# Serum Amyloid A: The "Other" Inflammatory Protein

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**Current Atherosclerosis Reports** 2006, **8**:62–68
Current Science Inc. ISSN 1523-3804


Inflammation long has been recognized as a hallmark of atherosclerotic lesions, but more recently attention has focused on chronic low-level elevations of specific plasma inflammatory proteins such as C-reactive protein (CRP) and serum amyloid A (SAA), which may not only represent markers of atherosclerosis risk but also participate directly in atherogenesis. This article briefly reviews evidence for and against potential roles of CRP as an atherosclerosis risk marker and in atherogenesis. The remainder of the article focuses on SAA, an inflammatory protein that is carried on, and may fundamentally alter the function of, high-density lipoprotein. Data are reviewed regarding the regulation of SAA by dietary cholesterol, obesity, and insulin resistance, and its potential role as an atherosclerosis mediator. Lying at the intersection of inflammation, dyslipidemia, obesity, and insulin resistance, SAA may play a key role in regulating the contributions of these processes to atherogenesis.

## Introduction

Inflammation long has been recognized as a hallmark of atherosclerotic lesions [1,2], but recently substantial attention has been paid to the role of serum proteins that are increased in response to inflammation. These proteins have been studied primarily as markers of atherosclerotic risk, but some also have been implicated as potential mediators of the atherosclerotic process. This review briefly discusses studies evaluating one of these "inflammatory" proteins, namely C-reactive protein (CRP), but then focuses primarily on serum amyloid A (SAA), which is a less well-studied protein that may serve as a link between atherosclerosis, inflammation, and obesity/insulin resistance.

## The Acute-phase Response

In acute inflammation, whether triggered by infection, direct tissue injury, or other diseases, circulating cytokines induce the liver to synthesize a number of proteins that attempt to respond to the insult. Many of these proteins play key roles, not only in the inflammatory and immune response to injury, but also in activating the fibrinolytic and complement systems. Some acute-phase proteins are carried in the plasma on circulating lipoproteins. Though the functions of many acute-phase proteins are well understood, this is not true of all proteins involved in the acute-phase response, particularly CRP and SAA, which may increase by as much as 1000-fold above their baseline levels in acute inflammation [3]. More recently, it has become apparent that levels of many acute-phase proteins, including CRP and SAA, may be chronically elevated in some individuals, albeit at levels much lower than those seen in the acute-phase response. Although acute elevations of these proteins are likely to have beneficial effects, chronic elevations might be deleterious. It is, therefore, of interest that levels of CRP and SAA may be chronically elevated in individuals with atherosclerosis, diabetes, obesity, insulin resistance, and rheumatologic diseases, all of which are associated with an increased risk of cardiovascular disease. Therefore, the potential significance of these proteins in chronic disease states, both as clinical markers and as pathologic mediators of cardiovascular disease, has undergone substantial scrutiny. This is particularly true for CRP, which several studies have associated with increased risk for ischemic heart disease events and stroke. In addition, while the liver classically has been considered the primary source of most acute-phase proteins, recent studies have demonstrated that other tissues might also be sources of these proteins, especially SAA. These observations have raised the exciting possibility that specific acute-phase proteins might be exploited for diagnostic or therapeutic benefits in chronic disease states such as atherosclerosis.

## C-Reactive Protein: The "Orthodox" Inflammatory Protein

C-reactive protein is the most studied of the acute-phase proteins and was named for its property of binding to the

C-polysaccharide of *Streptococcus pneumoniae*. CRP is a pentraxin, composed of five identical protein molecules noncovalently linked around a central protein core. CRP circulates in plasma unbound to lipoproteins, but it can bind to oxidized lipoproteins and apoptotic cells [4] as well as to aggregated low-density lipoprotein (LDL) in vitro [5,6]. In addition, CRP can bind complement and can bind to the Fc-gamma-II (CD32) receptor [6].

