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C-Reactive Protein and Asymmetric Dimethylarginine: Markers or Mediators in Cardiovascular Disorders?

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Department of Molecular and Applied Biology, School of Biosciences, University of Westminster, London, UK

Abstract: C-reactive protein (CRP) has received much attention as a cardiovascular risk factor and has been recommended to be used in screening to assist in predicting the occurrence of cardiovascular disorders. There are numerous association studies documenting changes in circulating CRP concentrations, there are, however, fewer studies providing evidence that CRP mediates the progression of cardiovascular pathologies. Elucidating the potential mechanisms for CRP has been confounded by recent reports that contaminants of CRP are partially responsible for observed effects.

In this review the use of CRP as a tool to predict cardiovascular disorders will be discussed alongside a more recently described cardiovascular risk factor asymmetric dimethylarginine (ADMA). An endogenously occurring nitric oxide synthase inhibitor, ADMA, is formed by the action of protein arginine methyltransferases and subsequent proteolysis and it is metabolised in vivo by the dimethylaminohydrolases (DDAH). The evidence available documenting the effects of CRP and ADMA, the regulatory mechanisms and the genetic influences, will be discussed in order to determine whether CRP and ADMA are mediators in the progression of cardiovascular disorders or merely useful biomarkers.

Key Words: C-reactive protein, asymmetric dimethylarginine, nitric oxide, cardiovascular risk factor, atherosclerosis, dimethylarginine dimethylaminohydrolase, protein arginine methyltransferase.

INTRODUCTION
ADMA and CRP as Cardiovascular Risk Factors

In 1992 Vallance et al. reported impaired nitric oxide synthesis and elevated concentrations of asymmetric dimethylarginine (ADMA) in patients with end stage renal failure and suggested that ADMA might contribute to the development of hypertension [1]. In a study of men in the Kuopio Ischaemic Heart Disease Risk Factor Study, ADMA concentrations exceeding 0.69 μmol/L in subjects with a history of heart disease were associated with a 4-fold risk of a coronary event [2] and in the same issue Zoccali et al. reported that in patients with end stage renal disease, raised concentrations of ADMA independently predicted mortality and cardiovascular outcome [3]. Since this time there have been many studies looking at the concentrations of ADMA and the correlation with cardiovascular risk, (these are summarised in Table 1 reviewed by [4]).

A panoply of studies have reported increased C-reactive protein (CRP) concentrations in cardiovascular related disorders and it is not the intention of this review to detail all of these, an overview is shown in Table 2. C-reactive protein had been characterised as an acute phase protein. One of the first reports of CRP as a cardiovascular risk factor came from observations that circulating concentrations of CRP were increased following myocardial infarction [5]. This was followed by a study of a cohort of patients with unstable angina where higher concentrations of CRP correlated with poor outcome [6]. Concentrations of plasma CRP above 3.6 mg/L were associated with a 2-fold risk of a coronary event characterised by myocardial infarction or sudden coronary death [7]. Higher concentrations of CRP in patients with pre-existing atherosclerosis were found to correlate with susceptibility to subsequent cardiovascular events [8], and, following myocardial infarction, CRP concentrations predicted adverse short term outcomes [9]. The relative risk of a coronary event when circulating CRP concentrations exceeded 3 mg/L was originally determined to be ~2.0 [10] but in a larger study this was revised to 1.45 [11].

Interest has been shown in the benefits of measuring risk factors, in addition to age, family history, exercise, smoking history, cholesterol levels and blood pressure identified originally in the Framingham study; which may further predict the risk of cardiovascular disorders.

Table 1. Summary of Cardiovascular Disorders which have Reported Changes in ADMA Levels. Elevated ADMA (0.6-4 μmol/L) Correlated with Lower NO Bioavailability; Reviewed by [4]. *Alzheimers is not a CV Disorder

