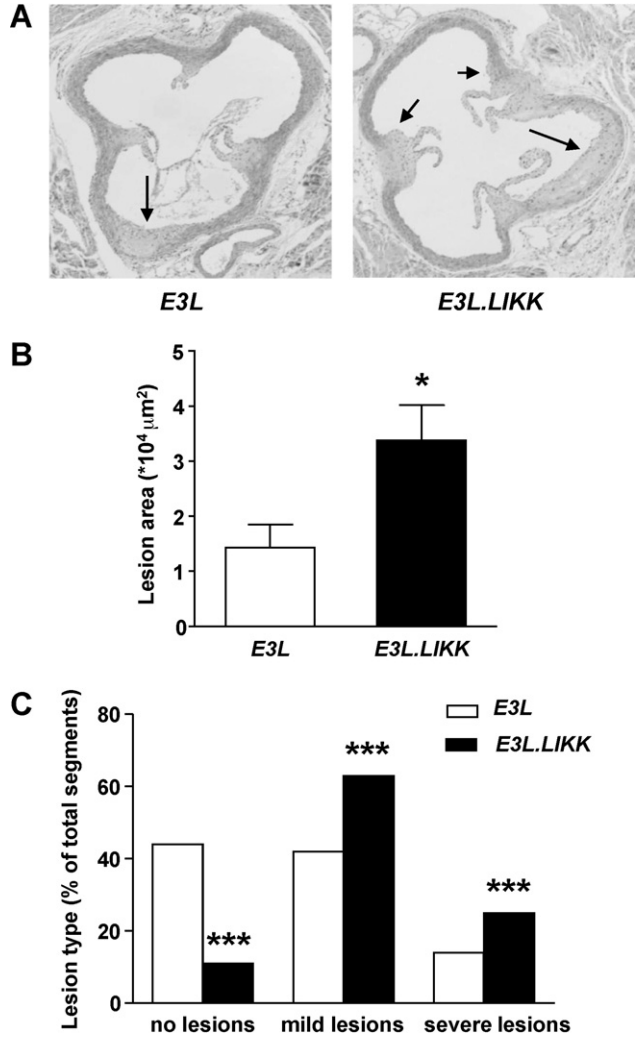

Fig. 4. *LIKK* aggravates atherosclerotic lesion area and severity. After 24 weeks of Western-type diet feeding, *E3L.LIKK* (black bars) and *E3L* (white bars) mice were sacrificed and cross-sections of aortic roots were stained with HPS. Representative pictures are shown. Arrows indicate lesions (A). Total lesion area was assessed in 4 sections of the aortic root (B) and lesion severity was determined separately in each of the 3 segments between the aortic valves of the 4 sections (C). Statistical analysis for lesion area was performed by Mann–Whitney *U* test, for lesion severity was determined by the χ^2 test. Values are means ± SEM; $n = 15$/group; $^{*}P < 0.05$, $^{***}P < 0.001$.

a tendency towards a mildly enhanced hepatic proinflammatory state and an elevated sensitivity to proinflammatory stimuli as compared to *E3L* littermates.

3.2. LIKK transiently enhances VLDL cholesterol levels

To assess the effect of hepatocyte-specific NF-κB activation on plasma lipid levels, TC, TG and PL concentrations were determined every 4 weeks in *E3L.LIKK* and *E3L* mice. *LIKK* expression caused a transient increase of plasma TC levels only at 8 weeks (+50%; $P < 0.0001$) and 12 weeks (+28%; $P < 0.05$) of Western-type diet feeding (Fig. 3A). Accordingly, the cumulative total cholesterol exposure was higher in *E3L.LIKK* than in *E3L* mice (+17%; $P < 0.05$; Fig. 3B). A similar transient increase was found for plasma TG and PL levels (Supplemental Fig. 5A and B).