**C-reactive protein as a marker of cardiovascular risk**

Recently, CRP has been studied extensively as a marker for atherosclerosis risk, and elevated levels have been associated with increased risk for important atherosclerosis endpoints, including myocardial infarction and stroke [7–10]. CRP levels are increased in a number of conditions that are associated with increased cardiovascular risk, including obesity [11,12•,13•], insulin resistance [13•,14–17], type 2 diabetes [16], and smoking [18]. More recently, increased dietary cholesterol intake has been shown to increase CRP levels in lean, insulin-sensitive subjects [13•]. However, CRP has been less firmly associated with atherosclerosis severity, at least as measured by electron beam computed tomography [19,20] or coronary angiography [21]. Recent studies have suggested that, following acute coronary syndromes, CRP levels give prognostic information beyond that provided by LDL levels alone [22,23]. However, these studies do not take into account the relationships of obesity and insulin resistance to CRP levels [13•,14,15], nor do they prove that CRP has incremental prognostic value over a composite of traditional risk factors that, in aggregate, may account for up to 94% of the population-attributable risk for coronary events [24]. In addition, a recent study by Danesh et al. [25], which included data from the Reykjavik Prospective Study as well as an updated meta-analysis of CRP levels in over 20,000 subjects, concluded that the additional risk associated with elevated CRP levels was less than that for hypercholesterolemia, systolic hypertension, or smoking. Finally, one recent study demonstrated that, even in the absence of changes in either medication or clinical status, nearly 40% of ischemic heart disease patients changed their CRP-based risk category [26] over 1 month of follow-up [27]. Thus, despite its apparent utility as a risk predictor in large groups, the marked inter-individual variability in CRP levels may limit its clinical utility as a tool for either predicting risk or following response to therapy in individual patients.

**C-reactive protein as a mediator of atherogenesis**

A number of recent studies also have suggested mechanisms by which CRP might participate directly in atherogenesis. CRP has been shown to have a number of potentially proatherogenic effects on endothelial effects in vitro. These include induction of leukocyte adhesion molecules [28], monocyte chemoattractant protein-1 [29], interleukin-8 [30], and plasminogen activator inhibitor-1 [31], as well as inhibition of tissue plasminogen activator [32] and of the vasodilators prostacyclin [33] and nitric oxide (NO) [34,35]. However, a more recent study reported that purified, native-form human CRP actually increased NO bioavailability in endothelial cells and isolated arterial rings [36]. Thus, some authorities have raised the concern that proinflammatory effects identified in some previous in vitro studies might have been due to use of CRP with low levels of contaminants, such as endotoxin, sodium azide, or CRP in non-native forms [37].

In vivo evidence of a direct proatherogenic role of CRP consists of one study in the apoE-deficient mouse model of atherogenesis, demonstrating a modest increase in atherosclerotic lesion area in male, but not in female, mice expressing a human CRP transgene. In contrast, three recent studies found no effect on atherosclerosis of overexpression of either a human [38••,39•] or rabbit [40•] CRP transgene. Thus, more recent in vivo studies do not support a role for CRP as a mediator of atherogenesis.

## Serum Amyloid A: The "Other" Inflammatory Protein

Serum amyloid A is a family of four homologous, amphipathic, alpha-helical proteins encoded for by genes located on chromosome 7 in mice and on chromosome 11 in humans. SAA includes SAA1 and SAA2, which are acute-phase proteins, and SAA4, which is expressed constitutively. Like CRP, hepatic expression of SAA1 and SAA2 is increased markedly in response to a variety of inflammatory stimuli [3,41]. In contrast, SAA4 is constitutively expressed by the liver [41]. Though clasically thought to be produced primarily by hepatocytes, both the acute-phase and constitutive forms of SAA have been shown to be expressed by endothelial cells, macrophages, and smooth muscle cells in human atherosclerotic plaques [42], and by cytokine-stimulated smooth muscle cells in vitro [42]. SAA3 is a truncated protein primarily expressed in mice by extrahepatic cells, including adipocytes and macrophages. SAA3 typically is not expressed in humans due to the presence of a premature stop codon in exon 2 [3,41]. SAA are transported in the plasma primarily on HDL particles, but also may be carried on triglyceride-rich very low-density lipoprotein (VLDL) particles, particularly in circumstances where SAA levels are elevated [3,41]. In general, serum levels of CRP and SAA are highly correlated in humans [12•,13•,43] and, like CRP, SAA levels are elevated in obesity [12•,13•,16,21,44], insulin resistance [12•,13•,45], and diabetes [16,45,46]. In multivariate analyses, CRP levels generally have correlated better with atherosclerosis risk than have levels of SAA [43], though a recent study has demonstrated better correlation of SAA levels than of CRP levels with angiographic coronary artery disease severity in women [21]. Thus, whereas SAA may not be as sensitive a marker of

atherosclerosis risk as CRP, it might prove to be a better marker of atherosclerosis severity. An important caveat is that SAA levels are subject to substantial inter-individual variation over time, as are levels of CRP [12•].

