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## **Identifying the anti-inflammatory response to lipid lowering therapy: a position paper from the working group on atherosclerosis and vascular biology of the European Society of Cardiology**

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### **Abstract**

Dysregulated lipid metabolism induces an inflammatory and immune response leading to atherosclerosis. Conversely, inflammation may alter lipid metabolism. Recent treatment strategies in secondary prevention of atherosclerosis support beneficial effects of both anti-inflammatory and lipid-lowering therapies beyond current targets. There is a controversy about the possibility that anti-inflammatory effects of lipid-lowering therapy may be either independent or not of a decrease in low-density lipoprotein cholesterol. In this Position Paper, we critically interpret and integrate the results obtained in both experimental and clinical studies on anti-inflammatory actions of lipid-lowering therapy and the mechanisms involved. We highlight that: (i) besides decreasing cholesterol through different mechanisms, most lipid-lowering therapies share anti-inflammatory and immunomodulatory properties, and the anti-inflammatory response to lipid-lowering may be relevant to predict the effect of treatment, (ii) using surrogates for both lipid metabolism and inflammation as biomarkers or vascular inflammation imaging in future studies may contribute to a better understanding of the relative importance of different mechanisms of action, and (iii) comparative studies of further lipid lowering, anti-inflammation and a combination of both are crucial to identify effects that are specific or shared for each treatment strategy.

### **1. Introduction**

Dyslipidaemia and inflammation are closely interconnected key drivers of atherosclerosis.<sup>1</sup> Dysregulated lipid metabolism induces an inflammatory and immune response in atherosclerosis, whereas the beneficial effects of low-density lipoproteins (LDLs) lowering on cardiovascular outcomes are associated with decreased inflammation. However, the controversy on anti-inflammatory effects of lipid-lowering therapy has created a large confusion around the mechanism by which these drugs exert beneficial actions. In particular, whether the improved cardiovascular outcome of statins solely reflects a decrease in LDL cholesterol, or whether lipid-independent anti-inflammatory actions prevail has been a matter of debate for a long time. In this regard, there is a discrepancy between the results obtained in experimental and clinical studies. Since novel treatment strategies in secondary prevention of atherosclerosis argue for beneficial effects of both further lipid-lowering<sup>2</sup> and anti-inflammation,<sup>3</sup> it is crucial to clarify whether further lipid-lowering therapy leads to a sufficient anti-inflammatory response, and whether adding specific anti-inflammatory therapy can achieve additional risk reduction on top of the most efficient lipid lowering.

The recent introduction of proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors represents an opportunity to deepen our understanding on the role of lipids on the immune and inflammatory responses in atherosclerosis. Likewise, the beneficial effects of IL-1 $\beta$

blockade<sup>3</sup> provided the proof of concept for anti-inflammation as a therapeutic strategy in cardiovascular disease. In addition, recent post hoc analyses point to inflammatory biomarkers for the prediction of cardiovascular outcomes in patients treated with PCSK9 inhibitors.<sup>4,5</sup> These findings have initiated a debate on whether personalized medicine can be anticipated based on either basal levels or changes in lipid levels and inflammatory biomarkers in response to lipid-lowering and/or anti-inflammatory treatments.

Thus, it is important to critically interpret and integrate the results obtained in both clinical and experimental studies to conclude on possible mechanisms behind these observations and to provide arguments for the design of future studies. This Position Paper will provide an overview of anti-inflammatory effects of lipid-lowering drugs, aiming to clarify the relation and the relevance of lipid-dependent and lipid-independent anti-inflammatory effects observed. In addition, this Position Paper underlines the mechanistic insights that translate into the observed outcomes and provides a consensus statement on how to identify the anti-inflammatory response to lipid lowering therapy.

## **2. Dyslipidaemia and immunity—cause or consequence?**

Increased LDL levels are a well-recognized cause of inflammation in atherosclerosis. However, the fact that immune cells may also affect lipid metabolism in atherogenesis is less well known and may have been overlooked.

