C-reactive protein and coronary heart disease: a critical review

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Modestly elevated baseline concentrations of C-reactive protein (CRP), the classical acute phase protein, are associated with the long-term risk of coronary heart disease in general populations, whilst the major acute phase response of CRP following myocardial infarction is associated with death and cardiac complications. The pathogenic and clinical significance of these associations is controversial. Here we critically review the evidence and describe large-scale epidemiological studies, novel experiments and possible specific therapies which will rigorously inform the debate. We distinguish between the potential pathogenicity of high acute phase circulating CRP concentrations in individuals with substantial tissue damage and modest but persistent increases in baseline values in generally healthy subjects.

Keywords: coronary heart disease, C-reactive protein.

Introduction

Inflammation has been proposed to contribute to different stages in the pathogenesis of coronary heart disease (CHD), including the lifelong process of atherosclerosis, the acute atherothrombotic event, which causes ischaemic necrosis in acute myocardial infarction (MI) and the myocardial damage following ischaemia [1–5]. C-reactive protein (CRP), the classical acute phase protein, is the most extensively studied systemic marker of inflammation [6]. Since the original demonstration of an association between even modest increases in baseline circulating CRP and subsequent cardiovascular outcomes in patients with unstable angina at the initial examination [7, 8], CRP has been the focus of intense investigation [5].

Several population-based prospective studies of CHD (defined in this paper as non fatal MI or coronary death) have reported on associations of subtle, prolonged increases in baseline CRP levels with CHD risk [9]. Also, CRP has been known for more than 25 years to bind to LDL [10, 11] and it has been detected in atherosclerotic plaques [12]. These observations have collectively raised the possibility that CRP may play a direct causal role in CHD (and, by implication, could be an important therapeutic target), although there is debate about the interpretation of both epidemiological and experimental observations [13–15]. There is also uncertainty about the separate issue of the value of measurement of CRP in the long-term prediction of cardiovascular outcomes [16, 17]. A completely distinct possibility is that, in contrast to modest long-term differences in baseline CRP values, the major acute phase response of CRP triggered by MI may contribute acutely to severity and outcome of the ischaemic lesion [18]. The objective of this article is to provide a critical review of the
available evidence on associations of CRP with CHD and to highlight emerging approaches that may help clarify the uncertainties described above.

**Structure of CRP**

C-reactive protein is a nonglycosylated circulating plasma protein which together with the distinct but closely related protein, serum amyloid P component (SAP), comprises the pentraxin family of proteins [19, 20], which belong to the lectin fold superfamily [21]. Each pentraxin is composed of five identical noncovalently associated subunits arranged with cyclic pentameric symmetry in a disc-like configuration and has characteristic calcium-dependent binding to specific ligands (Fig. 1). Each CRP protomer has the flattened β-jellyroll lectin fold and bears on one face, the B or binding face, a pocket which contains two calcium ions bound just 4 Å apart by coordination with protein carboxylate and amide side chains derived from loops that congregate on one face of the protomer core. These calcium atoms are essential for all physiological ligand binding by CRP and also markedly stabilize both the structure of the protomer [22] and the integrity of the native pentamer. Calcium is, of course, always present in the extracellular environment in vivo in which CRP exists, and inappropriate omission of calcium in handling CRP for in vitro experiments produces misleading observations.

In the absence of calcium, even if pH and ionic strength are physiological, CRP readily aggregates, loses its physiological ligand binding and the normal secondary effects thereof, dissociates into protomers which then readily misfold, and becomes susceptible to proteolytic cleavage. In marked contrast, native CRP in the presence of physiological conditions of calcium, pH and ionic strength is remarkably stable. Although isolated pure CRP can aggregate if subjected to violent turbulence, it is otherwise very stable, unaffected by repeated freeze-thawing, remains in its normal pentameric assembly and is highly resistant to proteolysis [23]. It does not dissociate into protomers even in the presence of the very strong anionic detergent, sodium dodecyl sulphate, unless boiled. CRP is also stable in serum which enhances greatly its utility as a clinical marker. In view of these robust observations, speculations about physiological or pathophysiological effects of denatured CRP subunits, so-called modified CRP, or of proteolytic cleavage fragments of CRP are unlikely to be correct and the evidence for existence of such moieties in vivo is unconvincing.