### Regulation of serum amyloid A

As noted previously, SAA levels generally correlate with levels of CRP in a variety of disease states in humans. However, in mice, only SAA levels, but not those of CRP, are regulated by inflammatory stimuli. For example, it was shown several years ago that feeding of an atherogenic diet containing fat, cholesterol, and cholate induces SAA expression in mice [47] through induction of nuclear factor-κB. More recently, it has been demonstrated that mild, chronic elevations of SAA can be found in both the apoE-deficient and LDL receptor–deficient mouse models of atherogenesis, even in mice fed a chow diet [48•]. Another recent study has demonstrated in LDL receptor–deficient mice that SAA levels are elevated by adding fat to a chow diet, and are elevated even more dramatically by the further addition of cholesterol [49••]. Interestingly, both studies demonstrated, either by immunoprecipitation in chow-fed mice [48•] or by fast protein liquid chromatography in chow-, fat-, or fat and cholesterol–fed mice [49••], that SAA was detected not only on high-density lipoprotein (HDL), but also on VLDL. These findings suggest that SAA may influence the roles of both HDL and VLDL in atherogenesis.

In humans, the relationship of dietary composition to SAA levels is more complex and appears to be mediated, at least in part, by both insulin resistance and obesity. The relationship of obesity [16,21,44] and insulin resistance [45] to elevated levels of SAA is well established. However, four recent studies have extended these observations. Three of these studies have demonstrated that dietary weight loss is associated with reduction in SAA levels [12•,50••,51••]. However, in one of these studies, the correlation of weight loss with reduction in SAA was found even in a group of obese women receiving a weight-loss diet that contained high levels of fat and cholesterol [12•]. Interestingly, in that study, decrease in SAA also correlated with improvement in basal insulin resistance [12•]. The fourth study [13•] differed in that it was not a weight-loss study but rather examined the effect of feeding four eggs per day for 1 month on plasma levels in insulin-sensitive and insulin-resistant groups of subjects. Surprisingly, egg feeding had no effect on SAA levels in the insulin-resistant groups, but dramatically increased SAA levels in insulin-sensitive subjects [13•]. Thus, in contrast to mice, in which feeding dietary fat and/or cholesterol raises SAA levels, short-term increases in dietary fat and cholesterol appear to raise SAA levels only in insulin-sensitive individuals. In contrast, whereas obese subjects and insulin-resistant subjects have elevated SAA levels at baseline, short-term increases in dietary fat and/or cholesterol do not further raise their plasma SAA levels. Specific mechanisms that might account for why obesity and insulin resistance inhibit diet-induced elevations in SAA levels are not known. One possibility is that dietary cholesterol absorption is inhibited in the presence of obesity [52] and/or insulin resistance [53]. Alternatively, it may be that the presence of adipose tissue macrophages, which recently have been shown to accumulate in obesity [54,55], may blunt the inflammatory response to dietary cholesterol by mechanisms as yet unknown.

### Extrahepatic sources of serum amyloid A

As noted previously, with the exception of SAA3, the liver classically has been considered the primary source of SAA protein expression in both normal and disease states. However, several years ago, Meek et al. [42] demonstrated that acute-phase and constitutive SAA also may be expressed by plaque cells, especially macrophage and smooth muscle foam cells. More recently, two important studies have challenged the commonly held notion that the liver is the major source of SAA in obesity, by demonstrating that adipocytes represent a major site of SAA expression in obese individuals [50••,51••]. In the first study, investigators found that 1) SAA expression is 20-fold higher in mature adipocytes than in stromal vascular cells of subcutaneous white adipose tissue (sWAT); 2) mRNA and adipocyte immunoreactivity for SAA were much higher in sWAT of obese as compared with lean subjects; and 3) sWAT mRNA levels (and plasma SAA levels) were decreased following dietary weight loss [50••]. In the second study, SAA mRNA and protein were detected in subcutaneous and omental adipose tissue, and SAA protein was localized to adipocytes by immunohistochemistry. Also, similar to the first study, diet-induced weight loss was associated with a reduction in adipose tissue SAA expression that correlated with reduction in plasma SAA levels [51••]. Finally, the second study also demonstrated by microarray analysis that omental and subcutaneous adipose tissue had substantially higher levels of SAA mRNA expression than did any other tissue studied, including liver [51••]. Taken together, these findings suggest that adipocyte SAA expression (and its regulation by obesity) may account for the strong correlation of SAA levels with obesity and insulin resistance. A summary of the potential sources for the increased levels of CRP and SAA seen in response to dietary cholesterol and obesity/insulin resistance are shown in Figure 1.