To determine which lipoproteins contribute to the transient elevated plasma TC levels, lipoproteins were size-fractionated by FPLC, and cholesterol was measured in the individual fractions. The transient increase in plasma TC levels at 8 weeks of Western-type diet feeding in *E3L.LIKK* mice was confined to (V)LDL, whereas at 16 weeks the lipoprotein distribution in the *E3L.LIKK* mice was similar to that of the *E3L* mice, in line with the plasma lipid levels (Fig. 3C). Consistent with our previous finding that expression of *LIKK* increased the VLDL production in male mice on chow diet [14], we found that expression of *LIKK* increased, albeit not significantly, the VLDL-TG production rate (+24%) (Supplemental Fig. 6A), and tended to increase the VLDL-apolipoprotein B (apoB) production rate (+33%) (Supplemental Fig. 6B). No differences were observed in the liver lipid content between *E3L.LIKK* and *E3L* mice (Supplemental Fig. 7A–E). Taken together, these findings indicate that hepatocyte-specific NF-κB activation results in a modest and transient increase in plasma lipid levels in *E3L* mice.

3.3. LIKK enhances atherosclerosis development

To investigate the effect of *LIKK* expression on atherosclerosis development, *E3L.LIKK* and *E3L* mice were sacrificed after 24 weeks of Western-type diet feeding, and lesion size and severity were measured in the aortic root. Representative pictures of both groups are shown in Fig. 4A. *E3L.LIKK* mice developed more than 2-fold

larger atherosclerotic lesions (+131%; $P < 0.05$; Fig. 4B) as compared to their *E3L* littermates. This increased lesion area coincided with more advanced lesion progression, since we found markedly fewer segments without atherosclerotic lesions (11% *vs.* 42%; $P < 0.001$) and more segments with mild (63% *vs.* 44%; $P < 0.001$) and severe lesions (26% *vs.* 14%; $P < 0.001$) as compared to *E3L* mice (Fig. 4C). Examples of mild and severe lesions are shown in Supplemental Fig. 8. These data indicate that chronic hepatocyte-specific NF-κB activation severely augments atherosclerosis development in *E3L* mice.

3.4. LIKK aggravates atherosclerotic lesion composition

We next evaluated whether *LIKK* expression would affect monocyte adherence and recruitment to the vascular wall, as well as the composition of the atherosclerotic lesions with respect to the macrophage, smooth muscle cell, and collagen content of the lesions. Adherence of monocytes to the vessel wall and the content of the chemokine monocyte chemoattractant protein-1 (MCP-1) of the atherosclerotic lesions was not significantly enhanced in *E3L.LIKK* mice as compared to *E3L* mice (Fig. 5A and B). *LIKK* expression did not affect the relative macrophage and collagen content of the lesions (Fig. 5C and E), but did result in an increased smooth muscle cell content of the lesions (+79%, $P < 0.05$; Fig. 5D).

3.5. Aggravated atherosclerosis development in E3L.LIKK mice does not solely depend on the transient increase in plasma cholesterol levels

In *E3L* mice on Western-type diet, the cumulative plasma cholesterol exposure is highly predictive for the atherosclerotic lesion area (unpublished data, J.F.P. Berbée, P.C.N. Rensen). To verify if the transient increase in plasma TC levels (Fig. 3) alone could account for the aggravation in atherosclerosis development observed in *E3L.LIKK* mice, or whether additional mechanism(s) could contribute, including the low-grade systemic inflammation, we assessed the correlation between the cumulative plasma total cholesterol exposure and the atherosclerotic lesion area of the *E3L.LIKK* and *E3L* mice. As expected, there was a significant positive logarithmic correlation between the atherosclerotic lesion area and the cumulative plasma cholesterol exposure in the control *E3L* mice (Supplemental Fig. 9A; $r^2 = 0.757$, $P = 0.002$). However, we did not observe such a correlation in the *E3L.LIKK* mice (Supplemental Fig. 9B; $r^2 = -0.250$, $P = 0.369$), indicating that in addition to the transient increase in plasma TC levels in *E3L.LIKK* mice, additional mechanism(s), most likely the increased sensitivity to proinflammatory stimuli, contributed to the aggravated atherosclerosis development in these mice.