<table>
<thead>
<tr>
<th>Cardiovascular disorder</th>
<th>Change in ADMA</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronic renal failure associated with hypertension</td>
<td>↑</td>
<td>[1,3,14,15]</td>
</tr>
<tr>
<td>Stroke</td>
<td>↑</td>
<td>[16,17]</td>
</tr>
<tr>
<td>Pulmonary hypertension</td>
<td>↑</td>
<td>[18-20]</td>
</tr>
<tr>
<td>Left ventricular hypertrophy</td>
<td>↑</td>
<td>[21,22]</td>
</tr>
<tr>
<td>Type II diabetes</td>
<td>↑</td>
<td>[23-25]</td>
</tr>
<tr>
<td>Pre-eclampsia</td>
<td>↑</td>
<td>[26,27]</td>
</tr>
<tr>
<td>Heart failure</td>
<td>↑</td>
<td>[28]</td>
</tr>
<tr>
<td>Ischaemia</td>
<td>↑</td>
<td>[29,30]</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>↑</td>
<td>[31,32]</td>
</tr>
<tr>
<td>Atherosclerosis</td>
<td>↑</td>
<td>[33,34]</td>
</tr>
<tr>
<td>Alzheimers*</td>
<td>↓</td>
<td>[35,36]</td>
</tr>
</tbody>
</table>

Table 2. Summary of Cardiovascular Disorders Where Changes in the Concentrations of CRP have been Measured

<table>
<thead>
<tr>
<th>Cardiovascular disorder</th>
<th>Change in CRP</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Angina</td>
<td>↑</td>
<td>[7,9,11]</td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>↑</td>
<td>[37,38]</td>
</tr>
<tr>
<td>Atherosclerosis</td>
<td>↑</td>
<td>[39-43]</td>
</tr>
<tr>
<td>Diabetes</td>
<td>↑</td>
<td>[44,45]</td>
</tr>
<tr>
<td>Stroke</td>
<td>↑</td>
<td>[8]</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td>↑</td>
<td>[46-51]</td>
</tr>
<tr>
<td>Systemic lupus erythematosus</td>
<td>↓</td>
<td>[52-54]</td>
</tr>
</tbody>
</table>

*Address correspondence to this author at the Department of Molecular and Applied Biology, School of Biosciences, University of Westminster, London W1W 6UW, UK; Tel: 020 79115000, Ext. 3877; E-mail: smithc@wmin.ac.uk
cular disease. The high throughput methods available to screen CRP levels in plasma are perhaps partly responsible for the recommendation by the American Heart Association that CRP concentrations should be measured routinely in patients to aid in diagnosis and outcome alongside the originally recommended Framingham criteria [12]. There have been some criticisms of this proposal as concentrations of CRP may range from 0.1-1000 mg/L depending upon the inflammatory status and this might impair the interpretation of CRP as a cardiovascular marker [13]. At present measurements of ADMA are not routinely used to predict cardiovascular outcome, this may be partially due to the lack of accessible high throughput methods for screening.

**ADMA & Atherosclerosis**

ADMA concentrations have been demonstrated to predict luminal occlusion [55], an important measure of atherogenesisis. In patients with end stage renal failure, increased intima-media thickness was reported to correlate with raised ADMA [33,56], and in isolated uterine arteries there was a correlation between raised ADMA and intimal hyperplasia; the latter study also suggested reduced ADMA metabolism by DDAH [34].

Regulation of circulating homocysteine is critical in maintaining normal cardiovascular function with hyperhomocysteinemia pre-disposing towards the progression of atheroma. In hypercholesterolemic rabbits there are increased plasma ADMA concentrations [31,57] correlating with decreased ADMA metabolism and DDAH activity [58]. Hypercholesterolemia increases oxidative stress and monocyte binding to human endothelial cells [59,60], ADMA might have effects upon endothelial adhesion molecules. In a further study of carotid intimal-thickness ADMA concentrations correlated with increases in soluble vascular cell adhesion molecule (sVCAM1), which has been described as an atherosclerotic marker [61]. Most of the studies of ADMA and atherogenesis are association studies and further work is needed to determine whether ADMA is mediating the changes observed in atherosclerosis.

**CRP & Atherosclerosis**

High concentrations of CRP have been measured in atherosclerotic plaques [41-43]. This is consistent with high levels of complement 3 also seen in these plaques reviewed [-62]. In the “Reverse ofAtherosclerosis with Aggressive Lipid Lowering; REVERSAL” trial lowering cholesterol and CRP reduced the progression of atheroma [40,63]. CRP binds the phosphocholine of low density lipoprotein (LDL), increases the expression of adhesion molecules and promotes the uptake of LDL by macrophages [64,65].

A model commonly used to investigate the effects of CRP is the ApoE knockout mouse, which is hypercholesterolemic and develops atherosclerotic lesions [66,67], transfected with human CRP. Conflicting results have been generated using these mice, some investigators have reported activation of complement, accelerated atherosclerosis and increased expression of angiotsin receptor-1, vascular cell adhesion molecule and collagen [68]. In contrast using the same murine model other investigators neither reported proinflammatory nor proatherogenic effects [69].