### **2.1 Lipid-induced immune responses**

Modified LDL (mLDL) increases the endothelial expression of adhesion molecules, chemokines, as well as costimulatory and pro-inflammatory molecules, such as CD40 and nuclear factor- $\kappa$ B (NF- $\kappa$ B),<sup>6,7</sup> which will promote the recruitment of inflammatory cells into the vascular wall. LDL accelerates monocyte to macrophage differentiation<sup>8</sup> and this macrophage activation involves innate immune receptors, which are strong inducers of inflammation and have an established impact on atherosclerosis.<sup>9</sup> For example, certain components of mLDL activate pattern recognition receptors, such as toll-like receptors (TLRs), triggering proinflammatory signals.<sup>10</sup> These TLRs in addition prime the NLRP3 inflammasome through its activation by cholesterol crystals.<sup>11</sup> Also, cholesterol-loaded smooth muscle cells acquire pro-inflammatory properties.<sup>12</sup> Importantly, LDL also accumulates in circulating monocytes eliciting a pro-migratory phenotype, supporting that the pro-inflammatory paradigm induced by LDL appears both locally in the vascular wall and systemically in the circulation.<sup>13</sup> B cells are involved in atherogenesis although their role is still not clear, with some data suggesting a protective action and others supporting a pro-atherogenic function, probably depending on the specific B-cell subset.<sup>14</sup>

In addition to innate immune cells, there is also evidence for an effect of lipids on adaptive immune responses. For example, stimulation of human CD4<sup>+</sup> T cells with lysophosphatidylcholine enhances the expression of interferon- $\gamma$  and CD40L, a molecule that binds to its receptor CD40 triggering the release of multiple inflammatory mediators.<sup>15</sup> Lipoprotein-derived lysophosphatidic acid enhances atherosclerosis by releasing chemokine CXCL1 from endothelium to recruit monocytes, and oxidized LDL increases metalloproteinase-9 (MMP-9) expression and NF- $\kappa$ B activity in human macrophages.<sup>7</sup> These data provide an essential mechanism by which lipids activate genes involved in immune responses in atherosclerosis.

High-density lipoprotein (HDL) has been said traditionally to induce atheroprotection. Bone marrow and splenic reservoirs of leucocytes accelerate atherosclerosis after myocardial infarction by liberating haematopoietic stem cells and progenitor cells.<sup>16</sup>

Conversely, HDL suppresses proliferation of these haematopoietic stem cells and myelopoiesis with anti-atherogenic effects mediated by ABCA1 (ATP-binding cassette transporter-1) and ABCG1 (ATP-binding cassette sub-family G member-1).<sup>17</sup> Importantly, the benefits of HDL can be lost in hypercholesterolaemia<sup>18</sup> and also in other conditions, such as chronic kidney disease.<sup>19</sup> In addition, despite these reported benefits, recent data suggest that extreme high HDL may be associated with high mortality in the general population.<sup>20</sup> Thus, more studies are needed to ascertain the mechanisms underlying these findings.

Dietary lipid modifications can also influence the immune response. In humans, NF- $\kappa$ B activity in circulating leucocytes is enhanced after a fat-enriched meal.<sup>21</sup> Accordingly, low-fat diet reduces high-sensitivity C-reactive protein (CRP) levels,<sup>22</sup> and Mediterranean diet decreases the expression of pro-inflammatory and prothrombotic genes.<sup>23</sup> Also, monocyte cholesterol content increases following oral fat ingestion, showing a pro-inflammatory phenotype, comprising increased CD11c/CD18 expression.<sup>24</sup> Then, besides LDL, triglyceride-rich particles, including cholesterol remnant particles, may equally contribute to pro-inflammatory changes.

In summary, lipid metabolism has profound effects on both innate and adaptive immunity via multiple mechanisms.