### Serum amyloid A in atherogenesis

The potential links between SAA, obesity, and insulin resistance, as well as the association of SAA with specific plasma lipoproteins, have stimulated recent interest in a potential role for SAA as a mediator of atherogenesis. In addition, SAA has been shown in vitro to have a number of effects that could potentially promote atherosclerosis, including mediating HDL binding to differentiated macrophages [56,57] and endothelial cells [57], and impairing the capacity of HDL to promote cholesterol efflux from macrophages [58]. Free SAA also has been shown in vitro to induce

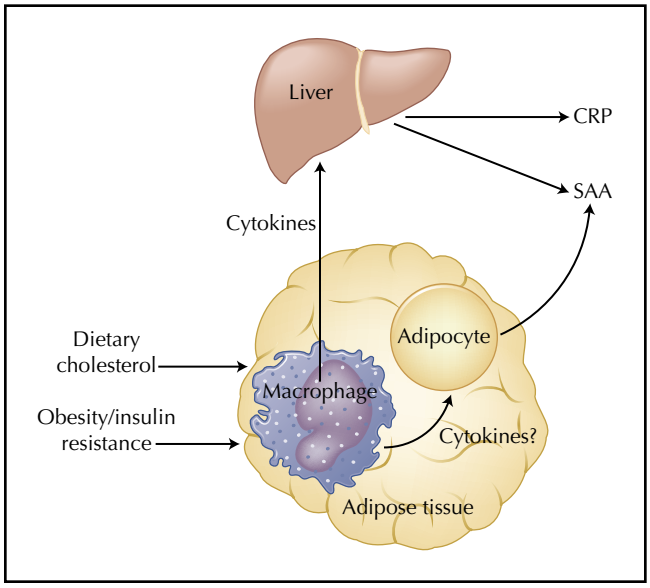


**Figure 1.** Sources of C-reactive protein (CRP) and serum amyloid A (SAA). Under the influence of dietary cholesterol and/or insulin resistance, adipose tissue accumulates macrophages, which in turn secrete cytokines that induce the liver to express both CRP and SAA. However, adipose tissue adipocytes may also themselves secrete SAA, possibly under the influence of locally secreted cytokines.

expression of extracellular matrix–degrading metalloproteinases [59,60] and to promote chemotaxis and adhesion of both monocytes [61] and T lymphocytes [62].

In addition, SAA may play major roles in lipid transport. As noted previously, SAA associates with both HDL and VLDL in plasma. The teleologic reason why SAA might associate with HDL in inflammatory states is not known, but one theory [56,58] is based on the observations that 1) SAA can displace apolipoprotein A-I from HDL particles [63]; and 2) as compared with HDL without SAA, SAA-containing HDL has decreased affinity for hepatocytes [56] and increased affinity for macrophages [56,58]. In this scheme, the presence of SAA changes HDL from a particle that removes cholesterol from peripheral tissues to the liver (so-called reverse cholesterol transport) to a particle that delivers cholesterol to peripheral tissues, in particular to sites of inflammation [56,58]. In contrast, a recent study has demonstrated that SAA2 (but not SAA1) contains a lipid transport activity in its amino-terminal region that promotes cholesterol efflux from cholesterol-laden macrophages in vitro [64].

Two recent studies [48•,49••] also have demonstrated that SAA might play a role in retention of HDL particles in atherosclerotic tissue by acting as a "bridging" molecule mediating binding of HDL to vascular proteoglycans. SAA has a number of positively charged amino acids in its carboxy-terminal region that have been implicated in proteoglycan binding [65]. The recent studies have shown that the presence of SAA on HDL particles increases its binding in vitro to perlecan [48•], a proteoglycan that accumulates in murine atherosclerotic lesions [66], and to biglycan [49••], another proteoglycan that accumulates in both murine [66] and human [67] atherosclerosis. Moreover, both studies co-localized SAA and apoA-I to perlecan-rich regions of murine atherosclerotic lesions, but not in perlecan-free, nonlesioned areas, providing in vivo evidence for a role for SAA in plaque HDL retention [48•,49••]. Importantly, one of these studies also showed that plasma levels of SAA, but not of cholesterol, correlated strongly with atherosclerotic lesion area [49••], further supporting a potential direct role for SAA in atherogenesis. However, firm confirmation of a role for SAA in atherogenesis will require direct testing in both transgenic and SAA-deficient animal models.

The retention of HDL on atherosclerotic extracellular matrix by SAA could promote atherogenesis in several ways. Firstly, HDL trapped in the atherosclerotic plaque would be unavailable for transport of cholesterol out of the plaque in the reverse cholesterol transport pathway. Secondly, HDL retained in plaque might be more susceptible to oxidation as well as to other chemical modifications such as nitrosylation [68], chlorination [69], and acrolein adducts [70], all of which may render HDL more atherogenic. In addition, SAA might also play a role in mediating plaque retention of VLDL, as both murine studies have confirmed that SAA also is present on VLDL particles [48•,49••].