**Is there an Association between ADMA and CRP?**

The number of studies looking at ADMA levels are a fraction of the number which have examined CRP. It is not surprising therefore that few studies have measured CRP and ADMA: Bae et al. found a positive correlation between ADMA and CRP plasma concentrations in patients with acute coronary syndrome [70] and Mamaci et al. reported a gain in the power of prediction when considering both ADMA and CRP plasma concentrations in a small cohort of dialysis patients [71].

In studies measuring the intima-media thickness both ADMA and CRP concentrations correlated with the intima thickness [33]. These researchers proposed that there might be a relationship between these risk factors. However, in another study investigating carotid intima-thickness, there was no correlation between plasma CRP and ADMA levels [61]. There is no functional evidence published to support a mechanistic association between these cardiovascular risk factors and further large scale clinical studies are required to determine if there is a true correlation between CRP and ADMA plasma concentrations.

**What are the Roles of ADMA?**

**Formation of nitric oxide**

Nitric oxide (NO) is an important signalling molecule. In the cardiovascular system it is vital in maintaining vascular tone [72] and in preventing leukocyte adhesion and platelet aggregation [73]. It has also been demonstrated to influence gene expression. NO is synthesised from arginine by nitric oxide synthases (NOS) in an oxygen-dependent reaction which utilises tetrahydrobiopterin as a cofactor [74,75] (Fig. 1). Limited substrate or cofactor availability leads to the generation of superoxide (O2•-) from NOS which may contribute to endothelial dysfunction [76,77]. There are two constitutively expressed NOS isoforms, endothelial (eNOS; NOSIII) and neuronal (nNOS, NOSII), and an inducible nitric oxide synthase (iNOS; NOSII). All NOS isoforms were reported to be inhibited by arginine analogues including L-N⁵-monomethylarginine (L-NMMA; [78,79]).

**Modulation of Nitric Oxide by Endogenously Occurring Methylarginines**

The molecules, asymmetric dimethylarginine (ADMA; N⁵G N⁵G dimethylarginine) and L-NMMA, were identified in human urine [80] and later evidence suggested that levels of ADMA rose in pathologies which included muscular dystrophy [81]. In 1992 Vanlance et al. recognised that the structure of ADMA closely resembled the NOS inhibitor L-NMMA [82] and ADMA was shown to dose-dependently attenuate the relaxation of nor-epinephrine pre-constricted aortic rings and this was an endothelium dependent effect. These investigators reported that plasma concentrations of ADMA were significantly elevated in patients with end-stage renal failure and this correlated with lower levels of nitric oxide [1]. This was the first indication that an endogenously occurring inhibitor of NOS could affect cardiovascular function. Both ADMA and L-NMMA are inhibitors of all 3 nitric oxide synthases (Fig. 1A), with inhibition constants in the range of 0.1-6.2 μmol/L [78,79,83].

The reaction catalysed by NOS is complex and it has been proposed that in the presence of methylarginines NOS may become uncoupled generating superoxide [77] (Fig. 1B). In eNOS knockout mice infused with ADMA, there were increased concentrations of superoxide and this correlated with increased staining for angiotensin converting enzyme [84]. In a study of dialysis patients, an association between ADMA concentrations and an eNOS polymorphism was reported [85], whether this might be an effect on NO production remains to be clarified.

Methyalted arginines are taken into cells through the y+ transporter and may also compete with arginine for cellular uptake [86]. Another endogenously occurring molecule, symmetric dimethylarginine (SDMA- N⁵G-N⁵G-dimethylarginine; structure shown in Fig. 1A), was found to have no effect on the production of nitric oxide [87], however SDMA is also transported by the y+ transporter and may compete with arginine under certain circumstances.

**Formation and Metabolism of Asymmetric Dimethylarginine**

The formation of dimethylarginines has been discussed previously [4]. Arginine residues in proteins are methylated post-translationally in vivo by a family of enzymes called protein arginine methyltransferases (PRMT: Table 3 and Fig. 2): the methyl group is donated by S-adenosylmethionine (SAM) with S-adenosylmethionine methyltransferases (PRMT: Table 3 and Fig. 2): the methyl group is donated by S-adenosylmethionine (SAM) with S-adenosylmethionine methyltransferases (PRMT: Table 3 and Fig. 2): the methyl group is donated by S-adenosylmethionine (SAM) with S-adenosylmethionine methyltransferases (PRMT: Table 3 and Fig. 2):
C-Reactive Protein and Asymmetric Dimethylarginine

Currently eight PRMT isoforms have been reported (Table 1): two of these can symmetrically methylate protein arginine residues [91-94]; five have been reported to asymmetrically methylate arginine residues [95-100]; and one member of the PRMT family has no known ability to methylate arginine [101]. The release of free methylarginines has been reported to occur following hydrolysis and proteolysis.