## **2.2 Inflammation modifies lipid metabolism**

Both innate and adaptive immune processes regulate lipid metabolism (Figure 1). Several innate immune cytokines accelerate hepatic steatosis by influencing fatty acid biosynthesis and oxidation.<sup>25</sup> Thus, subacute inflammation enhances the expression of sterol regulatory element-binding proteins (SREBPs),<sup>26</sup> the master regulators of lipid biosynthesis. Innate immune cytokines can also influence lipoprotein lipase in adipose and muscle tissue<sup>27,28</sup> leading to dysfunctional triglyceride clearance and increased plasma very-low density lipoprotein (VLDL) levels. Also, TLR activation inhibits cholesterol efflux by suppressing LXR (liver X receptor)-mediated induction of ABCA1 expression, thereby inhibiting reverse cholesterol transport.<sup>29</sup>

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Mechanisms through which inflammation may promote dyslipidaemia. Inflammation enhances SRBEPs (sterol regulatory element binding proteins). The interaction of co-stimulatory molecule LIGHT (lymphotoxin-related inducible ligand) with LT $\beta$ R (lymphotoxin  $\beta$  receptor), and the impairment of Tregs (T regulatory cells) responses decrease the expression of several lipases. TLR (Toll-like receptor) activation reduces reverse cholesterol transport through a suppression of ABCA1 expression. Finally, dysregulation of intestinal microbiome increases lipid levels via an enhanced permeability of the intestinal barrier.

The adaptive immune system can also influence lipoprotein metabolism. The interaction between the costimulatory molecule LIGHT (lymphotoxin-related inducible ligand) on T cells, and lymphotoxin  $\beta$  receptor (LT $\beta$ R) on hepatocytes decreases hepatic lipase activity, impairs VLDL and LDL turnover, increasing plasma lipids.<sup>30</sup> Similarly, enhanced hepatic inflammation due to impaired T regulatory cell (Treg) responses alters the expression of several lipid metabolism-related genes including sortilin-1 (Sort-1), lipoprotein lipase, hepatic lipase, and phospholipid transfer protein resulting in hypercholesterolaemia.<sup>31</sup> In this regard, low Treg numbers or decreased Treg/effector T

cell ratios are associated with cardiovascular disease.<sup>32</sup> In addition to these effects, during acute infections and sepsis, HDL gets dysfunctional and promotes inflammation.<sup>33,34</sup>

Interestingly, other chronic inflammatory diseases show increased triglycerides, small dense LDL, and lipoprotein (a) (Lp(a)), and decreased HDL levels.<sup>27</sup> In addition, a dysregulated intestinal microbiome with enhanced permeability of the intestinal barrier affects metabolic diseases<sup>35</sup> and atherosclerosis,<sup>36</sup> being associated with increased LDL, VLDL, and total cholesterol, and with more extensive atherosclerosis in the apoE<sup>-/-</sup> mouse model.<sup>37</sup> Also, intestinal inflammation may be associated to a decrease in trans-intestinal cholesterol efflux, increasing blood lipid levels.<sup>38</sup>

Altogether, these data indicate that the inflammatory and immune response affects lipid metabolism, establishing a vicious circle that promotes atherogenesis.

### **2.3 Effects of anti-inflammatory therapies on lipids and cardiovascular risk**

Anti-inflammatory therapies show a varied range of effects on lipids and cardiovascular risk. First, non-steroidal anti-inflammatory drugs are associated with an increased risk of cardiovascular events.<sup>39,40</sup> Glucocorticoids, the other widely used anti-inflammatory drugs, have been associated with increases in HDL/total cholesterol ratio,<sup>41</sup> but also with enhanced VLDL and triglyceride production.<sup>42</sup> Nevertheless, they show no effect on cardiovascular risk in secondary prevention.<sup>43</sup>

Anti-TNF therapy may lead to an increase in HDL without LDL changes,<sup>44</sup> or to a modest rise in total cholesterol,<sup>45</sup> although these effects may depend on the drug used. The impact of these changes on cardiovascular risk may be more complex, as anti-TNF therapies have been linked with a decreased number of cardiovascular events in rheumatoid arthritis patients. However, these results come mainly from observational studies,<sup>46</sup> and large randomized clinical trials are needed to confirm this effect.

