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

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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 dimethylarginine 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 panopoly 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 cardiovas-

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

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

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

Cardiovascular disorder	Change in CRP	Reference
Angina	↑	[7,9,11];
Myocardial infarction	↑	[37,38]
Atherosclerosis	↑	[39-43]
Diabetes	↑	[44,45],
Stroke	↑	[8]
Rheumatoid arthritis	↑	[46-51]
Systemic lupus erythematosus	↓	[52-54]

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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 lumen occlusion [55], an important measure of atherogene sis. 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 "Reversal of Atherosclerosis 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 angiotensin 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 Mallamaci *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 cardiovasculature 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 ($O_2^{\cdot-}$) from NOS which may contribute to endothelial dysfunction [76,77]. There are two constitutively expressed NOS isoforms, endothelial (eNOS; NOSIII) and neuronal (nNOS, NOSI), and an inducible nitric oxide synthase (iNOS; NOSII). All NOS isoforms were reported to be inhibited by arginine analogues including L-N^Gmonomethylarginine (L-NMMA; [78,79]).

Modulation of Nitric Oxide by Endogenously Occurring Methylarginines

The molecules, asymmetric dimethylarginine (ADMA; N^G N^G 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 Vallance *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.

Methylated 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- $N^GN^{G`}$–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-adenosylhomocysteine (SAH) a reaction by-product [88]. Evidence from *in vivo* loading of radiolabelled methionine indicated that the methyla-

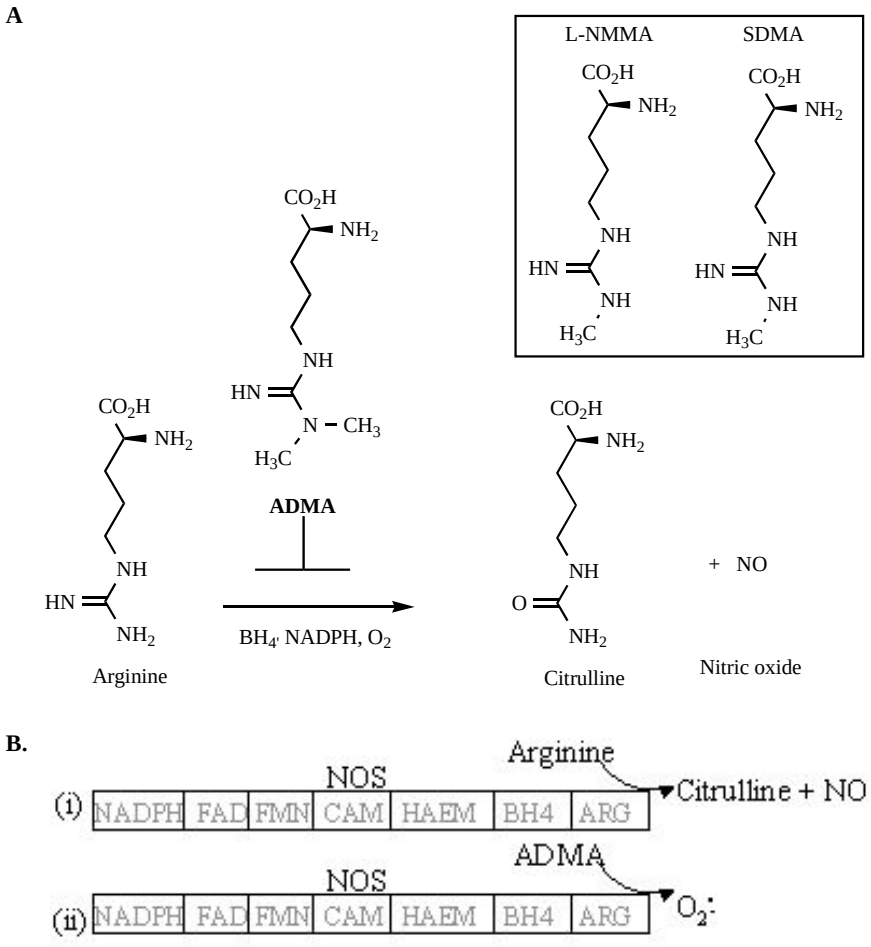

Fig. (1). Asymmetric dimethylarginine (ADMA) inhibits the formation of nitric oxide (NO).

A). Nitric oxide synthases catalyse the formation of NO from arginine in the presence of NADPH, O_2 and tetrahydrobiopterin (BH4). The structures of substrate arginine and product citrulline are shown with the inhibitor ADMA (N^GN^Gdimethylarginine). Inset box: structures for the endogenously occurring methylarginines L-N^G-monomethylarginine (L-NMMA) and $N^GN^{G'}$-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 (BH_4); superoxide ($O_2^{\bullet}$); arginine (ARG).

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

PRMT Type	Class	Chromosome	Arginine methylation	Localisation	Reference
Type 1	PRMT1	19q13	MMA, ADMA	Nucleus	[99,100]
	PRMT3	11p15.1	MMA, ADMA	Cytosol	[96,100]
	CARM1/PRMT4	12p13.32	MMA, ADMA	Nucleus	[102]
	PRMT6	1p13.3	MMA, ADMA	Nucleus	[97]
	PRMT8	12p13.3	MMA, ADMA	Membrane bound	[98]
	PRMT2	21q22.3	No apparent Arginine methylation	Nucleus	[101]
Type 2	PRMT5	14q11.2	MMA, SDMA	Cytosol	[92,103,104]
	PRMT7	16q22.1	MMA, SDMA, some ADMA	Nucleus & Cytosol	[93,94]

tion of arginine residues was irreversible and that degradation of the methylated proteins was extremely slow [89,90].

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 [^{14}C]labelled L-NMMA and ADMA excreted in urine were significantly lower than

Fig. (2). Formation and metabolism of ADMA.

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).

those of SDMA [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 6p21.3, close to the MHC region and dot-blot analyses revealed that DDAHII is located in vascularised and immune tissues [110]. Regulation of DDAHII is altered in development [110,111], perhaps reflecting a time when protein turnover and release of methylarginine is high.

CRP an Acute Phase Inflammatory Response Protein

CRP was described in 1930, after a protein in plasma from patients infected with *Streptococcus pneumoniae* was found to precipitate in the presence of the pneumococcus cell wall protein C-polysaccharide [112]. The gene encoding CRP has a single intron and has been localised to chromosome 1q21-q23 [113,114]. CRP is an acute phase inflammatory response protein that calcium-dependently precipitates in the presence of phosphocholine ligand; which is found in membrane proteins and bacterial polysaccharides [115]. Mutation studies have demonstrated that the Phe66 and Glu81 of CRP are critical for the CRP binding to phosphocholine [116]. CRP also binds to the to the immunoglobulin receptors, FcγRI and FcγRII, and subsequent signalling initiates a response from phagocytes [117,118].

Aggregated CRP or CRP complexed to ligands have been shown to activate complement (Fig. **3**); [119-122]. CRP activation of the classical complement pathway initiates opsonisation and the phagocytosis of bacteria (for review see [123]). Aggregated CRP has been shown to selectively bind plasma LDL and very low density lipoproteins (vLDL); this has been proposed as a mechanism by which CRP might recognise damaged cell membranes [64]. CRP also binds to degraded low density lipoproteins to activate complement and this property of CRP might be important in atherogenesis [65].

Aggregated CRP, or multivalent ligands to bound CRP, initiate interactions with C1q enabling activation of the classical complement pathway [120]. CRP may bind to a number of molecules including: chromatin, histones, fibronectin, small nuclear ribonucleotides, laminin and polycations. Chromatin binding to CRP has been suggested as a mechanism to explain how CRP might recognise and scavenge these proteins from damaged cells [124] (Fig. **3**).