Characterisation of the metabolism of ADMA began with observations made in rabbits that concentrations of [14C]-labelled L-NMMA and ADMA excreted in urine were significantly lower than

![Diagram](image-url)

**Fig. (1).** Asymmetric dimethylarginine (ADMA) inhibits the formation of nitric oxide (NO).

A). Nitric oxide synthases catalyse the formation of NO from arginine in the presence of NADPH, O₂ and tetrahydrobiopterin (BH₄). The structures of substrate arginine and product citrulline are shown with the inhibitor ADMA (N⁰⁰-dimethylarginine). Inset box: structures for the endogenously occurring methylarginines L-N⁰-monomethylarginine (L-NMMA) and N⁰⁰-N⁰-dimethylarginine (symmetric dimethylarginine –SDMA); L-NMMA but not SDMA inhibits NOS.

B). Putative mechanism for superoxide generation in the presence of ADMA. In the presence of arginine, nitric oxide synthases produce nitric oxide and citrulline (i), when methylarginines are present superoxide may be generated from NOS (ii). (Flavin adenine dinucleotide (FAD); flavin monophosphate (FMN); calmodulin (CAM); haemoglobin (HAEM); reduced nicotinamide adenine dinucleotide phosphate (NADPH); tetrahydrobiopterin (BH₄); superoxide (O₂⁻); arginine (ARG)).

**Table 3.** Summary of Type 1 and Type 2 PRMT isoforms. Chromosomal Localisation, as Published by The Welcome Trust Sanger Centre

<table>
<thead>
<tr>
<th>PRMT Type</th>
<th>Class</th>
<th>Chromosome</th>
<th>Arginine methylation</th>
<th>Localisation</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type 1</td>
<td>PRMT1</td>
<td>19q13</td>
<td>MMA, ADMA</td>
<td>Nucleus</td>
<td>[99,100]</td>
</tr>
<tr>
<td></td>
<td>PRMT3</td>
<td>11p15.1</td>
<td>MMA, ADMA</td>
<td>Cytosol</td>
<td>[96,100]</td>
</tr>
<tr>
<td></td>
<td>CARM1/PRMT4</td>
<td>12p13.32</td>
<td>MMA, ADMA</td>
<td>Nucleus</td>
<td>[102]</td>
</tr>
<tr>
<td></td>
<td>PRMT6</td>
<td>1p13.3</td>
<td>MMA, ADMA</td>
<td>Nucleus</td>
<td>[97]</td>
</tr>
<tr>
<td></td>
<td>PRMT8</td>
<td>12p13.3</td>
<td>MMA, ADMA</td>
<td>Membrane bound</td>
<td>[98]</td>
</tr>
<tr>
<td></td>
<td>PRMT2</td>
<td>21q22.3</td>
<td>No apparent Arginine methylation</td>
<td>Nucleus</td>
<td>[101]</td>
</tr>
<tr>
<td>Type 2</td>
<td>PRMT5</td>
<td>14q11.2</td>
<td>MMA, SDMA</td>
<td>Cytosol</td>
<td>[92,103,104]</td>
</tr>
<tr>
<td></td>
<td>PRMT7</td>
<td>16q22.1</td>
<td>MMA, SDMA, some ADMA</td>
<td>Nucleus &amp; Cytosol</td>
<td>[93,94]</td>
</tr>
</tbody>
</table>
the life of CRP is a few hours and the CRP levels in serum reflect rapid following the plasma concentration of the acute phase CRP. The half extent and persistence of inflammation can be determined by this may be through the activation of platelet factors [125]. The suggested as a mechanism to explain how CRP might recognise and tides, laminin and polycations. Chromatin binding to CRP has been including: chromatin, histones, fibronectin, small nuclear ribonucleo- 
interactions with C1q enabling activation of the classical comple-
CRP might recognise damaged cell membranes [64]. CRP also shown to selectively bind plasma LDL and very low density lipo-
classical complement pathway initiates opsonisation and the phago-
least of ADMA [105]. Dimethylarginine dimethylaminohydrolase (DDAH) was found to metabolise L-NMMA and ADMA to form methylamine or dimethylamine respectively and citrulline; DDAH does not metabolise SDMA. (Fig. 2; [106-107]. DDAH metabolises 250 μmol/L of the 300 μmol/L, generated daily, based upon urinary excretion of dimethylamine [108].
Two isoforms of DDAH have been identified [109]; human DDAHI was mapped to chromosome 1p22 and is more prevalent in the nervous system[110]. DDAHII was mapped to chromosome 6p2.13, close to the MHC region and dot-blot analyses revealed that DDAHIII is located in vascularised and immune tissues [110]. Regulation of DDAHIII is altered in development [110,111], perhaps reflecting a time when protein turnover and release of methyl-
Arginine residues on proteins are methylated by PRMTs with S-adenosylmethionine acting as a methyl donor. Free ADMA is released as the protein undergoes hydrolysis and proteolysis. DDAH metabolises ADMA to citrulline and dimethylamine. (Protein arginine methyltransferase (PRMT); S-adenosylhomocysteine (SAH), S-adenosylmethionine (SAM); dimethylarginine dimethylaminohydrolase (DDAH).