4. Discussion

NF-κB is regarded as a potential therapeutic target in atherosclerosis [3,4] and studying tissue- and cell-specific effects of NF-κB in atherogenesis will expand our knowledge in the comprehensive actions of NF-κB on atherosclerosis development. The present study demonstrates for the first time that chronic, hepatocyte-specific expression of IKKβ (*LIKK*) and subsequent activation of NF-κB aggravates atherosclerosis development in *E3L* mice. In addition, the atherosclerotic lesion composition with respect to the macrophage and collagen content was not affected by *LIKK*, but in line with the presence of more advanced lesions, the smooth muscle cell content was increased. Expression of *LIKK* resulted in transiently increased plasma cholesterol levels and an enhanced sensitivity to proinflammatory triggers, which both are likely to have contributed to the increased atherosclerotic lesion size and severity. Since lesion size and severity are often correlated in atherosclerosis studies with different murine models [13,17], the increased lesion severity in *E3L.LIKK* mice is likely to be mainly attributed to the larger size of the lesions.

Expression of *LIKK* in *E3L* mice increased the activation of the NF-κB pathway in the liver, in line with our previous report [14]. In addition, hepatic mRNA expression of inflammatory parameters was increased or tended to be increased in *E3L.LIKK* mice, indicating that inflammatory mediators at local tissue level were enhanced in *E3L.LIKK* mice. This enhanced activation of hepatocyte-specific NF-κB in *E3L.LIKK* mice, however, did not result in a significant increased systemic proinflammatory state under basal conditions as compared to their *E3L* littermates. Importantly, Cai et al. [12] demonstrated that in *LIKK* mice on a wild-type background, systemic levels of IL-6 were only mildly elevated, while IL-1β and TNFα levels were similar as in wild-type mice. Our results show that *LIKK* expression on an *E3L* background resulted in a less pronounced hepatic inflammatory state as compared to *LIKK* expression on a wild-type background as described by Cai et al. [12], as reflected in a smaller increase in active NF-κB (1.4- *vs.* 2.2-fold) and mRNA levels of proinflammatory cytokines levels in the liver. Furthermore, under basal conditions *E3L* mice have lower levels of active NF-κB present in the liver as compared to wild-type mice (unpublished data, J.A. van Diepen, M.C. Wong, P.J. Voshol). This implies that *E3L* mice have a lower chronic inflammatory state than wild-type mice, which could interfere with the proinflammatory effects caused by expression of *LIKK* in the present study. Also, in comparison with other murine atherosclerosis models, *e.g.* the *apoe*$^{-/-}$ and *ldlr*$^{-/-}$ mice, *E3L* mice display a milder phenotype with respect to hyperlipidemia and increased inflammation [18,19]. In the current study, basal circulating levels of some cytokines were at borderline of the detection limit of current assays (Supplemental Fig. 3) and, as expected, the cytokine levels increased approximately 5- to 3700-fold after LPS injection (Fig. 2). Furthermore, after stimulation with LPS, *E3L.LIKK* mice showed a tendency towards a higher systemic inflammatory state than *E3L* mice.

There is a strong interaction between inflammation and lipid metabolism [20]. For example, lowering inflammation using salicylate did not only reduced NF-κB activation, but concomitantly also reduced circulating cholesterol levels in *E3L* mice [21]. In line with this observation, in the present study we found higher plasma lipid levels at 8 weeks of Western-type diet feeding in female *E3L.LIKK* compared to *E3L* mice, which were confined to (V)LDL. We hypothesize that the increased lipid levels at this time point are accompanied by increased systemic inflammation, which is in line with previous findings showing that lipid metabolism and inflammation strongly influence each other [20]. A possible cause for the increased plasma lipid levels at 8 weeks of diet is therefore a more enhanced inflammation in the liver, possibly due to an increased activation of the NF-κB pathway in the liver.