Another biologic therapy, tocilizumab, an IL-6 receptor blocker, increases total cholesterol, LDL, and triglyceride levels, but shifts HDL particles towards an anti-inflammatory composition.<sup>47</sup> In contrast, the Canakinumab Antiinflammatory Thrombosis Outcome Study (CANTOS) demonstrated that IL-1 $\beta$  blockade with canakinumab 150 mg every three months reduces the incidence of nonfatal myocardial infarction, nonfatal stroke, or cardiovascular death [hazard ratio (HR) 0.85, 95% confidence interval (CI) 0.74–0.98; P = 0.021] without affecting lipid levels, except for a mild increase in triglycerides.<sup>3</sup>

Methotrexate has been associated with both, unfavourable and beneficial lipid changes and an improved macrophage cholesterol handling.<sup>48,49</sup> Regarding its effect on cardiovascular risk, the Cardiovascular Inflammation Reduction trial (CIRT) testing low dose methotrexate against placebo in secondary prevention has been discontinued earlier than scheduled (<https://www.forbes.com/sites/larryhusten/2018/05/21/nih-halts-large-cardiovascular-inflammation-reduction-cirt-trial/#4cc3a12c5b5f>) but the results are still pending.

Finally, in a study including 532 patients with stable coronary artery disease, colchicine reduced the incidence of acute coronary syndrome, out-of-hospital cardiac arrest, or noncardioembolic ischaemic stroke (HR 0.33, 95% CI 0.18–0.59; P < 0.001).<sup>50</sup> This effect is now being tested in two large clinical trials: the LoDoCo2 (<http://www.anzctr.org.au/TrialSearch.aspx?searchTxt=LoDoCo2&isBasic=True>) and the COLCOT studies (<https://clinicaltrials.gov/ct2/show/NCT02551094>).

Thus, studies of anti-inflammatory treatments have generated contradictory results in terms of their effects on lipids. Although the heterogeneous patient populations and different inflammatory targets studied preclude a definite conclusion, monitoring of lipid levels appears to be crucial in studies of anti-inflammation in cardiovascular prevention.

### **3. Statins and immunity: current status**

#### **3.1 Experimental data**

In *in vitro* studies statins inhibit the expression of adhesion molecules in both endothelial cells and monocytes,<sup>51</sup> as well as LDL-induced endothelial nitric oxide synthase down-regulation.<sup>52</sup> In addition, statins reduce NF- $\kappa$ B activation, and chemokine and MMP expression<sup>53,54</sup> and promote the expression of anti-inflammatory and cytoprotective molecules in endothelium.<sup>55</sup>

Statins also modulate the adaptive immune response by inhibiting the expression of major histocompatibility class II required for antigen presentation to effector T cells<sup>56</sup> and divert T cell differentiation to Tregs, that suppress pro-inflammatory responses of other immune cells and counteract pro-inflammatory IL-17-producing T cell differentiation (T<sub>h</sub>17).<sup>57</sup> Statins also up-regulate the expression of Kruppel-like factor 2 (KLF2) in mouse and human T cells, diminishing interferon- $\gamma$  expression,<sup>58</sup> as KLF2 controls the expression of molecules essential for naive T cell recirculation and maintenance of T cell quiescence.

#### **3.2 Evidence in humans**

Further proof of concept for anti-inflammatory effects of statins was provided by studies of patients scheduled to elective carotid endarterectomy randomized to either statins or no lipid-lowering therapy.<sup>59</sup> Plaques from patients under statins exhibited reduced NF- $\kappa$ B activity, decreased macrophage and T cell infiltrates, and less expression of proinflammatory mediators.<sup>59</sup> Moreover, statins diminish plasma levels of CRP, cytokines, and adhesion molecules<sup>60,61</sup> and decrease microparticle shedding from inflammatory cells.<sup>62</sup>

The anti-inflammatory effects of statins have been postulated to contribute to their clinical benefits. Accordingly, statins are especially effective when associated to a diminution in CRP levels.<sup>63</sup> Furthermore, it has been suggested that statins may be more effective in patients with high CRP levels.<sup>61,64,65</sup> However, this could be due to the higher cardiovascular risk of these patients and discordant data also exist.<sup>66</sup> Also, mendelian randomization analyses suggest that CRP concentration itself is unlikely to be a causal factor of coronary artery disease.<sup>67</sup> Thus, at present, CRP determination is not advised as its contribution to the existing methods of cardiovascular risk assessment seems to be small.<sup>68</sup>