**Species Variation in CRP Levels**

Whilst CRP is an evolutionary conserved protein, it is not an acute phase reactant in all mammals: in humans and rabbits CRP concentrations can rapidly rise from 0.1 mg/L to more than 3000-fold following acute phase stimulation. In mice levels are hardly affected by inflammation reaching only 2 mg/L [133] and the Syrian hamster does not have detectable levels of CRP. In rats, however, levels of CRP are constitutively high in serum (300-600 mg/L) rising to 900 mg/L following injury [127]. CRP has also been described in the horseshoe crab and in other cold blooded invertebrates [134].

**Effects of CRP on Nitric Oxide Production**

The use of commercially available sources of CRP without purification steps has been criticised [135] and focus has been placed upon the re-interpretation of the effects of CRP upon NO mediated biology. CRP was believed to attenuate NO production and downregulate eNOS expression [136, 137]. However, the levels of eNOS were not found to be altered in a murine transgenic models overexpressing CRP [68]. Clapp et al. examined the endotoxin specific effects of purified human CRP in vitro and in vivo and did not find inhibition of NO [138]. In contrast to previous reports these investigators found that CRP potentiated eNOS mediated relaxation and suggested CRP might affect the tetrahydrobiopterin pathway (NOS cofactor – see Fig. 1).
CRP reportedly augmented the cytokine activated NO production from iNOS [139]. However, conflicting results were observed in organ bath relaxation experiments using rabbit aortic rings and CRP from various sources, with commercial CRP alone eliciting vasorelaxation of the rings. The investigators controversially proposed that low levels of sodium azide (Na$_2$N$_3$), used as a preservative in commercial preparations, was responsible for reported vasodilatory effects of CRP [140]. Further experiments looking at vasorelaxation in rat aortic vessels and mesenteric arteries determined that CRP induced relaxation was attributable to sodium azide and purification of CRP to remove preservatives attenuated the vasodilatory effects of CRP [141]. Lafuente et al. demonstrated that the inhibitory effect of CRP on IL-1 induction of iNOS was mimicked by sodium azide and that purified CRP did not alter iNOS induction [142]. The presence of sodium azide may explain endothelial activation by CRP [143]; also the decreased migration, proliferation, and matrigel tube formation observed in endothelial cells since dialysed commercial CRP elicited no effect but vehicle buffer containing sodium azide had both anti-proliferative and proapoptotic effects [144]. The presence of LPS in commercial CRP has been identified and some pro-inflammatory effects attributed to CRP may be due to this contaminant [145].

Clearly the interpretation of the effects of CRP on the endothelium and NO production needs to be clarified and caution should be taken when analysing data generated using unpurified commercial preparations of CRP.

Regulation of ADMA Metabolism

ADMA levels are altered in many cardiovascular conditions and understanding the regulation of ADMA concentrations is important to understand underlying pathologies. In hypercholesterolemia plasma concentrations of ADMA are elevated [32]; oxidation of LDL is associated with hypercholesterolemia and in an in vitro model oxidised LDL increased PRMT activity and decreased DDAH activity [58]. A cysteine residue at the active site of DDAH has been proposed to be oxidised by homocysteine, this mechanism might account for lowered DDAH activity in hypercholesterolemia [146]. Erythropoietin therapy to treat anaemia has been reported to cause hypertension in some patients; and DDAH activity has been demonstrated to fall, oxidation of the active site cysteine was proposed to cause this effect [147].