The anti-inflammatory effects of CRP include increasing IL-10, IL-1 receptor antagonist, and lowering the production of IFNγ and TNF-α [123]. CRP has been reported to prevent platelet aggregation and this may be through the activation of platelet factors [125]. The extent and persistence of inflammation can be determined by following the plasma concentration of the acute phase CRP. The half life of CRP is a few hours and the CRP levels in serum reflect rapid changes associated with inflammation. Whether the raised concentrations of CRP seen in cardiovascular disorders are related to prolonged inflammatory responses or not is unclear, whilst myriad clinical studies have reported associations between CRP and cardiovascular disorders, mechanisms to prove or disprove a causal role for CRP in cardiovascular pathologies are poorly characterised.

CRP Structure

CRP is a pentameric protein with the five 21500 Da subunits arranged in a donut shape (Fig. **3**) and it is a member of the pentaxin family [126]. The structure of CRP has been reviewed in detail elsewhere [123,127].

Studies have shown that the downstream effects of CRP as a pentamer (native CRP) are different to those of monomeric CRP (mCRP – modified CRP; [128]. The modified monomeric CRP is proinflammatory and is associated with increased release of the inflammatory mediators IL-8, and monocyte chemoattractant protein-1 [129,130]. The effect of mCRP on IL-8 in neutrophils might be coupled to the formation of peroxynitrite [129]. In addition mCRP has been shown to increased ICAM-1 expression in endothelial cells [131]. Recently, in the ApoE knockout mouse, specific differences have been observed between native and modified CRP: with the modified CRP producing smaller plaques than native CRP[132]. These differences were related to the expression of adhesion molecules and the authors of this study have proposed that some varied reports of CRP in atherogenic studies might be due to the differential effects of native and modified CRP [132].

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 endothelium 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**).

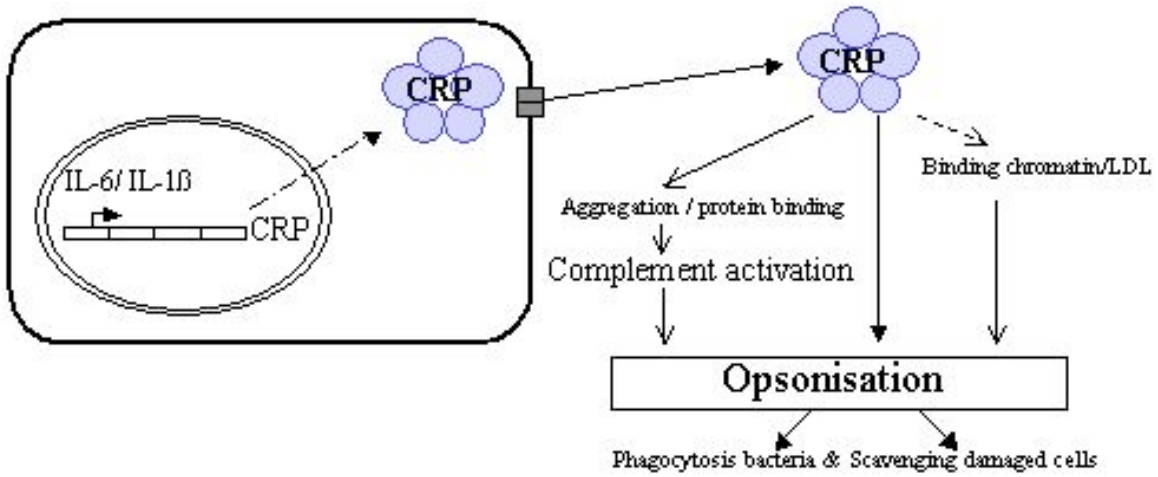

Fig. (3). Cartoon summarising CRP synthesis and actions as an acute phase protein.

CRP expression is co-operatively induced by IL-6 and IL-1ß. The pentameric CRP is transported from hepatocytes and recognises ligands, including phosphocholine, on proteins such as chromatin or low density lipoproteins. Either protein-bound CRP or CRP aggregates activate the complement cascade culminating in opsonisation and phagocytosis.

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 (NaN_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-I 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 affects 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 thiazolindinelione, rosiglitazone, reduced ADMA levels [151] and pioglitazone was shown to reduce ADMA levels by increasing DDAH activity in a rat model [152]. There are PPARγ response elements (binding site for thiazolindineliones) 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

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 be

Stimulus	DDAH	ADMA	NO	Reference
TNF-α	↓ protein / activity	↑	↓	[58]
Glucose	↓ protein / activity	↑	↓	[149]
Erythropoietin	↓ activity	↑	↓	[147]
IL-1β	↑ activity	↓	↑	[154]
Oestrogen	↑ activity	↓	↑	[155]
Retinoic acid	↑ DDAHII mRNA/protein/activity	↓	↑	[156]
Shear stress	↑ DDAHI expression	↓	↑	[157]

en Identified.

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/EBPß 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 orthophthaldialdehyde (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 DDAHI and have suggested that SNPs towards the 3' end of DDAHI 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

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, pathophysiological 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 mobilization 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 cardiovasculature. 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 infracted 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 dysfunction 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 chloroacetamidine [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 acquit 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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REFERENCES

[1] Vallance P, Leone A, Calver A, Collier J, Moncada S: Accumulation of an endogenous inhibitor of nitric oxide synthesis in chronic renal failure. Lancet 1992; 339: 572-575.

[2] Valkonen VP, Paiva H, Salonen JT, Lakka TA, Lehtimaki T, Laakso J, *et al.* Risk of acute coronary events and serum concentration of asymmetrical dimethylarginine. Lancet 2001; 358: 2127-2128.

[3] Zoccali C, Bode-Boger S, Mallamaci F, Benedetto F, Tripepi G, Malatino L, *et al.* Plasma concentration of asymmetrical dimethylarginine and mortality in patients with end-stage renal disease: a prospective study. Lancet 2001; 358: 2113-2117.

[4] Smith CL, Vallance P: Cardiovascular tests: use & limits of biochemical markers - therapeutic measurements of ADMA involved in cardiovascular disorders. Curr Pharm Des 2005; 11: 2177-2185.

[5] de Beer FC, Hind CR, Fox KM, Allan RM, Maseri A, Pepys MB: Measurement of serum C-reactive protein concentration in myocardial ischaemia and infarction. Br Heart J 1982; 47: 239-243.

[6] Liuzzo G, Biasucci LM, Gallimore JR, Grillo RL, Rebuzzi AG, Pepys MB, *et al.* The prognostic value of C-reactive protein and serum amyloid a protein in severe unstable angina. N Engl J Med 1994; 331: 417-424.

[7] Haverkate F, Thompson SG, Pyke SD, Gallimore JR, Pepys MB: Production of C-reactive protein and risk of coronary events in stable and unstable angina. European Concerted Action on Thrombosis and Disabilities Angina Pectoris Study Group. Lancet 1997; 349: 462-466.

[8] Ridker PM, Hennekens CH, Buring JE, Rifai N: C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. N Engl J Med 2000; 342: 836-843.

[9] Biasucci LM, Liuzzo G, Grillo RL, Caligiuri G, Rebuzzi AG, Buffon A, *et al.*Elevated levels of C-reactive protein at discharge in patients with unstable angina predict recurrent instability. Circulation 1999; 99: 855-860.

[10] Danesh J, Whincup P, Walker M, Lennon L, Thomson A, Appleby P, *et al.* Low grade inflammation and coronary heart disease: prospective study and updated meta-analyses. Bmj 2000; 321: 199-204.

[11] Danesh J, Wheeler JG, Hirschfield GM, Eda S, Eiriksdottir G, Rumley A, *et al.*C-reactive protein and other circulating markers of inflammation in the prediction of coronary heart disease. N Engl J Med 2004; 350: 1387-1397.

[12] Pearson TA, Mensah GA, Alexander W, Anderson JL, Cannon RO, Criqui M, *et al.* Markers of Inflammation and Cardiovascular Disease: Application

to Clinical and Public Health Practice: A Statement for Healthcare Professionals From the Centers for Disease Control and Prevention and the American Heart Association. Circulation, 2003; 107: 499 - 511.