The critical importance of appropriate handling of CRP is strikingly exemplified by recent observations

![Fig. 1 3D X-ray crystal structure of the human C-reactive protein (CRP) molecule with bound calcium (left) and of a phosphocholine molecule bound in the ligand binding pocket of one CRP protomer (right), showing the intermolecular interactions responsible for binding [187]. X-ray crystallographic structures provided by Dr. Simon Kolstoe.](image)
on the putative binding interaction between CRP and complement regulatory protein, factor H (fH). One of the two major polymorphic variants of fH is a highly significant risk factor for age-related macular degeneration [24–26], and the amino acid substitution (Y402H) is in the seventh short consensus repeat, the region of fH reported to bind to CRP [27]. Recent reports of binding between CRP and fH were all based on solid phase studies in which CRP was immobilized, usually from calcium-free solutions and thereby destabilized, by nonspecific adherence to plastic surfaces, conditions in which such immobilized proteins are inevitably at least partly denatured [28]. When these studies were repeated using CRP in the presence of calcium, no interaction with fH was detected (S. Hakobyan, C.L. Harris, C.W. van den Berg, M.C. Fernandez Alonso, E. Goicoechea de Jorge, S. Rodriguez de Cordoba, G. Rivas, P. Mangione, M.B. Pepys and B.P. Morgan, unpublished data). Further extensive controlled studies with fluid phase proteins and different configurations and methods of immobilization of CRP or fH confirmed that there was no detectable interaction between fH and native CRP either in solution or on a solid phase (S. Hakobyan, C.L. Harris, C.W. van den Berg, M.C. Fernandez Alonso, E. Goicoechea de Jorge, S. Rodriguez de Cordoba, G. Rivas, P. Mangione, M.B. Pepys and B.P. Morgan, unpublished data). Binding was only seen when the CRP was denatured and since, as noted above, evidence for the existence or at least persistence of denatured CRP in vivo is not compelling, a pathophysiologically relevant interaction between CRP and fH is questionable.

Ligand binding by CRP

C-reactive protein was discovered and named for its binding to pneumococcal somatic C-polysaccharide [29] in which it recognizes the phosphocholine residues which are present in this ribitol teichoic acid [30]. Phosphocholine is the natural ligand to which CRP binds with highest affinity and this key ligand is ubiquitous as the polar head group of phosphatidylcholine in cell membranes and plasma lipoproteins. Phosphocholine is also present in constituents of many bacteria, fungi and parasites and plants and the importance for mammalian biology of its recognition is exemplified by the fact that a significant proportion of the germline antibody specificities are directed at it. However, CRP does not bind to all materials containing phosphocholine as the residues must be ‘available’ or in an appropriate sterochemical configuration. Thus CRP binds to dead or damaged cells in which significant amounts of lyso-phosphatidyl choline are present, but not the surface of living healthy cells [31]. Binding of CRP to apoptotic cells is controversial and the most rigorous evidence suggests that CRP only binds to so-called late apoptotic cells which are effectively necrotic [32–34]. CRP also binds to oxidized phospholipids [33], platelet activating factor [35], modified LDL [36], β-VLDL, concentrated normal VLDL [37] and to small nuclear ribonucleoprotein particles (which do not contain phosphocholine) when these are exposed in dead or damaged cells [38, 39].

Binding of CRP to its macromolecular ligands has many of the same effects as binding of antibodies to antigens, thus CRP precipitates soluble ligands, aggregates particulate ligands and activates the classical complement pathway [40–42]. By analogy with antibodies, it is therefore possible that CRP might contribute both to host defence against infection and enhancement of inflammatory tissue damage.

Functions of human CRP

Despite many claims and assertions in the literature, neither the normal functions of human CRP nor its possible role in disease is known. This is because no deficiency or even structural polymorphism of human CRP has yet been reported, nor is any drug or other therapeutic manoeuvre yet available which specifically inhibits or depletes human CRP in vivo. The effects of absence, lack of function or inhibition of human CRP have thus so far not been tested.