There is a correlation between insulin resistance and raised ADMA levels [24,148] and glucose has been shown to decrease DDAH and increase ADMA levels [149]. ADMA levels have fallen in hyperglycaemic patients treated with the diabetic drug metformin [150]. In addition treatment with the thiazolidinedione, rosiglitazone, reduced ADMA levels [151] and pioglitazone was shown to reduce ADMA levels by increasing DDAH activity in a rat model [152]. There are PPARy response elements (binding site for thiazolidinediones) in the DDAHII promoter [153]. Further studies are required to characterise the role of DDAH in the progression of Type II diabetes.

Blood flow may exert an affect on DDAH expression, low blood flow in the heart correlated with increased DDAHI expression and shear stress has been reported to alter PRMT activity [158] as well as activating eNOS [159]. Finally, there have been reports that oestrogen replacement reduced plasma concentrations of ADMA [160], oestrogen has been demonstrated to increase DDAH activity and to reduce concentrations of ADMA [155]. Reports of various stimuli on DDAH expression or activity have been summarised in Table 4, however some of these observations have been made from cursory in vitro experiments and more rigorous studies will be needed to elucidate regulatory mechanisms of DDAH.

Angiogenesis

The pro-angiogenic all-trans-Retinoic acid has been shown to increase DDAHII expression and lower ADMA concentrations as well as increasing the DDAHII promoter activity [156]. These effects are possibly mediated by a PPAR/RXR (-927) site identified in the DDAHII promoter [153]. DDAH overexpression increases tube formation in matrigel assays [161] and increases neovascularisation when overexpressed in tumour cells [162]; the angiogenic effects of DDAH may be mediated in part by increased expression of VEGF [161,162]. More recently it has been shown that DDAH overexpression affected the hypoxia of tumours [163]. An overexpressing DDAH transgenic mouse has been described by Cooke et al. which has lower blood pressure than controls [164]. Angiogenic studies with these animals have supported the evidence that DDAH overexpression reduces ADMA concentrations and increases angiogenesis [165].

Regulation of CRP

A major site of CRP synthesis is the liver and CRP is released from hepatocytes into the plasma; CRP is also found in lymphocytes, neurons and monocytes as well as in atherosclerotic plaques. Early studies of the CRP promoter in human hepatocytes revealed
the presence of responsive elements to interleukin 6 (IL-6) and hepatocyte specific nuclear proteins [166,167]. Interleukin 6 (IL-6) is the major regulator of CRP but interleukin-1β (IL-1β) acts in a co-operative manner to stimulate CRP [168]. IL-6 may be acting through STAT3 and C/EBPβ which are in close proximity within the CRP promoter [169]. Variations in basal CRP levels which are seen in the population are proposed to arise from OCT-1 and NFκB activation of CRP [170].

There is interest in the effects of currently approved therapeutics to modulate CRP concentrations in cardiovascular disorders: statin treatments lower LDL and have also been reported to lower CRP levels [40,171,172] (for review see [171,173]). Anti-TNF-α therapy (Infliximab) has been demonstrated to lower both serum CRP and IL-6 levels in patients with a systemic inflammatory response [174]. Modification of the renin-angiotensin system may also reduce concentrations of both CRP and complement [175].

### Measuring Levels of ADMA & CRP

#### ADMA measurement

Several methods are available for measuring ADMA in a variety of samples and has been discussed in a previous issue of CPD [4]. Since this publication an ELISA has become available to measure ADMA which is able to differentiate between the asymmetrically labelled and symmetrically labelled methylarginines despite their identical molecular weights [176]. The advantages of this high throughput method are immediately apparent, however, when compared to HPLC, currently recognised as the gold standard for ADMA analysis [82], the reproducibility of the ELISA has been questioned [177].

HPLC analysis of ADMA involves pre-treatment of the samples using solid phase extraction followed by derivatisation with orthophthalaldialdehyde (OPA) reagent [178]. Alternative measurements of ADMA have coupled this OPA derivatisation and HPLC method separation to liquid mass spectrometry and reported values similar to other investigators (0.355 ± 0.066 μM ADMA and 0.46 ± 0.092 μM for SDMA) [179].