[13] Levinson SS, Miller JJ, Elin RJ: Poor predictive value of high-sensitivity C-reactive protein indicates need for reassessment. Clin Chem 2004; 50: 1733-1735.

[14] Matsuoka H, Itoh S, Kimoto M, Kohno K, Tamai O, Wada Y, *et al.* Asymmetrical dimethylarginine, an endogenous nitric oxide synthase inhibitor, in experimental hypertension. Hypertension 1997; 29: 242-247.

[15] Surdacki A, Nowicki M, Sandmann J, Tsikas D, Boeger RH, Bode-Boeger SM, *et al.*Reduced urinary excretion of nitric oxide metabolites and increased plasma levels of asymmetric dimethylarginine in men with essential hypertension. J Cardiovasc Pharmacol 1999; 33: 652-658.

[16] Wanby P, Teerlink T, Brudin L, Brattstrom L, Nilsson I, Palmqvist P, *et al.* Asymmetric dimethylarginine (ADMA) as a risk marker for stroke and TIA in a Swedish population. Atherosclerosis 2005.

[17] Yoo JH, Lee SC: Elevated levels of plasma homocyst(e)ine and asymmetric dimethylarginine in elderly patients with stroke. Atherosclerosis 2001; 158: 425-430.

[18] Kielstein JT, Bode-Boger SM, Hesse G, Martens-Lobenhoffer J, Takacs A, Fliser D, *et al.* Asymmetrical dimethylarginine in idiopathic pulmonary arterial hypertension. Arterioscler Thromb Vasc Biol 2005; 25: 1414-1418.

[19] Pullamsetti S, Kiss L, Ghofrani HA, Voswinckel R, Haredza P, Klepetko W, *et al.* Increased levels and reduced catabolism of asymmetric and symmetric dimethylarginines in pulmonary hypertension. Faseb J 2005; 19: 1175-1177.

[20] Gorenflo M, Zheng C, Werle E, Fiehn W, Ulmer HE: Plasma levels of asymmetrical dimethyl-L-arginine in patients with congenital heart disease and pulmonary hypertension. J Cardiovasc Pharmacol 2001; 37: 489-492.

[21] Zoccali C, Mallamaci F, Maas R, Benedetto FA, Tripepi G, Malatino LS, *et al.* Left ventricular hypertrophy, cardiac remodeling and asymmetric dimethylarginine (ADMA) in hemodialysis patients. Kidney Int 2002; 62: 339-345.

[22] Achan V, Broadhead M, Malaki M, Whitley G, Leiper J, MacAllister R, *et al.* Asymmetric dimethylarginine causes hypertension and cardiac dysfunction in humans and is actively metabolized by dimethylarginine dimethylaminohydrolase. Arterioscler Thromb Vasc Biol 2003; 23: 1455-1459.

[23] Chan NN, Chan JC: Asymmetric dimethylarginine (ADMA): a potential link between endothelial dysfunction and cardiovascular diseases in insulin resistance syndrome? Diabetologia 2002; 45: 1609-1616.

[24] Mittermayer F, Mayer BX, Meyer A, Winzer C, Pacini G, Wagner OF, *et al.* Circulating concentrations of asymmetrical dimethyl-L-arginine are increased in women with previous gestational diabetes. Diabetologia 2002; 45: 1372-1378.

[25] Fard A, Tuck CH, Donis JA, Sciacca R, Di Tullio MR, Wu HD, *et al.* Acute elevations of plasma asymmetric dimethylarginine and impaired endothelial function in response to a high-fat meal in patients with type 2 diabetes. Arterioscler Thromb Vasc Biol 2000; 20: 2039-2044.

[26] Maas R, Boger RH, Schwedhelm E, Casas JP, Lopez-Jaramillo P, Serrano N, *et al.* Plasma concentrations of asymmetric dimethylarginine (ADMA) in Colombian women with pre-eclampsia. Jama 2004; 291: 823-824.

[27] Savvidou MD, Hingorani AD, Tsikas D, Frolich JC, Vallance P, Nicolaides KH: Endothelial dysfunction and raised plasma concentrations of asymmetric dimethylarginine in pregnant women who subsequently develop pre-eclampsia. Lancet 2003; 361: 1511-1517.

[28] Usui M, Matsuoka H, Miyazaki H, Ueda S, Okuda S, Imaizumi T: Increased endogenous nitric oxide synthase inhibitor in patients with congestive heart failure. Life Sci 1998; 62: 2425-2430.

[29] Duan J, Murohara T, Ikeda H, Katoh A, Shintani S, Sasaki K, *et al.* Hypercholesterolemia inhibits angiogenesis in response to hindlimb ischemia: nitric oxide-dependent mechanism. Circulation 2000; 102: III370-376.

[30] Masuda H, Tsujii T, Okuno T, Kihara K, Goto M, Azuma H: Accumulated endogenous NOS inhibitors, decreased NOS activity, and impaired cavernosal relaxation with ischemia. Am J Physiol Regul Integr Comp Physiol 2002; 282: R1730-1738.

[31] Boger RH, Bode-Boger SM, Brandes RP, Phivthong-ngam L, Bohme M, Nafe R, *et al.* Dietary L-arginine reduces the progression of atherosclerosis in cholesterol-fed rabbits: comparison with lovastatin. Circulation 1997; 96: 1282-1290.

[32] Boger RH, Bode-Boger SM, Szuba A, Tsao PS, Chan JR, Tangphao O, *et al.* Asymmetric dimethylarginine (ADMA): a novel risk factor for endothelial dysfunction: its role in hypercholesterolemia. Circulation 1998; 98: 1842-1847.

[33] Zoccali C, Benedetto FA, Maas R, Mallamaci F, Tripepi G, Malatino LS, *et al.* Asymmetric dimethylarginine, C-reactive protein, and carotid intima-media thickness in end-stage renal disease. J Am Soc Nephrol 2002; 13: 490-496.

[34] Loyaga-Rendon RY, Sakamoto S, Beppu M, Aso T, Ishizaka M, Takahashi R, *et al.* Accumulated endogenous nitric oxide synthase inhibitors, enhanced arginase activity, attenuated dimethylarginine dimethylaminohydrolase activity and intimal hyperplasia in premenopausal human uterine arteries. Atherosclerosis 2005; 178: 231-239.

[35] Selley ML: Increased concentrations of homocysteine and asymmetric dimethylarginine and decreased concentrations of nitric oxide in the plasma of patients with Alzheimer's disease. Neurobiol Aging 2003; 24: 903-907.

[36] Abe T, Tohgi H, Murata T, Isobe C, Sato C: Reduction in asymmetrical dimethylarginine, an endogenous nitric oxide synthase inhibitor, in the cerebrospinal fluid during aging and in patients with Alzheimer's disease. Neurosci Lett 2001; 312: 177-179.

[37] Lagrand WK, Niessen HW, Wolbink GJ, Jaspars LH, Visser CA, Verheugt FW, *et al.* C-reactive protein colocalizes with complement in human hearts during acute myocardial infarction. Circulation 1997; 95: 97-103.

[38] Griselli M, Herbert J, Hutchinson WL, Taylor KM, Sohail M, Krausz T, *et al.* C-reactive protein and complement are important mediators of tissue damage in acute myocardial infarction. J Exp Med 1999; 190: 1733-1740.

[39] Koenig W, Sund M, Frohlich M, Fischer HG, Lowel H, Doring A, *et al.* C-Reactive protein, a sensitive marker of inflammation, predicts future risk of coronary heart disease in initially healthy middle-aged men: results from the MONICA (Monitoring Trends and Determinants in Cardiovascular Disease) Augsburg Cohort Study, 1984 to 1992. Circulation 1999; 99: 237-242.

[40] Nissen SE: Effect of intensive lipid lowering on progression of coronary atherosclerosis: evidence for an early benefit from the Reversal of Atherosclerosis with Aggressive Lipid Lowering (REVERSAL) trial. Am J Cardiol 2005; 96: 61F-68F.