The immunomodulatory effects of statins are also supported by data on clinical outcomes. Statins decrease the cytotoxicity of natural killer cells, and the incidence of coronary vasculopathy and rejection with dynamic impairment after a cardiac transplant.<sup>69</sup> Other studies have also found a lower incidence of coronary artery disease and intimal thickening without reducing the incidence of cardiac rejection.<sup>70</sup> Conversely, in patients with kidney transplantation, there are no consistent data confirming this immunomodulatory effect.<sup>71,72</sup>

In addition, imaging vascular inflammation by techniques such as PET (positron emission tomography) using FDG (18F-fluorodeoxyglucose) has evidenced that statins achieve a decrease of vascular inflammation consistent with the reduction of the cardiovascular risk observed in clinical trials.<sup>73</sup> This is relevant, as a lack of reduction in arterial FDG uptake

by anti-inflammatory therapies has been associated with an absence of clinical benefit.<sup>74</sup> In conclusion, both experimental and clinical data support that statins have anti-inflammatory and immunomodulatory properties. Nevertheless, a legitimate question is whether these actions could be, at least in part, independent of their lipid-lowering effects.

### **3.3 Lipid-independent anti-inflammatory and immunomodulatory effects of statins**

Some intermediate compounds of the mevalonate pathway that is blocked by statins to decrease cholesterol synthesis, are implicated in post-translational modifications of key proteins<sup>75</sup> involved in important cell functions. Among them, small G proteins<sup>75</sup> play a role in cell signal transduction, and their blockade in atherosclerotic cells might interfere with atherogenesis irrespective of the inhibition of cholesterol synthesis.<sup>75</sup> In this regard, statins have anti-inflammatory effects *in vitro* in the absence of changes in lipid concentrations.<sup>53</sup> In animal models, they have stronger anti-inflammatory effects than diet modification in spite of less decrease in cholesterol levels.<sup>76</sup> Moreover, they reduce inflammation in models of inflammatory disorders likely unrelated to lipids.<sup>77–79</sup>

In humans, however, data are less clear. In the Atorvastatin in Rheumatoid Arthritis (TARA) trial, atorvastatin modestly decreased inflammation, with a diminution of  $-0.5$  (95% CI  $-0.75$  to  $-0.25$ ) in the disease activity score, when compared with  $0.03$  (95% CI  $-0.23$  to  $0.28$ ) in the placebo group ( $P = 0.004$ ),<sup>80</sup> an effect that could be lipid-independent. However, in another study ezetimibe, that decrease LDL through a different mechanism, achieved also a mild but significant reduction in the activity score of  $-0.55 \pm 1.01$  ( $P = 0.002$ ) that was similar to that obtained by simvastatin ( $-0.67 \pm 0.91$ ;  $P = 0.002$ ).<sup>81</sup> Accordingly, PCSK9-antibodies reduce the pro-inflammatory changes in circulating monocytes, coinciding with a marked decrease in monocyte-cholesterol content.<sup>13</sup> Then, LDL lowering may be the predominant factor explaining anti-inflammatory effects in humans. In fact, risk reduction of major vascular events is similar in statin and non-statin therapies [ $0.77$  (95% CI  $0.71$ – $0.84$ ) and  $0.77$  (95% CI  $0.75$ – $0.79$ ) per  $38.7$  mg/dL LDL decrease, respectively;  $P < 0.001$  for both].<sup>82</sup>

Thus, although basic research suggests that statins have lipid-independent anti-inflammatory effects, this seems difficult to confirm in humans. This discrepancy may reflect the biodistribution of statins. In this regard, a nano-particulate formulation statin packaged in HDL particles exerts potent anti-inflammatory effects in plaques, as it delivers the statin into the plaque macrophages directly.<sup>83,84</sup> In contrast, oral statins in equal doses hardly affect plaque inflammation, illustrating that the first-pass clearance of statins by the liver precludes a strong anti-inflammatory effect *in vivo*.