The notable evolutionary and phylogenetic conservation of both the existence and the structure of the CRP molecule [20, 43, 44] suggest that it is likely to have a beneficial role and in view of its ligand binding specificity and secondary effects, this role could...
be related to both innate immunity against infection and the appropriate safe handling and disposal of damaged autologous cells and lipids. However, testing these possibilities experimentally is extremely challenging because despite considerable structural similarity, the CRP molecules in different species show major differences in normal concentration, in behaviour as acute phase proteins, fine ligand binding specificity and secondary effects after ligand binding. Extrapolation from animals to man is therefore difficult and requires that putatively conserved functions must be compatible with these major differences. Furthermore, effects of heterologous CRP in vivo, following administration or transgenic expression of human CRP in animals, are not necessarily genuine effects of human CRP in humans. Finally, any function proposed for human CRP must be consistent with the remarkable speed and dynamic range of its plasma concentration, which can rise by over 1000-fold in 24–48 h after a strong acute stimulus such as sepsis or acute MI, and can fall with a half time of about 24 h when the stimulus is removed. These dramatic changes are not associated with any local or systemic vascular or inflammatory effects in patients other than those related to the pathology or treatment, which respectively triggered or alleviated the acute phase response. The fact that injection of even huge doses of isolated pure authentic human CRP into healthy animals has no adverse, inflammatory or tissue damaging effects is consistent with these long well-established clinical observations.

Synthesis of CRP

The circulating CRP is produced by hepatocytes. Reports of extra-hepatic CRP synthesis by some other cell types have not always been reproducible [45, 46], and although possibly relevant to potential local effects of CRP, the contribution to plasma concentrations can only be minute with respect to that from the liver. Hepatic CRP synthesis is under exquisitely sensitive transcriptional regulation via pro-inflammatory cytokines including interleukin (IL)-6, IL-1 and tumour necrosis factor-α so that almost any form of tissue injury, infection or inflammation, and indeed also most forms of adverse nonphysiological ‘stress’, are associated with increased circulating CRP values [47]. CRP is apparently cleared from the plasma and catabolized exclusively by hepatocytes [48] and the plasma half-life in humans of about 19 h is the same in all individuals regardless of the presence of disease or the circulating concentration of CRP. The sole determinant of the plasma concentration is therefore the synthesis rate [49].

It is critically important for any interpretation of the significance and utility of CRP values to distinguish between modest increments in the very low normal baseline values of CRP and the massive rises of several orders of magnitude which occur very rapidly following major stimuli, such as sepsis, acute trauma or tissue necrosis, and which may persist for months or years in individuals with chronic active inflammatory and tissue damaging diseases, such as chronic infections, rheumatoid arthritis, Crohn’s disease, lymphoma and many others. Furthermore the almost completely nonspecific nature of the CRP response means that it is neither possible nor medically appropriate to interpret CRP values in an individual in the absence of full clinical information on that person, including history, physical examination and full results of all available investigations [6].

Concentration of circulating CRP in healthy normal individuals and in general populations

In healthy normal subjects CRP is a trace plasma protein. In the first substantial study of such individuals, the median value in 468 British Caucasian volunteer blood donors aged 18–63 years was 0.8 mg L⁻¹, the ninetieth percentile of the distribution was 3 mg L⁻¹ and the ninety-ninth percentile was 10 mg L⁻¹ [50]. Similar distributions have been reported from the general adult population of USA [51] and Northern Europe [52] although values are slightly higher overall in these unselected populations, more extreme high values are present and concentrations tend to increase slightly with age. The markedly skewed distribution of CRP values is typically normalized by log transformation. In USA 50% of individuals have baseline CRP greater than 2 mg L⁻¹ and 33% are between 3 and 10 mg L⁻¹ [51]. Compared with people of
Northern European ancestry, higher baseline CRP values of CRP have been observed in people of Afro-Caribbean origin residing in North America and lower values in people of Japanese or Chinese ancestry [53, 54]. In the indigenous Japanese, baseline CRP values are about one-tenth of those seen in individuals of European ancestry [55] but the reasons for these differences are uncertain. In healthy individuals followed serially with monthly samples, most CRP values cluster at a level typical for that individual and generally within the range of 0.1–3 mg L\(^{-1}\), but with occasional spikes unrelated to any obvious clinical pathology. About 50% of the individual variance in baseline CRP concentration is genetic and largely attributable to noncoding polymorphisms in the CRP gene (see below). The other major determinant, independent of genetic factors, is the level of adiposity, especially central abdominal obesity [56], reflecting production of pro-inflammatory cytokines by macrophages associated with such adipose tissue and by the adipocytes themselves [57] and possibly even some CRP production by adipocytes [58]. The year-to-year within-person variability of CRP in populations of initially apparent healthy adults is comparable with those seen for levels of blood pressure or serum cholesterol (Fig. 2a) and some other circulating markers of inflammation (e.g. fibrinogen, leucocyte count) [59–61]. For example, the intraclass correlation coefficient for CRP levels (calculated from two different measurements obtained from the same individuals a few years apart) is about 0.6 [62].