[41] Reynolds GD, Vance RP: C-reactive protein immunohistochemical localization in normal and atherosclerotic human aortas. Arch Pathol Lab Med 1987; 111: 265-269.

[42] Rowe IF, Walker LN, Bowyer DE, Soutar AK, Smith LC, Pepys MB: Immunohistochemical studies of C-reactive protein and apolipoprotein B in inflammatory and arterial lesions. J Pathol 1985; 145: 241-249.

[43] van der Meer IM, de Maat MP, Bots ML, Breteler MM, Meijer J, Kiliaan AJ, *et al.* Inflammatory mediators and cell adhesion molecules as indicators of severity of atherosclerosis: the Rotterdam Study. Arterioscler Thromb Vasc Biol 2002; 22: 838-842.

[44] Chambers JC, Eda S, Bassett P, Karim Y, Thompson SG, Gallimore JR, *et al.* C-reactive protein, insulin resistance, central obesity, and coronary heart disease risk in Indian Asians from the United Kingdom compared with European whites. Circulation 2001; 104: 145-150.

[45] Greenfield JR, Samaras K, Jenkins AB, Kelly PJ, Spector TD, Gallimore JR, *et al.* Obesity is an important determinant of baseline serum C-reactive protein concentration in monozygotic twins, independent of genetic influences. Circulation 2004; 109: 3022-3028.

[46] Devlin J, Gough A, Huissoon A, Perkins P, Holder R, Reece R, *et al.* The acute phase and function in early rheumatoid arthritis. C-reactive protein levels correlate with functional outcome. J Rheumatol 1997; 24: 9-13.

[47] Mallya RK, de Beer FC, Berry H, Hamilton ED, Mace BE, Pepys MB: Correlation of clinical parameters of disease activity in rheumatoid arthritis with serum concentration of C-reactive protein and erythrocyte sedimentation rate. J Rheumatol 1982; 9: 224-228.

[48] Mallya RK, Hind CR, Berry H, Pepys MB: Serum C-reactive protein in polymyalgia rheumatica. A prospective serial study. Arthritis Rheum 1985; 28: 383-387.

[49] Mallya RK, Young BJ, Pepys MB, Hamblin TJ, Mace BE, Hamilton EB: Anti-keratin antibodies in rheumatoid arthritis: frequency and correlation with other features of the disease. Clin Exp Immunol 1983; 51: 17-20.

[50] Spector TD, Hart DJ, Nandra D, Doyle DV, Mackillop N, Gallimore JR, *et al.* Low-level increases in serum C-reactive protein are present in early osteoarthritis of the knee and predict progressive disease. Arthritis Rheum 1997; 40: 723-727.

[51] van Leeuwen MA, van Rijswijk MH, Sluiter WJ, van Riel PL, Kuper IH, van de Putte LB, *et al.* Individual relationship between progression of radiological damage and the acute phase response in early rheumatoid arthritis. Towards development of a decision support system. J Rheumatol 1997; 24: 20-27.

[52] Szalai AJ, Alarcon GS, Calvo-Alen J, Toloza SM, McCrory MA, Edberg JC, *et al.* Systemic lupus erythematosus in a multiethnic US Cohort (LUMINA). XXX: association between C-reactive protein (CRP) gene polymorphisms and vascular events. Rheumatology (Oxford) 2005; 44: 864-868.

[53] Szalai AJ, McCrory MA, Cooper GS, Wu J, Kimberly RP: Association between baseline levels of C-reactive protein (CRP) and a dinucleotide repeat polymorphism in the intron of the CRP gene. Genes Immun 2002; 3: 14-19.

[54] Russell AI, Cunninghame Graham DS, Shepherd C, Roberton CA, Whittaker J, Meeks J, *et al.* Polymorphism at the C-reactive protein locus influences gene expression and predisposes to systemic lupus erythematosus. Hum Mol Genet 2004; 13: 137-147.

[55] Miyazaki H, Matsuoka H, Cooke JP, Usui M, Ueda S, Okuda S, *et al.* [Endogenous nitric oxide synthase inhibitor: a novel marker of atherosclerosis]. J Cardiol 1999; 33: 105-106.

[56] Zoccali C: Endothelial damage, asymmetric dimethylarginine and cardiovascular risk in end-stage renal disease. Blood Purif 2002; 20: 469-472.

[57] Bode-Boger SM, Boger RH, Kienke S, Junker W, Frolich JC: Elevated L-arginine/dimethylarginine ratio contributes to enhanced systemic NO production by dietary L-arginine in hypercholesterolemic rabbits. Biochem Biophys Res Commun 1996; 219: 598-603.

[58] Ito A, Tsao PS, Adimoolam S, Kimoto M, Ogawa T, Cooke JP: Novel mechanism for endothelial dysfunction: dysregulation of dimethylarginine dimethylaminohydrolase. Circulation 1999; 99: 3092-3095.

[59] Boger RH, Bode-Boger SM, Tsao PS, Lin PS, Chan JR, Cooke JP: An endogenous inhibitor of nitric oxide synthase regulates endothelial adhesiveness for monocytes. J Am Coll Cardiol 2000; 36: 2287-2295.

[60] Chan JR, Boger RH, Bode-Boger SM, Tangphao O, Tsao PS, Blaschke TF, *et al.* Asymmetric dimethylarginine increases mononuclear cell adhesiveness in hypercholesterolemic humans. Arterioscler Thromb Vasc Biol 2000; 20: 1040-1046.

[61] Nanayakkara PW, Teerlink T, Stehouwer CD, Allajar D, Spijkerman A, Schalkwijk C, *et al.* Plasma asymmetric dimethylarginine (ADMA) concentration is independently associated with carotid intima-media thickness and plasma soluble vascular cell adhesion molecule-1 (sVCAM-1) concentration in patients with mild-to-moderate renal failure. Kidney Int 2005; 68: 2230-2236.

[62] Hirschfield GM, Pepys MB: C-reactive protein and cardiovascular disease: new insights from an old molecule. Qjm 2003; 96: 793-807.

[63] Nissen SE, Tuzcu EM, Schoenhagen P, Crowe T, Sasiela WJ, Tsai J, *et al.* Statin therapy, LDL cholesterol, C-reactive protein, and coronary artery disease. N Engl J Med 2005; 352: 29-38.

[64] de Beer FC, Soutar AK, Baltz ML, Trayner IM, Feinstein A, Pepys MB: Low density lipoprotein and very low density lipoprotein are selectively bound by aggregated C-reactive protein. J Exp Med 1982; 156: 230-242.

[65] Bhakdi S, Torzewski M, Klouche M, Hemmes M: Complement and atherogenesis: binding of CRP to degraded, nonoxidized LDL enhances complement activation. Arterioscler Thromb Vasc Biol 1999; 19: 2348-2354.

[66] Zhang SH, Reddick RL, Piedrahita JA, Maeda N: Spontaneous hypercholesterolemia and arterial lesions in mice lacking apolipoprotein E. Science 1992; 258: 468-471.

[67] Plump AS, Smith JD, Hayek T, Aalto-Setala K, Walsh A, Verstuyft JG, *et al.* Severe hypercholesterolemia and atherosclerosis in apolipoprotein E-deficient mice created by homologous recombination in ES cells. Cell 1992; 71: 343-353.

[68] Paul A, Ko KW, Li L, Yechoor V, McCrory MA, Szalai AJ, *et al.* C-reactive protein accelerates the progression of atherosclerosis in apolipoprotein E-deficient mice. Circulation 2004; 109: 647-655.

[69] Hirschfield GM, Gallimore JR, Kahan MC, Hutchinson WL, Sabin CA, Benson GM, *et al.* Transgenic human C-reactive protein is not proatherogenic in apolipoprotein E-deficient mice. Proc Natl Acad Sci U S A 2005; 102: 8309-8314.

[70] Bae SW, Stuhlinger MC, Yoo HS, Yu KH, Park HK, Choi BY, *et al.* Plasma asymmetric dimethylarginine concentrations in newly diagnosed patients with acute myocardial infarction or unstable angina pectoris during two weeks of medical treatment. Am J Cardiol 2005; 95: 729-733.