### **4. Non-statin LDL-lowering drugs and inflammation**

The relationship between lipids and inflammation is supported by the observation that non-statin lipid-lowering drugs also have anti-inflammatory effects. In this regard, ezetimibe also diminishes plaque inflammation in models of atherosclerosis.<sup>85</sup> Accordingly, in patients at high cardiovascular risk, ezetimibe reduces plasma levels of inflammatory markers.<sup>86</sup> Similarly to data reported with statins, achievement of both CRP and LDL prespecified targets in patients receiving ezetimibe and statin combination therapy is associated with better outcomes than reaching only LDL target levels.<sup>87</sup> Then, non-statin LDL-lowering drugs also show anti-inflammatory properties despite working through different mechanisms, suggesting that lipid reduction plays a key role in these effects. In this regard, recent data show that lipid reduction is associated to a modulation of the inflammatory response irrespective of the lipid-lowering therapy used.<sup>88</sup>

Specifically, the reduction obtained in CRP levels with statins and with their combination with ezetimibe is proportional to the reduction observed in LDL levels.<sup>88</sup>

## **5. PCSK9 inhibition**

### **5.1 PCSK9: an endogenous inhibitor of the LDL receptor**

PCSK9 mediates intracellular degradation of the hepatic LDL receptor (LDLR).<sup>89</sup> Once secreted by the hepatocyte, PCSK9 binds to the extra-cellular EGF-A domain of the LDLR, leading to the internalization of the LDLR-PCSK9 complex through clathrin-coated pits.<sup>90</sup> In addition, PCSK9 could also enhance LDLR degradation by an intra-cellular pathway not requiring PCSK9 secretion.<sup>91</sup> In this regard, the S127R PCSK9 gain-of-function variant leads to autosomal dominant hypercholesterolaemia without PCSK9 secretion.<sup>92</sup>

PCSK9 monoclonal antibodies block the extracellular PCSK9 pathway reducing LDL, triglyceride, cholesterol and Lp(a) plasma levels, and increasing HDL and ApoA1 levels.<sup>2</sup> These data translate into a decrease in the incidence of cardiovascular events in secondary prevention<sup>2</sup> (<http://www.acc.org/latest-in-cardiology/clinical-trials/2018/03/09/08/02/odyssey-outcomes>).

### **5.2 PCSK9 and inflammation**

The fact that the recently developed PCSK9 inhibitors do not reduce plasma CRP<sup>13</sup> has led to the idea that they may not have anti-inflammatory effects (Figure 2). However, many data indicate that these drugs share anti-inflammatory actions with other lipid-lowering drugs. Even more, PCSK9 itself could have pro-inflammatory effects.

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Role of PCSK9 in sepsis and inflammation. Hepatic PCSK9 expression is induced by TNF $\alpha$  hepatocyte nuclear factor-1 $\alpha$  (HNF-1 $\alpha$ ) and lipopolysaccharide (LPS). Once in the plasma, PCSK9 binds to the LDL receptor facilitating LDL degradation in lysosomes, thus decreasing clearance of LDL-bound LPS. Extracellular PCSK9 also enhances the expression of pro-inflammatory markers, decreases macrophages in atheroma, and the expression of LDLR and ABCA1 in these cells. It also forms a complex with Lp(a). The intracellular function of PCSK9 in inflammation remains unknown. GNB, gram-negative bacteria; mab, monoclonal antibody.

The transcriptional regulation of PCSK9 suggests that it may have a role in inflammation. PCSK9 expression is induced by hepatocyte nuclear factor-1 $\alpha$  (HNF-1 $\alpha$ ), which regulates the expression of acute phase pro-inflammatory proteins.<sup>93</sup> Also, lipopolysaccharide induces early hepatic and renal PCSK9 mRNA expression in mice,<sup>94</sup> as well as in endothelial and vascular smooth muscle cells.<sup>95</sup> Finally, TNF $\alpha$  increases PCSK9 expression in vitro in cultured macrophages.<sup>96</sup> In humans, plasma PCSK9 concentrations increase in sepsis,<sup>97</sup> trauma,<sup>98</sup> and in acute coronary syndromes. Furthermore, they are positively associated with white blood cell count, fibrinogen,<sup>99</sup> and CRP<sup>100</sup> in coronary patients. To date, there are no data reporting the effects of anti-inflammatory molecules on PCSK9 levels.