The term ‘high-sensitivity’ or ‘highly sensitive’ CRP, abbreviated as hs-CRP, has been widely adopted in recent literature. It refers to measurement of CRP in serum or plasma samples using immunoassay methods with sufficient sensitivity to quantify CRP throughout its normal range in contrast to older less sensitive commercial assays which had detection limits in the range 2–10 mg L\(^{-1}\) and were suitable for measurement of acute phase responses of CRP rather than baseline values. It is very important to recognize that the analyte designated as hs-CRP is just CRP itself, not anything new or different and in particular is not a novel analyte with any special relationship to cardiovascular disease. hs-CRP is the same exquisitely

![Fig. 2](image-url) Direct comparisons of C-reactive protein, several established cardiovascular risk factors and emerging markers in relation to: (a) within-person variability over 12 years (expressed as the regression dilution ratio\(^*\)) and (b) odds ratios for coronary heart disease (adjusted for established risk factors\(^\dagger\)). \(^*\)Regression dilution ratio calculated using Rosner’s multivariate regression method, adjusted for baseline age, sex, smoking history, diabetes history, total cholesterol, log triglycerides, systolic blood pressure, and body mass index. \(^\dagger\)Compared with not current smokers. \(^\ddagger\)Odds ratios (top third vs. bottom third) are adjusted for cohort, age, sex, period of recruitment, smoking status, history of diabetes, total cholesterol, log triglycerides, systolic blood pressure and body mass index. Data source: data have been derived from the 19 000-participants Reykjavik cohort study and collated from the following publications: [62, 102, 104, 105].

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sensitive and entirely nonspecific systemic marker of infection, inflammation, tissue damage and/or almost any form of adverse nonphysiological stress as the CRP, which has been extensively studied and used clinically for over 75 years. Precisely the same constraints apply to use of its values, notably that these cannot be interpreted in the absence of full clinical information on the individual patient at the time the sample was taken [6]. An individual with an apparent baseline CRP value of, say 7 mg L$^{-1}$, should not just be told that they have an increased risk of cardiovascular disease and be advised to alter their lifestyle accordingly, when the increased CRP concentration might reflect any of a very wide range of other serious diseases which have not yet declared themselves, for example, Hodgkin’s disease or renal carcinoma or any other condition which is often occult for some time before clinical presentation [6].

Long-term prospective epidemiological studies of CRP and CHD

In 1996, the first population-based prospective study reported on associations of ‘baseline’ CRP levels with CHD outcomes [63]. By 2008, over 40 further such studies had reported [62–101], typically involving middle-aged populations of European ancestry. A literature-based meta-analysis was published in 2004 involving 22 population-based prospective studies with a total of 7068 incident CHD cases with a mean follow-up of 12 years. The odds ratio for CHD adjusted for several established risk factors was 1.6 (95% CI: 1.5–1.7) in a comparison of people with baseline CRP levels in the top third compared with those in the bottom third of the population distribution (corresponding to values of about 2.4 mg L$^{-1}$ vs. 1 mg L$^{-1}$) [62]. This odds ratio is similar in magnitude to those reported for some other nonspecific circulating markers of inflammation (e.g. fibrinogen [60], IL-6 [102], leucocyte count [103]) and some other emerging biochemical risk markers (e.g. triglycerides [104], lipoprotein (a) [105]) (Fig. 2b). By contrast, an odds ratio of 2.0 (1.6–2.5) for CHD was reported in a previous review of the 11 initial prospective studies of CRP [71] and even more extreme odds ratios have been reported in some earlier individual studies. This attenuation in CHD risk associated with CRP levels during the course of its study is likely, at least in part, to reflect the decrease in scope for random error and for selective publication in larger and later hypothesis-testing studies. It is also possible, however, that there may be important differences across studies in features related to population characteristics, assay procedures and statistical methods used. To help address such uncertainties more reliably, the Emerging Risk Factors Collaboration (ERFC) has collated and harmonized individual data from participants in 48 population-based prospective studies of CRP involving a total of about 120 000 participants and about 9500 CHD outcomes [9]. In contrast with literature-based reviews (which have access to only published or limited aggregated data), detailed pooled analyses involving primary data should provide: (i) precise analyses of CRP-CHD associations under a range of various circumstances (such as at different ages, in women and men and at different levels of risk factors), (ii) reliable characterization of the shape of any dose–response relationship curves, (iii) consistent approaches to adjust for possible confounding factors, (iv) correction for within-person variability in levels of CRP and of possible confounding factors and (v) detailed investigation of potential sources of heterogeneity.