[71] Mallamaci F, Tripepi G, Cutrupi S, Malatino LS, Zoccali C: Prognostic value of combined use of biomarkers of inflammation, endothelial dysfunction, and myocardiopathy in patients with ESRD. Kidney Int 2005; 67: 2330-2337.

[72] Rees DD, Palmer RM, Moncada S: Role of endothelium-derived nitric oxide in the regulation of blood pressure. Proc Natl Acad Sci U S A 1989; 86: 3375-3378.

[73] Alheid U, Frolich JC, Forstermann U: Endothelium-derived relaxing factor from cultured human endothelial cells inhibits aggregation of human platelets. Thromb Res 1987; 47: 561-571.

[74] Pollock JS, Forstermann U, Mitchell JA, Warner TD, Schmidt HH, Nakane M, *et al.* Purification and characterization of particulate endothelium-derived relaxing factor synthase from cultured and native bovine aortic endothelial cells. Proc Natl Acad Sci U S A 1991; 88: 10480-10484.

[75] Gross SS, Levi R: Tetrahydrobiopterin synthesis. An absolute requirement for cytokine-induced nitric oxide generation by vascular smooth muscle. J Biol Chem 1992; 267: 25722-25729.

[76] Vasquez-Vivar J, Hogg N, Martasek P, Karoui H, Pritchard KA, Jr., Kalyanaraman B: Tetrahydrobiopterin-dependent inhibition of superoxide generation from neuronal nitric oxide synthase. J Biol Chem 1999; 274: 26736-26742.

[77] Pou S, Keaton L, Surichamorn W, Rosen GM: Mechanism of superoxide generation by neuronal nitric-oxide synthase. J Biol Chem 1999; 274: 9573-9580.

[78] Gross SS, Stuehr DJ, Aisaka K, Jaffe EA, Levi R, Griffith OW: Macrophage and endothelial cell nitric oxide synthesis: cell-type selective inhibition by NG-aminoarginine, NG-nitroarginine and NG-methylarginine. Biochem Biophys Res Commun 1990; 170: 96-103.

[79] Rees DD, Palmer RM, Schulz R, Hodson HF, Moncada S: Characterization of three inhibitors of endothelial nitric oxide synthase in vitro and in vivo. Br J Pharmacol 1990; 101: 746-752.

[80] Reporterer M, Corbin JL: N G,N G,-dimethylarginine in myosin during muscle development. Biochem Biophys Res Commun 1971; 43: 644-650.

[81] Lou MF: Human muscular dystrophy: elevation of urinary dimethylarginines. Science 1979; 203: 668-670.

[82] Vallance P, Leone A, Calver A, Collier J, Moncada S: Endogenous dimethylarginine as an inhibitor of nitric oxide synthesis. J Cardiovasc Pharmacol 1992; 20 Suppl 12: S60-62.

[83] Olken NM, Rusche KM, Richards MK, Marletta MA: Inactivation of macrophage nitric oxide synthase activity by NG-methyl-L-arginine. Biochem Biophys Res Commun 1991; 177: 828-833.

[84] Suda O, Tsutsui M, Morishita T, Tasaki H, Ueno S, Nakata S, *et al.* Asymmetric dimethylarginine produces vascular lesions in endothelial nitric oxide synthase-deficient mice: involvement of renin-angiotensin system and oxidative stress. Arterioscler Thromb Vasc Biol 2004; 24: 1682-1688.

[85] Testa A, Spoto B, Tripepi G, Mallamaci F, Malatino L, Fatuzzo P, *et al.* The GLU298ASP variant of nitric oxide synthase interacts with asymmetric dimethyl arginine in determining cardiovascular mortality in patients with end-stage renal disease. J Hypertens 2005; 23: 1825-1830.

[86] Schmidt K, Klatt P, Mayer B: Uptake of nitric oxide synthase inhibitors by macrophage RAW 264.7 cells. Biochem J 1994; 301 (Pt 2): 313-316.

[87] MacAllister RJ, Whitley GS, Vallance P: Effects of guanidino and uremic compounds on nitric oxide pathways. Kidney Int 1994; 45: 737-742.

[88] Casellas P, Jeanteur P: Protein methylation in animal cells. I. Purification and properties of S-adenosyl-L-methionine: protein (arginine) N-methyltransferase from Krebs II ascites cells. Biochim Biophys Acta 1978; 519: 243-254.

[89] Miyake M, Kakimoto Y: Synthesis and degradation of methylated proteins of mouse organs: correlation with protein synthesis and degradation. Metabolism 1976; 25: 885-896.

[90] Small DH, Carnegie PR: In vivo methylation of an arginine in chicken myelin basic protein. J Neurochem 1982; 38: 184-190.

[91] Branscombe TL, Frankel A, Lee JH, Cook JR, Yang Z, Pestka S, *et al.* PRMT5 (Janus kinase-binding protein 1) catalyzes the formation of symmetric dimethylarginine residues in proteins. J Biol Chem 2001; 276: 32971-32976.

[92] Rho J, Choi S, Seong YR, Cho WK, Kim SH, Im DS: Prmt5, which forms distinct homo-oligomers, is a member of the protein-arginine methyltransferase family. J Biol Chem 2001; 276: 11393-11401.

[93] Miranda TB, Miranda M, Frankel A, Clarke S: PRMT7 is a member of the protein arginine methyltransferase family with a distinct substrate specificity. J Biol Chem 2004; 279: 22902-22907.

[94] Lee JH, Cook JR, Yang ZH, Mirochnitchenko O, Gunderson SI, Felix AM, *et al.* PRMT7, a new protein arginine methyltransferase that synthesizes symmetric dimethylarginine. J Biol Chem 2005; 280: 3656-3664.

[95] Chen SL, Loffler KA, Chen D, Stallcup MR, Muscat GE: The coactivator-associated arginine methyltransferase is necessary for muscle differentiation: CARM1 coactivates myocyte enhancer factor-2. J Biol Chem 2002; 277: 4324-4333.

[96] Frankel A, Clarke S: PRMT3 is a distinct member of the protein arginine N-methyltransferase family. Conferral of substrate specificity by a zinc-finger domain. J Biol Chem 2000; 275: 32974-32982.

[97] Frankel A, Yadav N, Lee J, Branscombe TL, Clarke S, Bedford MT: The novel human protein arginine N-methyltransferase PRMT6 is a nuclear enzyme displaying unique substrate specificity. J Biol Chem 2002; 277: 3537-3543.

[98] Lee J, Sayegh J, Daniel J, Clarke S, Bedford MT: PRMT8, a new membrane-bound tissue-specific member of the protein arginine methyltransferase family. J Biol Chem 2005; 280: 32890-32896.

[99] Tang J, Frankel A, Cook RJ, Kim S, Paik WK, Williams KR, Clarke S, Herschman HR: PRMT1 is the predominant type I protein arginine methyltransferase in mammalian cells. J Biol Chem 2000; 275: 7723-7730.

[100] Tang J, Gary JD, Clarke S, Herschman HR: PRMT 3, a type I protein arginine N-methyltransferase that differs from PRMT1 in its oligomerization, subcellular localization, substrate specificity, and regulation. J Biol Chem 1998; 273: 16935-16945.

[101] Qi C, Chang J, Zhu Y, Yeldandi AV, Rao SM, Zhu YJ: Identification of protein arginine methyltransferase 2 as a coactivator for estrogen receptor alpha. J Biol Chem 2002; 277: 28624-28630.

[102] Chen D, Ma H, Hong H, Koh SS, Huang SM, Schurter BT, Aswad DW, Stallcup MR: Regulation of transcription by a protein methyltransferase. Science 1999; 284: 2174-2177.

[103] Boisvert FM, Cote J, Boulanger MC, Cleroux P, Bachand F, Autexier C, *et al.* Symmetrical dimethylarginine methylation is required for the localization of SMN in Cajal bodies and pre-mRNA splicing. J Cell Biol 2002; 159: 957-969.