We recently reported that male *E3L.LIKK* mice on chow diet also showed enhanced (V)LDL levels as a result of an increased hepatic VLDL-TG production rate [14], and found in the current study a trend towards an enhanced VLDL-apoB production in female *E3L.LIKK* mice on Western-type diet, with a similar effect-size. Possible reasons for the less apparent increase of VLDL-TG production in females compared to males are differences in gender and/or diet. Although the increase in VLDL-TG production is more apparent in male *E3L.LIKK* mice, we used female mice in the present study. The main reason for this is that female *E3L* mice are more susceptible to develop atherosclerosis. In order for male *E3L* mice to become similarly atherosclerosis-prone they need to be fed Western-type diets not only with higher percentages of cholesterol, but also containing cholate. In addition, fructose was added to the drinking water to further raise their (V)LDL-cholesterol levels [22]. The increase in (V)LDL levels in females in the current study was only transient at 8 weeks of Western-type diet feeding and disappeared at 16

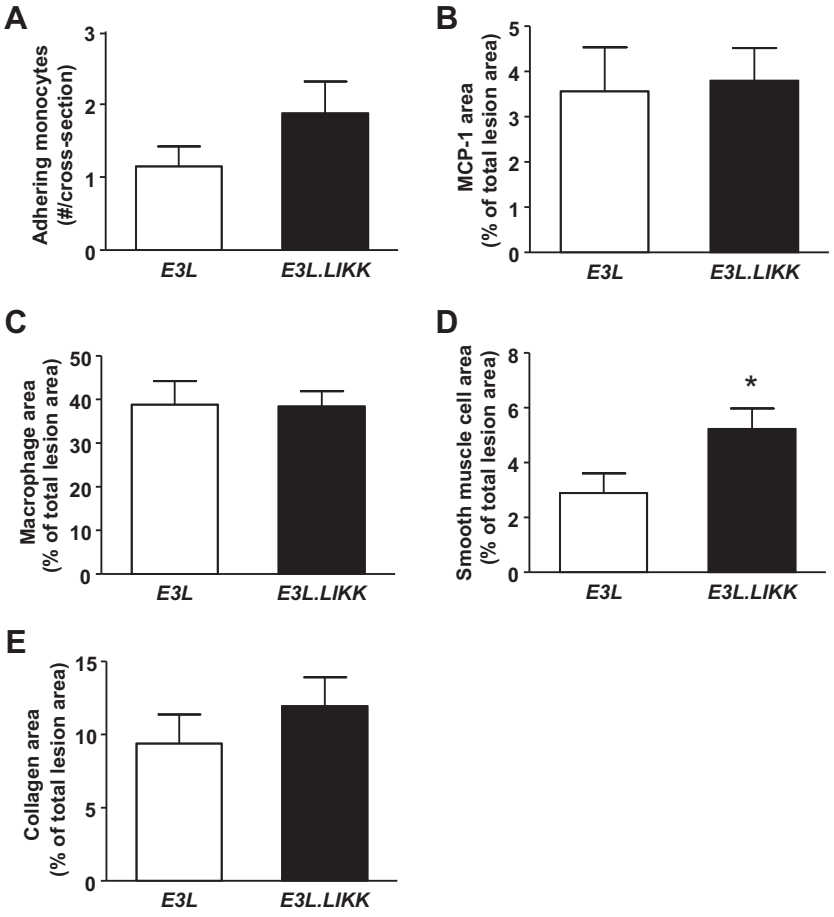

Fig. 5. *LIKK* induces more advanced atherosclerotic lesions. In the sections obtained as described in Fig. 4, the number of adhering monocytes (A), monocyte chemoattractant protein-1 (MCP-1) content (B), macrophage content (C), smooth muscle content (D) and collagen content (E) was determined. Values are means ± SEM; $n = 15$/group; $^{*}P < 0.05$, $^{**}P < 0.01$.

weeks. Since no differences in plasma lipid levels and hepatic mRNA expression of genes involved in lipid metabolism were detected between both groups at 24 weeks of diet, the increased VLDL-TG production at 8 weeks of diet is likely to be transient. At present, we cannot explain the transient nature of this increase in (V)LDL levels, but it may be the result of a progressive negative feedback mechanism to reduce the hepatic VLDL production which takes place during long-term Western-type diet feeding.