Even analyses in the ERFC may not, however, distinguish reliably whether CRP is mainly a causal risk factor in CHD or mainly a marker of established cardiovascular risk factors to which it is correlated, or mainly a marker of subclinical disease or some combination of these possibilities. The potential scope for confounding in studies of CRP is suggested by observations that individuals with higher than average levels of CRP tend to have higher values of blood pressure, pulse-wave velocity, central arterial blood pressure, proatherogenic lipid fractions (LDL, apolipoprotein-B), higher body mass index and levels of abdominal obesity and a greater prevalence of diabetes and metabolic syndrome and smoking and lower socio-economic status and birth weight [106–109]. CRP is inversely associated with a range of potentially protective risk factors in CHD, such as physical activity, HDL cholesterol, apolipoprotein-AI and...
consumption of fruits and vegetables [100, 110]. CRP levels are also associated with levels of several other emerging biochemical risk markers of uncertain relevance to CHD, such as fibrinogen, adiponectin and triglycerides [88, 100]. The third National Health and Nutrition Examination Survey reported that in up to about two-thirds of Americans, a CRP level greater than 3 mg L\(^{-1}\) could be attributable to elevations in at least one established cardiovascular risk factor such as those named above [111]. The many potentially relevant correlates of CRP make it difficult, therefore, to determine to what extent the observed associations of CRP with CHD risk are independent from established and emerging coronary risk markers. Statistical adjustment for confounding factors is potentially limited because not all relevant confounders have been (or can be) measured in a study. Moreover, even measured confounders may be incompletely adjusted for because allowances are typically not made for within-person variability in levels of confounders (e.g. blood pressure, serum lipid concentrations). Alternatively, statistical overadjustment – the correction for markers in any causal pathway between CRP levels and CHD risk – could in principle obscure a potentially important aetiological relationship. In practice, however, it is difficult to judge the likelihood of over adjustment given current uncertainties about whether CRP exerts any vascular effects, as described in the next section.

**Experimental studies and animal models of CRP and atherogenesis**

The possibility that CRP might have proatherogenic actions was first suggested in 1982 by the discovery of its specific binding to LDL and VLDL [10, 11] and was supported by its detection in atherosclerotic plaque [12]. Even before this it was well established that CRP can activate the classical complement pathway [40, 41] and is therefore potentially pro-inflammatory. However, compelling evidence for a role of CRP in atherosclerosis has not emerged despite many reports describing a very large range of pro-inflammatory, pro-thrombotic, vasoactive and thus potentially pro-atherogenic and pro-atherothrombotic effects of CRP preparations on various cell types in vitro. Almost none of these reports, most of which use commercial preparations of CRP, have described any characterization of the integrity or purity of the protein, and very few included any controls. None of the early studies removed either the toxic sodium azide, present in all commercial preparations as a bacteriostatic, or considered the inevitable presence of bacterial endotoxin (lipopolysaccharide) in CRP produced by recombinant *Escherichia coli*. Careful studies with authentic pure CRP isolated from human material and with recombinant CRP produced by mammalian cells, and thus free of such biologically active contamination, do not confirm claims for wide-ranging direct cellular actions of CRP [13, 112–119].