[104] Boulanger MC, Miranda TB, Clarke S, Di Fruscio M, Suter B, Lasko P, *et al.* Characterization of the Drosophila protein arginine methyltransferases DART1 and DART4. Biochem J 2004; 379: 283-289.

[105] McDermott JR: Studies on the catabolism of Ng-methylarginine, Ng, Ng-dimethylarginine and Ng, Ng-dimethylarginine in the rabbit. Biochem J 1976; 154: 179-184.

[106] Ogawa T, Kimoto M, Sasaoka K: Purification and properties of a new enzyme, NG,NG-dimethylarginine dimethylaminohydrolase, from rat kidney. J Biol Chem 1989; 264: 10205-10209.

[107] Ogawa T, Kimoto M, Sasaoka K: Occurrence of a new enzyme catalyzing the direct conversion of NG,NG-dimethyl-L-arginine to L-citrulline in rats. Biochem Biophys Res Commun 1987; 148: 671-677.

[108] Achan V, Broadhead M, Malaki M, Whitley G, Leiper J, MacAllister R, *et al.* Asymmetric dimethylarginine causes hypertension and cardiac dysfunction in humans and is actively metabolized by dimethylarginine dimethylaminohydrolase. Arterioscler Thromb Vasc Biol. 2003; 23: 1455-9.

[109] Leiper JM, Santa Maria J, Chubb A, MacAllister RJ, Charles IG, Whitley GS, *et al.* Identification of two human dimethylarginine dimethylaminohydrolases with distinct tissue distributions and homology with microbial arginine deiminases. Biochem J 1999; 343 Pt 1: 209-214.

[110] Tran CT, Fox MF, Vallance P, Leiper JM: Chromosomal localization, gene structure, and expression pattern of DDAH1: comparison with DDAH2 and implications for evolutionary origins. Genomics 2000; 68: 101-105.

[111] Arrigoni FI, Vallance P, Haworth SG, Leiper JM: Metabolism of asymmetric dimethylarginines is regulated in the lung developmentally and with pulmo-