Dyslipidemia is regarded as the classical risk factor for atherosclerosis development. The transiently enhanced total cholesterol levels, resulting in a modest increase (+17%) in cumulative total cholesterol exposure upon *LIKK* expression, thus likely contributed to the enhanced atherosclerosis development. Previous diet-induced atherosclerosis studies in *E3L* mice have consistently demonstrated that there is a positive logarithmic relation between the cumulative cholesterol exposure during the study and the atherosclerotic lesion area (J.F.P. Berbée, P.C.N. Rensen, unpublished data). In agreement with these previous observations, we did observe such a significant logarithmic relation in *E3L* mice but not in *E3L.LIKK* mice. This suggests that the increase in atherosclerotic lesion area in *E3L.LIKK* mice can only partly be attributed to the transiently enhanced plasma cholesterol levels and that additional mechanisms are involved.

Inflammation is the second main risk factor for atherosclerosis. Enhanced extravascular or systemic inflammation, by the periodontal pathogen *Porphyromonas gingivalis* [23] or by repeated administration of LPS [24], respectively, promotes atherosclerosis development. In addition, in humans, low-grade systemic inflammation is associated with enhanced risk of coronary artery disease [25,26]. It is thus likely that, as discussed above, the increased sensitivity for proinflammatory triggers such as LPS in *E3L.LIKK* mice also directly contributed to the enhanced atherosclerotic lesion formation.

We excluded higher circulating levels of proinflammatory Ly6C-hi monocytes as being another possible contributor to the aggravated atherosclerosis development in *E3L.LIKK* mice. Adhesion of monocytes to endothelial cells and subsequent migration into the vessel wall is one of the crucial steps in atherosclerotic lesion formation. Ly6C-hi monocytes are more prone to adhere to activated endothelium than Ly6C-lo monocytes and are, therefore, associated with enhanced atherosclerosis development [27]. We found that *E3L.LIKK* mice had similar levels of circulating subsets of monocytes as compared to their *E3L* controls, which is consistent with the observed similar number of adhering monocytes to the vascular wall.

In line with the enhanced atherosclerosis development that we observed in *E3L.LIKK* mice, Luchtefeld et al. [11] have reported that gp130-deficient mice with defective IL-6 signaling specifically in hepatocytes, develop less atherosclerosis, indicating that modulation of hepatic inflammation can have profound effects on atherogenesis. These studies also underscore that enhanced inflammation in the liver, *e.g.* due to viral hepatitis or steatohepatitis, may augment atherosclerosis development. Indeed, in several clinical studies, such hepatic pathological conditions are associated with an elevated occurrence of CVD [28–30]. Even after adjustment for classical risk factors for CVD, such as LDL cholesterol levels,

chronic hepatitis C infection was still significantly associated with increased atherosclerosis in a cross-sectional study [30]. Together, these findings suggest that there is a direct effect of hepatic inflammation on atherosclerosis development, independent of systemic lipid levels. Moreover, they suggest that in addition to the currently used lipid-targeted drugs such as statins, reducing NF-κB activity in the liver may be a promising additive therapeutic strategy against atherosclerosis development.

In conclusion, we have shown that hepatocyte-specific activation of NF-κB leads to larger and more advanced atherosclerotic lesions. Our studies furthermore suggest that both the transient elevated (V)LDL cholesterol levels as well as the increased sensitivity to proinflammatory stimuli are most likely responsible for this aggravating effect on atherosclerosis. These findings contribute to the present understanding of the role of the liver, and more specifically the role of hepatic NF-κB, in atherosclerosis development and may help to develop new innovative anti-atherosclerotic strategies.

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Conflict of interest

None.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.atherosclerosis.2011.06.055.

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