There is now an extensive and controversial literature which extends to *in vivo* studies involving either injection of CRP in different species or transgenic expression of CRP in mice. Injection of even enormous doses (40 mg kg\(^{-1}\)) of purified authentic human CRP into mice and rats neither elicited inflammation nor produced any clinical ill effects (including changes in blood pressure) [120]. This important observation is consistent with the fact that human CRP concentrations can cover a 10 000-fold range from less than 50 \(\mu\)g L\(^{-1}\) to more than 500 mg L\(^{-1}\) in the acute phase response and this is very unlikely to be compatible with significant effects of CRP on vascular tone, activation of inflammatory cells, triggering of coagulation or any of the other purported signalling functions lately ascribed to CRP on the basis of *in vitro* studies with commercial CRP preparations. Although there is one report of enhanced atherosclerosis in apolipoprotein E knockout mice expressing transgenic human CRP [121] other and larger studies show no such effect [122–124] nor any pro-inflammatory or pro-thrombotic action even in aged atherosclerotic animals [125]. In the more humanized model of atherosclerosis in LDL receptor knockout mice expressing apoB100, transgenic human CRP was atheroprotective [126]. Indeed, the presence of CRP in human atheroma is no less likely *a priori* to be atheroprotective than it is to be atherogenic [36]. Extrapolation to humans from *in vivo* experimental animal studies of CRP function is not possible because, despite the considerable phylogenetic conservation of CRP structure, there are very substantial differences...
between CRPs of different species with respect to fine
details of ligand recognition, secondary effects of
ligand binding including complement activation,
normal concentrations and behaviour as acute phase
reactants [44, 127, 128]. Introduction of human CRP
into animals, wherein the protein is interacting with
xenogeneic molecules, cells, physiological and patho-
physiological processes, cannot be assumed to be a
robust test for functions of human CRP in humans.
Despite the claims to the contrary, the only rigorous
conclusion at present is that neither the physiological
nor the pathophysiological functions of human CRP
are yet known.

To address these questions, work is in progress in the
Pepys laboratory in three areas:

1 C-reactive protein knockout mice have been created
using C57BL/6 embryonic stem cells in order to have
genetically homogenous pure line C57BL/6 mice defi-
cient in CRP. These mice are fertile and so far have an
apparently normal life span. Characterization of their
phenotype in relation to atherogenesis and other chal-
lenges will elucidate the functions of mouse CRP and
may be informative about the human protein, even
though the two have radically different concentrations
and behaviour as acute phase proteins. Others have
also lately reported production of a mouse CRP knock-
out but not its genetic background [129].

2 Clinical ‘good manufacturing practice’ grade
human CRP has been isolated and purified from
human plasma pooled from over 6000 comprehen-
sively validated normal healthy donors. This is a con-
siderable undertaking in view of the normal plasma
concentration of CRP of less than 1 mg L\(^{-1}\) and will
be only the second reported human CRP preparation
made from healthy individuals [49] rather than from
diseased individuals mounting an acute phase
response or produced in recombinant bacteria or cells.
It is thus entirely physiological in addition to being
highly purified, completely free of exposure to bacte-
rial endotoxin or other contamination, sterile, nonpyr-
ogenic, fully structurally and functionally intact and
approved by the UK regulatory authorities for admin-
istration to human subjects. This preparation will be
used to test for effects of human CRP itself, on cells
in vitro, on animals in vivo and finally in human vol-
unteers, unrelated to any possible contamination or
alteration in the native protein. This unique reagent
will be made available for replication studies by oth-
ers and should make a major contribution to resolu-
tion of the present controversies.

3 The first specific CRP inhibitor drug, 1,6 (bis)-
phosphocholine hexane [120] and related compounds
are being developed for clinical testing. Although they
are not orally bioavailable, have a very short half-life,
and will thus be suitable only for short-term acute
use, they should be informative about the role of CRP
following acute MI or in other acute clinical condi-
tions characterized by tissue damage and a major
acute phase CRP response. Development of new
drugs able to specifically block CRP function for sus-
tained periods will be required to establish robustly
the role of human CRP in health and disease, as
described in the final section of this review.