nary hypertension induced by hypobaric hypoxia. Circulation 2003; 107: 1195-1201.
[112] Tillett WSF, T. Jr: Serological reactions in pneumonia with a nonprotein somatic fraction of pneumococcus. J Exp Med 1930; 52: 561-585.
[113] Whitehead AS, Bruns GA, Markham AF, Colten HR, Woods DE: Isolation of human C-reactive protein complementary DNA and localization of the gene to chromosome 1. Science 1983; 221: 69-71.
[114] Floyd-Smith G, Whitehead AS, Colten HR, Francke U: The human C-reactive protein gene (CRP) and serum amyloid P component gene (APCS) are located on the proximal long arm of chromosome 1. Immunogenetics 1986; 24: 171-176.
[115] Volanakis JE, Kaplan MH: Specificity of C-reactive protein for choline phosphate residues of pneumococcal C-polysaccharide. Proc Soc Exp Biol Med 1971; 136: 612-614.
[116] Thompson D, Pepys MB, Wood SP: The physiological structure of human C-reactive protein and its complex with phosphocholine. Structure Fold Des 1999; 7: 169-177.
[117] Tebo JM, Mortensen RF: Characterization and isolation of a C-reactive protein receptor from the human monocytic cell line U-937. J Immunol 1990; 144: 231-238.
[118] Bharadwaj D, Stein MP, Volzer M, Mold C, Du Clos TW: The major receptor for C-reactive protein on leukocytes is fcgamma receptor II. J Exp Med 1999; 190: 585-590.
[119] Kaplan MH, Volanakis JE: Interaction of C-reactive protein complexes with the complement system. I. Consumption of human complement associated with the reaction of C-reactive protein with pneumococcal C-polysaccharide and with the choline phosphatides, lecithin and sphingomyelin. J Immunol 1974; 112: 2135-2147.
[120] Volanakis JE, Kaplan MH: Interaction of C-reactive protein complexes with the complement system. II. Consumption of guinea pig complement by CRP complexes: requirement for human C1q. J Immunol 1974; 113: 9-17.
[121] Claus DR, Siegel J, Petras K, Skor D, Osmand AP, Gewurz H: Complement activation by interaction of polyanions and polycations. III. Complement activation by interaction of multiple polyanious and polycations is the presence of C-reactive protein. J Immunol 1977; 118: 83-87.
[122] Siegel J, Rent R, Gewurz H: Interactions of C-reactive protein with the complement system. I. Protamine-induced consumption of complement in acute phase sera. J Exp Med 1974; 140: 631-647.
[123] Volanakis JE: Human C-reactive protein: expression, structure, and function. Mol Immunol 2001; 38: 189-197.
[124] Robey FA, Jones KD, Tanaka T, Liu TY: Binding of C-reactive protein to chromatin and nucleosome core particles. A possible physiological role of C-reactive protein. J Biol Chem 1984; 259: 7311-7316.
[125] Fiedel BA, Gewurz H: Effects of C-reactive protein on platelet function. I. Inhibition of platelet aggregation and release reactions. J Immunol 1976; 116: 1289-1294.
[126] Shrive AK, Cheetham GM, Holden D, Myles DA, Turnell WG, Volanakis JE, *et al.* Three dimensional structure of human C-reactive protein. Nat Struct Biol 1996; 3: 346-354.
[127] Black S, Kushner I, Samols D: C-reactive Protein. J Biol Chem 2004; 279: 48487-48490.
[128] Motie M, Brockmeier S, Potempa LA: Binding of model soluble immune complexes to modified C-reactive protein. J Immunol 1996; 156: 4435-4441.
[129] Khreiss T, Jozsef L, Potempa LA, Filep JG: Loss of pentameric symmetry in C-reactive protein induces interleukin-8 secretion through peroxynitrite signaling in human neutrophils. Circ Res 2005; 97: 690-697.
[130] Pasceri V, Cheng JS, Willerson JT, Yeh ET: Modulation of C-reactive protein-mediated monocyte chemoattractant protein-1 induction in human endothelial cells by anti-atherosclerosis drugs. Circulation 2001; 103: 2531-2534.
[131] Khreiss T, Jozsef L, Potempa LA, Filep JG: Conformational rearrangement in C-reactive protein is required for proinflammatory actions on human endothelial cells. Circulation 2004; 109: 2016-2022.
[132] Schwedler SB, Amann K, Wernicke K, Krebs A, Nauck M, Wanner C, *et al.* Native C-reactive protein increases whereas modified C-reactive protein reduces atherosclerosis in apolipoprotein E-knockout mice. Circulation 2005; 112: 1016-1023.
[133] Pepys MB, Baltz M, Gomer K, Davies AJ, Doenhoff M: Serum amyloid P-component is an acute-phase reactant in the mouse. Nature 1979; 278: 259-261.
[134] Robey FA, Liu TY: Limulin: a C-reactive protein from Limulus polyphemus. J Biol Chem 1981; 256: 969-975.
[135] Pepys MB: CRP or not CRP? That is the question. Arterioscler Thromb Vasc Biol 2005; 25: 1091-1094.
[136] Venugopal SK, Devaraj S, Yuhanna I, Shaul P, Jialal I: Demonstration that C-reactive protein decreases eNOS expression and bioactivity in human aortic endothelial cells. Circulation 2002; 106: 1439-1441.
[137] Verma S, Wang CH, Li SH, Dumont AS, Fedak PW, Badiwala MV, *et al.* A self-fulfilling prophecy: C-reactive protein attenuates nitric oxide production and inhibits angiogenesis. Circulation 2002; 106: 913-919.
[138] Clapp BR, Hirschfield GM, Storry C, Gallimore JR, Stidwill RP, Singer M, *et al.* Inflammation and endothelial function: direct vascular effects of human C-reactive protein on nitric oxide bioavailability. Circulation 2005; 111: 1530-1536.
[139] Ikeda U, Maeda Y, Yamamoto K, Shimada K: C-Reactive protein augments inducible nitric oxide synthase expression in cytokine-stimulated cardiac myocytes. Cardiovasc Res 2002; 56: 86-92.
[140] van den Berg CW, Taylor KE, Lang D: C-reactive protein-induced in vitro vasorelaxation is an artefact caused by the presence of sodium azide in commercial preparations. Arterioscler Thromb Vasc Biol 2004; 24: e168-171.
[141] Swafford AN, Jr., Bratz IN, Knudson JD, Rogers PA, Timmerman JM, Tune JD, Dick GM: C-reactive protein does not relax vascular smooth muscle: effects mediated by sodium azide in commercially available preparations. Am J Physiol Heart Circ Physiol 2005; 288: H1786-1795.
[142] Lafuente N, Azcutia V, Matesanz N, Cercas E, Rodriguez-Manas L, Sanchez-Ferrer CF, *et al.* Evidence for sodium azide as an artifact mediating the modulation of inducible nitric oxide synthase by C-reactive protein. J Cardiovasc Pharmacol 2005; 45: 193-196.
[143] Taylor KE, Giddings JC, van den Berg CW: C-reactive protein-induced in vitro endothelial cell activation is an artefact caused by azide and lipopolysaccharide. Arterioscler Thromb Vasc Biol 2005; 25: 1225-1230.
[144] Liu C, Wang S, Deb A, Nath KA, Katusic ZS, McConnell JP, *et al.* Proapoptotic, antimigratory, antiproliferative, and antiangiogenic effects of commercial C-reactive protein on various human endothelial cell types in vitro: implications of contaminating presence of sodium azide in commercial preparation. Circ Res 2005; 97: 135-143.
[145] Pepys MB, Hawkins PN, Kahan MC, Tennent GA, Gallimore JR, Graham D, *et al.* Proinflammatory Effects of Bacterial Recombinant Human C-Reactive Protein Are Caused by Contamination With Bacterial Products, Not by C-Reactive Protein Itself. Circ Res 2005.
[146] Stuhlinger MC, Tsao PS, Her JH, Kimoto M, Balint RF, Cooke JP: Homocysteine impairs the nitric oxide synthase pathway: role of asymmetric dimethylarginine. Circulation 2001; 104: 2569-2575.
[147] Scalera F, Kielstein JT, Martens-Lobenhoffer J, Postel SC, Tager M, *et al.* Erythropoietin increases asymmetric dimethylarginine in endothelial cells: role of dimethylarginine dimethylaminohydrolase. J Am Soc Nephrol 2005; 16: 892-898.
[148] Zsuga J, Gesztelyi R, Torok J, Keki S, Bereczki D: Asymmetric dimethylarginine: A molecule responsible for the coexistence of insulin resistance and atherosclerosis via dual nitric oxide synthase inhibition. Med Hypotheses 2005; 65: 1091-1098.
[149] Lin KY, Ito A, Asagami T, Tsao PS, Adimoolam S, Kimoto M, *et al.* Impaired nitric oxide synthase pathway in diabetes mellitus: role of asymmetric dimethylarginine and dimethylarginine dimethylaminohydrolase. Circulation 2002; 106: 987-992.
[150] Asagami T, Abbasi F, Stuelinger M, Lamendola C, McLaughlin T, Cooke JP, Reaven GM, Tsao PS: Metformin treatment lowers asymmetric dimethylarginine concentrations in patients with type 2 diabetes. Metabolism 2002; 51: 843-846.
[151] Stuhlinger MC, Abbasi F, Chu JW, Lamendola C, McLaughlin TL, Cooke JP, *et al.* Relationship between insulin resistance and an endogenous nitric oxide synthase inhibitor. Jama 2002; 287: 1420-1426.
[152] Wakino S, Hayashi K, Tatematsu S, Hasegawa K, Takamatsu I, Kanda T, *et al.* Pioglitazone lowers systemic asymmetric dimethylarginine by inducing dimethylarginine dimethylaminohydrolase in rats. Hypertens Res 2005; 28: 255-262.
[153] Jones LC, Tran CT, Leiper JM, Hingorani AD, Vallance P: Common genetic variation in a basal promoter element alters DDAH2 expression in endothelial cells. Biochem Biophys Res Commun 2003; 310: 836-843.
[154] Ueda S, Kato S, Matsuoka H, Kimoto M, Okuda S, Morimatsu M, *et al.* Regulation of cytokine-induced nitric oxide synthesis by asymmetric dimethylarginine: role of dimethylarginine dimethylaminohydrolase. Circ Res 2003; 92: 226-233.
[155] Holden DP, Cartwright JE, Nussey SS, Whitley GS: Estrogen stimulates dimethylarginine dimethylaminohydrolase activity and the metabolism of asymmetric dimethylarginine. Circulation 2003; 108: 1575-1580.
[156] Achan V, Tran CT, Arrigoni F, Whitley GS, Leiper JM, Vallance P: all-trans-Retinoic acid increases nitric oxide synthesis by endothelial cells: a role for the induction of dimethylarginine dimethylaminohydrolase. Circ Res 2002; 90: 764-769.
[157] Laussmann T, Janosi RA, Fingas CD, Schlieper GR, Schlack W, Schrader J, *et al.* Myocardial proteome analysis reveals reduced NOS inhibition and enhanced glycolytic capacity in areas of low local blood flow. Faseb J 2002; 16: 628-630.
[158] Osanai T, Saitoh M, Sasaki S, Tomita H, Matsunaga T, Okumura K: Effect of shear stress on asymmetric dimethylarginine release from vascular endothelial cells. Hypertension 2003; 42: 985-990.
[159] Rizzo V, McIntosh DP, Oh P, Schnitzer JE: In situ flow activates endothelial nitric oxide synthase in luminal caveolae of endothelium with rapid caveolin dissociation and calmodulin association. J Biol Chem 1998; 273: 34724-34729.
[160] Teerlink T, Neele SJ, de Jong S, Netelenbos JC, Stehouwer CD: Oestrogen replacement therapy lowers plasma levels of asymmetrical dimethylarginine in healthy postmenopausal women. Clin Sci (Lond) 2003; 105: 67-71.
[161] Smith CL, Birdsey GM, Anthony S, Arrigoni FI, Leiper JM, Vallance P: Dimethylarginine dimethylaminohydrolase activity modulates ADMA levels, VEGF expression, and cell phenotype. Biochem Biophys Res Commun 2003; 308: 984-989.

[162] Kostourou V, Robinson SP, Cartwright JE, Whitley GS: Dimethylarginine dimethylaminohydrolase I enhances tumour growth and angiogenesis. Br J Cancer 2002; 87: 673-680.

[163] Kostourou V, Troy H, Murray JF, Cullis ER, Whitley GS, Griffiths JR, *et al.* Overexpression of dimethylarginine dimethylaminohydrolase enhances tumor hypoxia: an insight into the relationship of hypoxia and angiogenesis in vivo. Neoplasia 2004; 6: 401-411.

[164] Dayoub H, Achan V, Adimoolam S, Jacobi J, Stuehlinger MC, Wang BY, *et al.* Dimethylarginine dimethylaminohydrolase regulates nitric oxide synthesis: genetic and physiological evidence. Circulation 2003; 108: 3042-3047.

[165] Jacobi J, Sydow K, von Degenfeld G, Zhang Y, Dayoub H, Wang B, *et al.* Overexpression of dimethylarginine dimethylaminohydrolase reduces tissue asymmetric dimethylarginine levels and enhances angiogenesis. Circulation 2005; 111: 1431-1438.

[166] Majello B, Arcone R, Toniatti C, Ciliberto G: Constitutive and IL-6-induced nuclear factors that interact with the human C-reactive protein promoter. Embo J 1990; 9: 457-465.

[167] Toniatti C, Demartis A, Monaci P, Nicosia A, Ciliberto G: Synergistic transactivation of the human C-reactive protein promoter by transcription factor HNF-1 binding at two distinct sites. Embo J 1990; 9: 4467-4475.