While PCSK9 expression in macrophages is debatable, data suggest that it modulates LDLR expression in these cells, either through a paracrine<sup>101</sup> and/or autocrine mechanism<sup>102</sup> and also stimulates the expression of scavenger receptors (principally LOX-1) and ox-LDL uptake in these cells.<sup>96</sup> Furthermore, PCSK9 silencing reduces oxLDL-induced cytokine expression in THP-1 derived macrophages through NF- $\kappa$ B



inhibition<sup>103</sup> and decreases TLR4 expression and NF- $\kappa$ B activation in oxLDL-treated macrophages.<sup>104</sup> Conversely, overexpression of human PCSK9 in lipopolysaccharide-stimulated macrophages promotes the expression of pro-inflammatory markers, while inhibiting anti-inflammatory molecules.<sup>102</sup> Similarly, PCSK9 overexpression in TNF $\alpha$ -primed macrophages enhances the expression of scavenger receptors.<sup>96</sup> Bone marrow transplantation from mice expressing human PCSK9 (hPCSK9tg) in apoE knockout mice generated a chimeric model expressing hPCSK9 only in macrophages.<sup>102</sup> Interestingly, despite not modifying lipid levels, transplanted animals showed a LDLR-dependent increased number of Ly6Chi inflammatory monocytes within atherosclerotic lesions and spleens, and a reduction of LDLR expression in macrophages.<sup>102</sup> Furthermore, PCSK9 decreases ABCA1 expression, thereby reducing cholesterol efflux in macrophages at least partly in a LDLR-dependent manner.<sup>105</sup> In addition, human recombinant PCSK9 induces the expression of monocyte chemoattractant protein-1, IL6 and other pro-inflammatory cytokines in both THP-1 derived and human primary macrophages.<sup>106</sup> Altogether, these data suggest that PCSK9 may locally regulate atherosclerotic plaque inflammation. Accordingly, the ATHEROREMO-IVUS study found that circulating PCSK9 levels were positively associated with the extent of necrotic core in atheroma, independently of LDL levels in patients with acute coronary syndrome.<sup>107</sup> Moreover, PCSK9 inhibition with alirocumab in APOE\*3 Leiden.CETP mice, decreases macrophage and necrotic core content and increases vascular smooth muscle cells and collagen content.<sup>108</sup>

Regarding the effects of PCSK9 inhibition on inflammation in humans, it reduces Lp(a) levels,<sup>109</sup> a molecule that circulates bound to PCSK9 in plasma,<sup>110</sup> and promotes inflammation and oxidative stress and coagulation. Also, PCSK9-antibody therapy markedly reduces monocyte inflammatory phenotype in patients with familial hypercholesterolaemia, without any change in plasma hsCRP concentration.<sup>13</sup> A similar lack of effect on hsCRP levels has been described in large cardiovascular outcomes trials.<sup>4</sup> This emphasizes the potential for anti-inflammatory effects without reduction in the liver-derived acute phase reactant hsCRP.<sup>111</sup>

### **5.3 PCSK9 and septic shock**

Beyond its lipid-lowering action, as lipopolysaccharide circulates bound to LDL, up-regulation of hepatic LDLR by PCSK9 inhibition has been suggested to result in increased lipopolysaccharide clearance (Figure 2), a decreased inflammatory response, and improved survival following sepsis in mice<sup>112,113</sup> although, in a recent study, PCSK9 inhibition failed to reduce LPS-induced mortality in mice.<sup>114</sup> Importantly, humans with PCSK9 loss-of-function variants also exhibit improved clinical outcomes during septic shock.<sup>115</sup> Finally, enhanced plasma PCSK9 levels during sepsis are associated with multiple organ failure.<sup>97</sup> Based on these results, clinical trials are planned to assess the effect of PCSK9 inhibition outcomes during sepsis.