Randomized trials of statins in relation to CRP and
CHD

The Justification for the Use of Statins in Primary
Prevention: an Intervention Trial Evaluating Rosuvast-
atin (JUPITER) randomized about 18 000 participants
without evidence of cardiovascular disease, who had
relatively high baseline levels of CRP (2 mg L\(^{-1}\) or
greater) and relatively low levels of LDL cholesterol
(i.e. <3.36 mmol L\(^{-1}\), to receive either rosvastatin
20 mg once daily or placebo [130, 131]. Based on a
recommendation from an independent data and safety
monitoring board, the trial was stopped early in Feb-
uary 2008 because there was unequivocal evidence
of a reduction in cardiovascular morbidity and mortal-
ity in patients treated with rosvastatin compared
with placebo (http://www.cardiosource.com/rapidnews
summaries/summary.asp?SumID=318). The JUPITER
findings extend the known benefits of statins beyond
those previously demonstrated in a meta-analysis of
individual results on about 90 000 patients that
showed cardioprotective effects across a wide range
of predominantly intermediate-to-high risk individuals
[132].
The findings of the JUPITER trial, which have not yet been published in detail, have raised several questions. First, is the measurement of CRP and LDL cholesterol an optimum approach to target statin medication in individuals at low-to-intermediate risk of cardiovascular disease? This topic is considered in a subsequent section of this review in relation to measurement of CRP for the purposes of risk stratification. Second, might statins confer at least part of their benefits through lowering CRP (or related anti-inflammatory activities)? This question arises because statins are known to lower baseline CRP concentrations by about 20% [133]. Unfortunately, this hypothesis is difficult to confirm or refute reliably in randomized trials of statins because these agents so potently lower the plasma concentration of LDL cholesterol, a major known causative factor in CHD. A third question suggested by the JUPITER findings is: might the cardioprotective benefits of statins be proportionally greater in people with higher baseline CRP levels than those with lower CRP levels (i.e. effect-modification of statin effectiveness), given the possibility that statins may have relevant anti-inflammatory activities? [133–136]. The JUPITER trial itself may not be the optimum study to test this hypothesis because, as noted above, it was restricted to participants with CRP levels 2 mg L⁻¹ or greater. This question can, however, be readily addressed by measurement of CRP levels in the stored serum samples of several existing large statin trials that recruited patients without reference to baseline CRP levels. For example, such analyses of CRP are expected to emerge from the Heart Protection Study, which randomized about 20 000 high-risk patients to 40 mg simvastatin or placebo and has recorded several thousand cardiovascular outcomes during follow-up [137, 138].

**Genetic studies**

An emerging approach to evaluate any causal relevance of subtle, prolonged increases in CRP levels to CHD involves genetic epidemiology [139–141]. ‘Mendelian randomization’ experiments attempt to minimize confounding and avoid reverse association bias by measurement of common polymorphisms or haplotypes in regulatory regions of the CRP gene that have been reliably associated with differences in circulating CRP concentration (but not with any known change in CRP function). According to Mendel’s second law [142], the inheritance of genetic variants should be subject to the random assortment of maternal and paternal alleles at the time of gamete formation. So, if CRP levels actually increase the risk of CHD, then carriage of alleles (or haplotypes) that expose individuals to a long-term elevation of CRP should confer an increased risk of CHD outcomes in proportion to the difference in CRP levels attributable to the allele. Because of the randomized allocation of alleles from parents to offspring, potential confounders should be distributed amongst the genotypic classes, and any bias because of reverse causation should be avoided because genotypes are fixed at conception and not prone to modification by the onset of disease [143, 144] (Fig. 3). This approach has been applied to the study of other emerging risk markers, including plasma levels of fibrinogen and homocysteine [139, 141]. The potential limitations of Mendelian randomization analyses include the need for very large sample sizes because most genotypes have only

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**Fig. 3** Conceptual parallels between a randomized controlled trial and a hypothetical Mendelian randomization experiment to judge the causal relevance of a biomarker associated with coronary heart disease (CHD) risk.
modest effects on concentrations of biochemical markers and, at least in principle, the scope for residual confounding by unrecognized pleiotropic effects of genotypes and by developmental adaptation (‘canalization’) [140].