Thus, the association of SAA with HDL (and VLDL) in chronic disease states, including insulin resistance, obesity, and diabetes could account, at least in part, for the association of these diseases with increased risk for atherosclerosis. Some of the multiple potential mechanisms by which SAA might stimulate atherogenesis are shown in Figure 2.

### Serum amyloid A as a therapeutic target

If SAA is a mediator of atherosclerosis, what therapies may decrease its expression? In general, many therapies that decrease circulating CRP levels also decrease those of SAA, including weight loss [12•,50••,51••], statin treatment [71,72], and improvement in insulin resistance through weight loss [12•] or treatment with peroxisome proliferator activated receptor gamma (PPARγ) activators [45,73]. In addition, because dietary fat and cholesterol appear to increase SAA levels in lean subjects [13•] but not in individuals who are obese [12•,13•] and/or insulin-resistant [13•], decreasing intake of these dietary components might reduce SAA levels in lean individuals. However, the effects of these therapies are not exclusive to SAA. Therapies that specifically target SAA, such as inhibiting its proteoglycan binding domain (as has been done for apoB) [74], have not been tested.

## Conclusions

Acute-phase proteins, in particular CRP and SAA, appear to be good predictors of cardiovascular disease in large

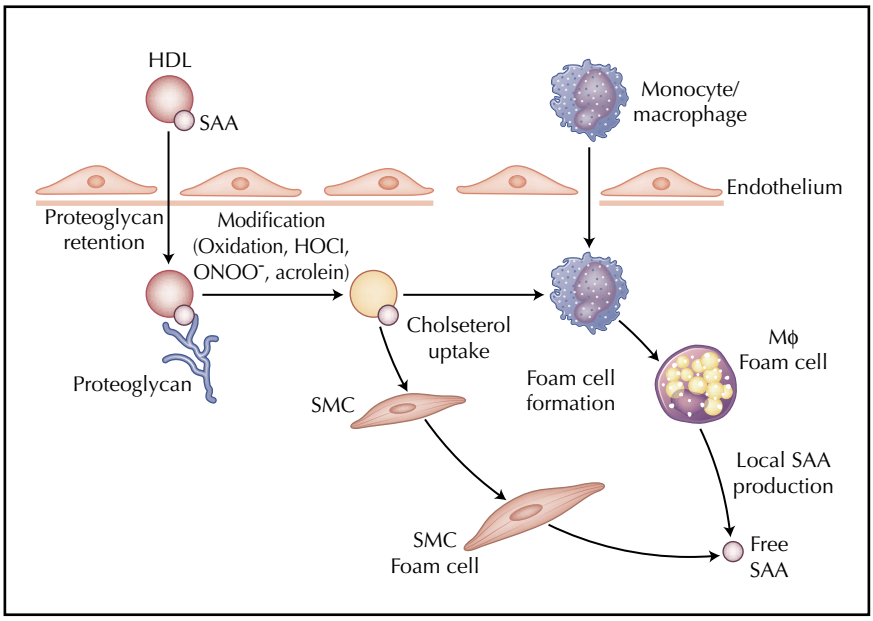


**Figure 2.** Potential roles of serum amyloid A (SAA) in atherogenesis. When it associates with high-density lipoprotein (HDL), SAA can mediate binding of HDL to vessel wall proteoglycans. The trapped SAA containing HDL then could undergo oxidation or other chemical modifications by locally produced hypochlorous acid (HOCl), peroxynitrite ($ONOO^-$), and/or acrolein, which impair the ability of HDL to remove cellular cholesterol. SAA also can directly increase HDL binding to plaque cells. Together, these changes could result in a marked increase in net cellular cholesterol accumulation, thereby leading to macrophage (Mφ) and smooth muscle cell (SMC) foam cell formation. These activated plaque cells then could secrete free SAA, which in turn may further accelerate plaque development by inducing expression of matrix-degrading metalloproteinases and promoting monocyte and T lymphocyte chemotaxis and adhesion.

epidemiologic studies, though SAA has been less well studied than CRP. The roles of these molecules as mediators of atherogenesis are less clear, particularly for CRP, as three recent transgenic animal studies of CRP have failed to demonstrate any proatherogenic effect. SAA, through its effects on HDL metabolism and regulation by dietary cholesterol, obesity, and insulin resistance, lies at the intersection of inflammation, dyslipidemia, and metabolic syndrome. As a consequence, the potential role of SAA as an atherosclerosis mediator is provocative, though it still needs to be rigorously tested in animal models. If SAA is shown definitively to increase atherosclerosis risk, specific targeting of this inflammatory molecule may offer therapeutic approaches beyond classical risk reduction.

## Acknowledgment

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