### **5.4 Vascular actions of PCSK9**

PCSK9 is expressed in many other tissues than the liver, including atherosclerotic plaques,<sup>101,116</sup> and vascular areas of low shear stress, that are prone to develop atherosclerosis.<sup>95</sup> PCSK9 expression is found mainly in vascular smooth muscle cells,<sup>101</sup> and to a lesser extent in endothelial cells.<sup>95</sup> PCSK9 deficiency reduces neointimal formation following injury of the carotid artery in mice beyond cholesterol lowering by decreasing vascular smooth muscle cell migration and proliferation rate.<sup>117</sup>

Recent epidemiological studies have demonstrated that plasma PCSK9 levels are associated with carotid atherosclerosis<sup>118</sup> independently of LDL. However, other data challenge the idea of a lipid-independent effects of PCSK9 inhibitors. For instance, plasma PCSK9 levels have not proven consistently to predict cardiovascular events.<sup>100,119,120</sup> Moreover, in the GLAGOV trial a linear relationship was found between the regression of atheroma and the decrease of LDL achieved with evolocumab.<sup>121</sup> Finally, the risk reduction observed with PCSK9 inhibitors in clinical trials, seems to be fully explained by the decrease of LDL achieved, suggesting that the anti-atherosclerotic benefit of this therapy is directly related to LDL reduction.<sup>122</sup>

However, inflammation may affect the response to PCSK9 inhibition as suggested by very recent post hoc analyses of PCSK9 trials.<sup>4</sup> First, patients with high CRP at baseline obtain greater benefit with PCSK9 inhibitors.<sup>4</sup> Second, patients with persistent high CRP levels after initiating treatment with statins and PCSK9 inhibitors have a worse prognosis.<sup>5</sup> Although the risk reductions obtained in these trials seem to be fully explained by the decrease in LDL cholesterol, a more marked beneficial response to PCSK9 inhibition may hence be predicted in patients with high inflammatory levels.

### **Consensus statements**

- 1 Lipid reduction is associated with modulation of the inflammatory and immune responses irrespective of the lipid-lowering therapy used. Despite decreasing cholesterol through different mechanisms, most lipid-lowering therapies, including dietary interventions, share anti-inflammatory, and immunomodulatory properties. This observation provides strong evidence that lipid lowering per se causes alterations in inflammation and immunity. Some lipid-lowering drugs also directly target lipid-independent pathways to reduce inflammation in experimental and exploratory studies. However, a contribution of these effects to cardiovascular outcomes is unclear, and further studies are required to address this question. However, regardless of the mechanism involved, an anti-inflammatory response to lipid-lowering may be of clinical importance to predict the effect of treatment.
- 2 Using surrogates for both lipid metabolism and inflammation as biomarkers in future studies may contribute to a better understanding of the relative importance of different mechanisms of action. Given the strong association between inflammation, lipids and atherosclerosis, assessment of the inflammatory response to lipid-lowering interventions could be helpful to establish optimal dose and type of lipid-lowering therapy in cardiovascular prevention. There is still an unmet need for new biomarkers and further validation of existing biomarkers that more closely reflect the inflammatory activity in atherosclerosis before such approach can be implemented.<sup>123</sup> In this regard, at present, CRP determination is not advised as it adds small value to the existing methods of cardiovascular risk assessment.<sup>68</sup> Also, imaging vascular inflammation by techniques such as PET show promise to assess the anti-inflammatory effect of lipid-lowering therapies. Furthermore, we raise the notion of lipid monitoring in studies of anti-inflammatory therapies.
- 3 Comparative studies of further lipid lowering, anti-inflammation and a combination of both will be crucial to identify effects that are specific or shared for each treatment strategy. Current experimental and clinical research evidence discussed in the present Position Paper can be used to design a head-to-head comparison of the

potential beneficial effects of additional anti-inflammation or lipid lowering therapies or the combination of both regimens to current medical standards in secondary prevention. In this context, personalized medicine could be anticipated based on predictive factors for a beneficial response to lipid lowering and/or inflammatory levels in secondary prevention.

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