#### CRP Measurement

The size of CRP has enabled the successful production of antisera and immunoassays are major tools to measure CRP plasma concentrations. Reliable immunoassays to detect CRP were developed as early as 1978. Utilising the fluorescently labelled antibodies, these assays had detection limits as low as 20 μg/L [180]. High-throughput automated radioligand binding assays utilising the pneumococcal C-polysaccharide have also been described with sensitivity as low as 1 μg/L [181]. The efficient high-throughput methods available to accurately and sensitively measure CRP perhaps reflect why levels have been so well characterised in disease states.

### Genetic Influences on the Levels of ADMA and CRP

#### Polymorphisms Observed in Enzymes Regulating ADMA Levels

The earliest report about single nucleotide polymorphisms (SNP) in DDAH, identified a functional polymorphism in the DDAHII promoter at -871 (6G>7G) and was found in approximately 1% of the population [153]. Since this time another group have reported on several SNPs in DDAH and have suggested that SNPs towards the 3’ end of DDAH might be associated with pre-eclampsia, the high blood pressure observed in the maternal vasculature during pregnancy, although these were not significant with multiple testing criteria [182]. ADMA concentrations have previously been shown to predict the onset of pre-eclampsia [27]. Larger genetic epidemiological studies are needed to reveal whether these DDAH polymorphisms have causal effects on ADMA concentrations. Although there are PRMT SNPs reported in the databases particularly for PRMT3 (http://www.ncbi.nlm.nih.gov/SNP/), there are at present no reports about functional polymorphisms in PRMTs and it remains to be seen whether any emerge which might influence ADMA concentrations.

#### Polymorphisms of CRP

In contrast to the limited number of studies examining the effects of polymorphisms on ADMA levels, there are numerous reports detailing a correlation between CRP genotype and CRP concentrations. The reported CRP polymorphisms do not seem to elicit an effect upon the CRP amino acid sequence encoded but do affect CRP concentrations. Higher basal concentrations of CRP were described in healthy adults with a +1444 C>T polymorphism in the 3’ UTR [183] and carriers of this +1444T polymorphism had higher concentrations of CRP than +1444C individuals following a moderate inflammatory response [184]. In the CARDIA study, several CRP promoter variants were strongly associated with CRP concentrations and these variants were demonstrated to affect promoter-reporter function in vitro [185]. The effects of genotype on CRP concentrations appear to be independent of other traditional cardiovascular risk factors [185].

Polymorphisms in the CRP gene which increase the concentrations of CRP have been suggested to predict the occurrence of arterial thrombosis [186]. Functional polymorphisms of the human CRP gene have also shown decreased concentrations of CRP in the circulation and increased risk of developing systemic lupus erythematosus [52-54]. The lower levels of CRP are thought to contribute to a decreased clearance of damaged cell membranes and to enhance production of autoantibodies.

CRP levels are influenced by IL-6 and IL-1β therefore polymorphisms in genes encoding for IL-6 [187] and IL-1β [188] might also influence the levels of CRP. There is conflicting evidence about TNF-α polymorphisms and the effects on plasma CRP concentrations [187,189]. Carriers of a genetic variant in the Toll-like

### Table 4. Effects of Stimuli Able to Alter DDAH Activity. Where it has been Demonstrated that the Change was Due to a Specific DDAH Isoform, this has been

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>DDAH</th>
<th>ADMA</th>
<th>NO</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF-α</td>
<td>↓ protein / activity</td>
<td>↑</td>
<td>↓</td>
<td>[58]</td>
</tr>
<tr>
<td>Glucose</td>
<td>↓ protein / activity</td>
<td>↑</td>
<td>↓</td>
<td>[149]</td>
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<td>Erythropoietin</td>
<td>↓ activity</td>
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<td>[147]</td>
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<td>↑ activity</td>
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<td>↑ activity</td>
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<td>[155]</td>
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<tr>
<td>Retinoic acid</td>
<td>↑ DDAHII mRNA/protein/activity</td>
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<td>[156]</td>
</tr>
<tr>
<td>Shear stress</td>
<td>↑ DDAH expression</td>
<td>↓</td>
<td>↑</td>
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<th>ADMA</th>
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receptor 4 allele (Asp299Gly), associated with an impaired inflammatory response, are reported to have lower CRP concentrations [190]. Further large scale studies are needed to determine whether polymorphisms of genes involved in cytokine signalling elicit significant prolonged effects on CRP plasma concentration.