[168] Ganter U, Arcone R, Toniatti C, Morrone G, Ciliberto G: Dual control of C-reactive protein gene expression by interleukin-1 and interleukin-6. Embo J 1989; 8: 3773-3779.

[169] Zhang D, Sun M, Samols D, Kushner I: STAT3 participates in transcriptional activation of the C-reactive protein gene by interleukin-6. J Biol Chem 1996; 271: 9503-9509.

[170] Voleti B, Agrawal A: Regulation of basal and induced expression of C-reactive protein through an overlapping element for OCT-1 and NF-kappaB on the proximal promoter. J Immunol 2005; 175: 3386-3390.

[171] Ridker PM, Rifai N, Clearfield M, Downs JR, Weis SE, Miles JS, *et al.* Measurement of C-reactive protein for the targeting of statin therapy in the primary prevention of acute coronary events. N Engl J Med 2001; 344: 1959-1965.

[172] Wang TJ, Larson MG, Levy D, Benjamin EJ, Kupka MJ, Manning WJ, *et al.* C-reactive protein is associated with subclinical epicardial coronary calcification in men and women: the Framingham Heart Study. Circulation 2002; 106: 1189-1191.

[173] Willerson JT, Ridker PM: Inflammation as a cardiovascular risk factor. Circulation 2004; 109: II2-10.

[174] Booth AD, Jayne DR, Kharbanda RK, McEniery CM, Mackenzie IS, Brown J, *et al.* Infliximab improves endothelial dysfunction in systemic vasculitis: a model of vascular inflammation. Circulation 2004; 109: 1718-1723.

[175] Shagdarsuren E, Wellner M, Braesen JH, Park JK, Fiebeler A, Henke N, *et al.* Complement activation in angiotensin II-induced organ damage. Circ Res 2005; 97: 716-724.

[176] Schulze F, Wesemann R, Schwedhelm E, Sydow K, Albsmeier J, Cooke JP, *et al.* Determination of asymmetric dimethylarginine (ADMA) using a novel ELISA assay. Clin Chem Lab Med 2004; 42: 1377-1383.

[177] Valtonen P, Karppi J, Nyyssonen K, Valkonen VP, Halonen T, Punnonen K: Comparison of HPLC method and commercial ELISA assay for asymmetric dimethylarginine (ADMA) determination in human serum. J Chromatogr B Analyt Technol Biomed Life Sci 2005.

[178] Teerlink T, Nijveldt RJ, de Jong S, van Leeuwen PA: Determination of arginine, asymmetric dimethylarginine, and symmetric dimethylarginine in human plasma and other biological samples by high-performance liquid chromatography. Anal Biochem 2002; 303: 131-137.

[179] Martens-Lobenhoffer J, Bode-Boger SM: Measurement of asymmetric dimethylarginine (ADMA) in human plasma: from liquid chromatography estimation to liquid chromatography-mass spectrometry quantification. Eur J Clin Pharmacol 2005: 1-8.

[180] Siboo R, Kulisek E: A fluorescent immunoassay for the quantification of C-reactive protein. J Immunol Methods 1978; 23: 59-67.

[181] De Beer FC, Shine B, Pepys MB: Radiometric ligand binding assay for C-reactive protein. Complexed C-reactive protein is not detectable in acute phase serum. Clin Exp Immunol 1982; 50: 231-237.

[182] Akbar F, Heinonen S, Pirskanen M, Uimari P, Tuomainen TP, Salonen JT: Haplotypic association of DDAH1 with susceptibility to pre-eclampsia. Mol Hum Reprod 2005; 11: 73-77.

[183] Brull DJ, Serrano N, Zito F, Jones L, Montgomery HE, Rumley A, *et al.* Human CRP gene polymorphism influences CRP levels: implications for the prediction and pathogenesis of coronary heart disease. Arterioscler Thromb Vasc Biol 2003; 23: 2063-2069.

[184] D'Aiuto F, Casas JP, Shah T, Humphries SE, Hingorani AD, Tonetti MS: C-reactive protein (+1444C>T) polymorphism influences CRP response following a moderate inflammatory stimulus. Atherosclerosis 2005; 179: 413-417.

[185] Carlson CS, Aldred SF, Lee PK, Tracy RP, Schwartz SM, Rieder M, *et al.* Polymorphisms within the C-reactive protein (CRP) promoter region are associated with plasma CRP levels. Am J Hum Genet 2005; 77: 64-77.

[186] Zee RY, Ridker PM: Polymorphism in the human C-reactive protein (CRP) gene, plasma concentrations of CRP, and the risk of future arterial thrombosis. Atherosclerosis 2002; 162: 217-219.

[187] D'Aiuto F, Parkar M, Brett PM, Ready D, Tonetti MS: Gene polymorphisms in pro-inflammatory cytokines are associated with systemic inflammation in patients with severe periodontal infections. Cytokine 2004; 28: 29-34.

[188] Eklund C, Lehtimaki T, Hurme M: Epistatic effect of C-reactive protein (CRP) single nucleotide polymorphism (SNP) +1059 and interleukin-1B SNP +3954 on CRP concentration in healthy male blood donors. Int J Immunogenet 2005; 32: 229-232.

[189] Araujo F, Pereira AC, Mota GF, Latorre Mdo R, Krieger JE, Mansur AJ: The influence of tumor necrosis factor -308 and C-reactive protein G1059C gene variants on serum concentration of C-reactive protein: evidence for an age-dependent association. Clin Chim Acta 2004; 349: 129-134.

[190] Kolek MJ, Carlquist JF, Muhlestein JB, Whiting BM, Horne BD, Bair TL, *et al.*Toll-like receptor 4 gene Asp299Gly polymorphism is associated with reductions in vascular inflammation, angiographic coronary artery disease, and clinical diabetes. Am Heart J 2004; 148: 1034-1040.

[191] Smith CL, Anthony S, Hubank M, Leiper JM, Vallance P: Effects of ADMA upon Gene Expression: An Insight into the Pathophysiological Significance of Raised Plasma ADMA. PLoS Med 2005; 2: e264.

[192] Thum T, Tsikas D, Stein S, Schultheiss M, Eigenthaler M, Anker SD, Suppression of endothelial progenitor cells in human coronary artery disease by the endogenous nitric oxide synthase inhibitor asymmetric dimethylarginine. J Am Coll Cardiol 2005; 46: 1693-1701.

[193] Danesh J, Muir J, Wong YK, Ward M, Gallimore JR, Pepys MB: Risk factors for coronary heart disease and acute-phase proteins. A population-based study. Eur Heart J 1999; 20: 954-959.

[194] Cross JM, Donald AE, Kharbanda R, Deanfield JE, Woolfson RG, MacAllister RJ: Acute administration of L-arginine does not improve arterial endothelial function in chronic renal failure. Kidney Int 2001; 60: 2318-2323.

[195] Hand MF, Haynes WG, Webb DJ: Hemodialysis and L-arginine, but not D-arginine, correct renal failure-associated endothelial dysfunction. Kidney Int 1998; 53: 1068-1077.

[196] MacAllister RJ, Parry H, Kimoto M, Ogawa T, Russell RJ, Hodson H, *et al.* Regulation of nitric oxide synthesis by dimethylarginine dimethylaminohydrolase. Br J Pharmacol 1996; 119: 1533-1540.

[197] Rossiter S, Smith CL, Malaki M, Nandi M, Gill H, Leiper JM, *et al.* Selective substrate-based inhibitors of mammalian dimethylarginine dimethylaminohydrolase. J Med Chem 2005; 48: 4670-4678.

[198] Stone EM, Schaller TH, Bianchi H, Person MD, Fast W: Inactivation of two diverse enzymes in the amidinotransferase superfamily by 2-chloroacetamidine: dimethylargininase and peptidylarginine deiminase. Biochemistry 2005; 44: 13744-13752.

[199] Knipp M, Braun O, Vasak M: Searching for DDAH inhibitors: S-nitroso-L-homocysteine is a chemical lead. J Am Chem Soc 2005; 127: 2372-2373.