**Is ADMA a Marker for Cardiovascular Disorders or is it a Mediator?**

There is now compelling data that ADMA is a biomarker for the progression of cardiovascular disease and there is some data emerging that ADMA may be functionally important. Concentrations of circulating methylarginines in plasma were not thought to rise to levels which could affect NO synthesis in vivo. However, pathophysiological concentrations of ADMA were found to alter the expression of several genes in endothelial cells, including those related to bone morphogenetic proteins and PRMT3 (which is involved in arginine methylation) [191] and pathway mapping of the microarray data suggested that ADMA alter genes involved in cell-cycle regulation, cell proliferation, transcriptional regulation and metabolism [191]. In the eNOS knockout mouse, pathophysiologically concentrations of ADMA were shown to increase expression of angiotensin-converting enzyme and increase atherosclerotic lesion size [84]. There are also reports that low levels of ADMA might inhibit endothelial progenitor cell mobilisation and differentiation [192].

**Is CRP a Mediator or Marker for Cardiovascular Disease?**

Concentrations of CRP in humans escalate several 1000-fold in inflammation, without deleterious effects on the cardiovascular system. Persistently raised concentrations of CRP, above 3 mg/L, however have been independently linked to cardiovascular risk [7,193]. Does CRP contribute to the pathophysiology accompanying cardiovascular disorders or is it an acute marker of the inflammation associated with cardiovascular disease?

Complement bound to CRP has been measured in infarcted regions of the heart [37]. In a model of ischaemic injury, human CRP was observed to activate complement and enhance myocardial damage, this effect was ameliorated by cobra venom factor, which disrupts complement [38]. CRP may also play a role in increasing vascular calcification and has been reported to correlate to the coronary calcification score independently of other risk factors [172].

The exact mechanisms by which CRP mediates the progression of cardiovascular disorders remain elusive: the long term study of carriers of functional CRP polymorphisms and/or the development of specific CRP inhibitors are likely to be valuable tools designed to clarify these mechanisms.

**Potential Therapeutic Modulation of ADMA and CRP Targets for Altering ADMA Levels**

Since the bioeffects of ADMA are assumed to be altering NOS activity and nitric oxide bioavailability, arginine supplementation was predicted to overcome ADMA inhibition. There have been mixed results using arginine supplementation to improve endothelial function associated with chronic renal failure [194,195].

At the present time the formation of ADMA has not been comprehensively characterised and therefore targeting the metabolism of methylarginines appears to be the better route for altering concentrations of ADMA. Until recently the characterisation of DDAH had been limited by the availability of specific inhibitors. A panel of new inhibitors have been described which were based upon the original 4124W structure described by MacAllister [196] with IC50s approaching 20 μmol/L and no effect on NOS activity [197]. As a therapeutic target these inhibitors also demonstrated that ADMA concentrations could be raised both in vitro and in vivo [197]. Other potential DDAH inhibitors include chloroacetamide [198] and S-nitroso-L-homocysteine [199], however, these inhibitors have not yet been demonstrated to elicit a rise in ADMA concentrations in in vivo models.

**Targets for Altering CRP Concentrations**

Whilst CRP concentrations are predictive of outcome for patients with myocardial infarction and angina [7,11], there is still disagreement about whether CRP has a causal role in pathology; particularly that associated with the progression of atheroma. The development of specific CRP inhibitors would benefit investigators in elucidating a role for CRP. Although there is evidence that CRP levels are lowered following treatment with statins [40,63,130,171,172] specific CRP inhibitors are needed. Potential targets might include CRP transport through FcγRI and FcγRII receptors [117,118].

**CONCLUSION**

CRP is an established marker for cardiovascular disease; however despite the numerous association studies which have characterised the circulating concentrations of CRP, evidence is still lacking which proves a mechanistic role for CRP. Further genetic-epidemiological studies characterising functional CRP variants and the development of specific CRP inhibitors will help to either accept CRP as a causal agent in cardiovascular pathology or to elucidate its pathophysiological role.

ADMA concentrations are also altered in numerous cardiovascular disorders but before it can reach the prominence of CRP, as a biomarker to diagnose cardiovascular events; more efficient screening methods are needed. The utilisation of inhibitors to disrupt ADMA metabolism and further studies of DDAH transgenic animals are needed to provide valuable evidence about the potential involvement of this pathway in the pathogenesis of cardiovascular complications.

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C-Reactive Protein and Asymmetric Dimethylarginine


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C-Reactive Protein and Asymmetric Dimethylarginine