C-reactive protein concentration is a heritable trait [56, 145–148] and several single nucleotide polymorphisms (SNPs) within the CRP gene have now been identified (Fig. 4) [149]. Twelve common SNPs with minor allele frequency of >5% are present in a 6 kb region encompassing the gene in people of European ancestry and there are around 22 SNPs in subjects of African descent. In Europeans, the 12 SNPs are distributed amongst just four common haplotypes that account for approximately 95% of all chromosomes. A number of studies have reported consistent associations with CRP concentration of individual SNPs or subsets of SNPs, some of which have been typed for their ability to capture haplotype diversity (tagging SNPs) [108, 150–152]. For example, a combined analysis of 25 reports involving a total of around 31 000 participants of European descent has yielded a difference in CRP levels of 0.12–0.96 mg L\(^{-1}\) per allele, depending on the SNP studied, a difference that equates to between 0.3 and 0.8 standard deviations of the CRP distribution. A meta-analysis of genetic studies involving a new Bayesian method (enabling integration of information across studies that had typed a partially overlapping set of CRP SNPs) has provided evidence of four functional SNPs at the CRP locus that influence its circulating concentration [149]. Several initial studies have reported that although these CRP genotypes (and haplotypes) are clearly associated with CRP levels, they are not materially associated with any of a large panel of established or emerging cardiovascular risk markers, suggesting that these variants may well be unbiased proxies for CRP and suitable for use in Mendelian randomization analyses (Table 1) [108, 151, 153–155]. Although such studies (which have collectively involved a few thousand CHD cases) have generally reported lack of strong association of these genetic variants with CHD risk, they have not been sufficiently powerful to realistically confirm or refute any moderate effect of CRP levels on CHD such as a 10–20% increase in risk per standard deviation increase in levels. As sample size calculations suggest that at least 15 000 CHD cases and a similar number of controls may well be needed to provide

### CRP levels by CRP gene variant

<table>
<thead>
<tr>
<th>CRP Gene variant</th>
<th>No. Studies (individuals)</th>
<th>Traditional meta-analyses Estimate (95% confidential intervals)</th>
<th>Bayesian model Estimate (95% credible intervals)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs1800947 (+1059G→C)*</td>
<td>10 (11 045)</td>
<td>(-0.38 (-0.50, -0.25))</td>
<td>(-0.26 (-0.46, -0.11))</td>
</tr>
<tr>
<td>rs1205 (+2302G→A)</td>
<td>10 (16 942)</td>
<td>(-0.35 (-0.41, -0.28))</td>
<td>(-0.52 (-0.68, -0.36))</td>
</tr>
<tr>
<td>rs1417938 (+194A→T)</td>
<td>5 (7460)</td>
<td>0.17 (0.03, 0.31)</td>
<td>N/A</td>
</tr>
<tr>
<td>rs2794521 (−717A→G)</td>
<td>6 (5803)</td>
<td>0.12 (−0.10, 0.35)</td>
<td>N/A</td>
</tr>
<tr>
<td>rs1130864 (+1444C→T)</td>
<td>19 (21 674)</td>
<td>0.19 (0.14, 0.25)</td>
<td>(-0.34 (0.20, -1.17))</td>
</tr>
<tr>
<td>rs3091244 (−286C→T/A)</td>
<td>7 (7786)</td>
<td>0.20 (0.15, 0.25)</td>
<td>N/A</td>
</tr>
<tr>
<td>rs3093077 (+489T→G)</td>
<td>4 (6619)</td>
<td>0.48 (0.33, 0.62)</td>
<td>0.59 (0.37, 0.95)</td>
</tr>
<tr>
<td>rs3093059 (−757T→C)</td>
<td>3 (3475)</td>
<td>0.58 (0.37, 0.78)</td>
<td>N/A</td>
</tr>
</tbody>
</table>

**Fig. 4** The effect of eight common polymorphisms in the human CRP gene on CRP concentration (mg L\(^{-1}\)). Information was obtained from published and unpublished studies, and analyses were based on traditional meta-analysis using an additive model as well as Bayesian meta-analysis. The figure is reproduced from Fig. 4 in Verzilli et al. Am J Hum Genet, 2008; 82: 859–872, p 865. N/A refers to SNPs excluded from the Bayesian model.
such informative analyses, the CRP Coronary Disease Genetics Collaboration (CCGC) has been established [156]. This collaboration currently involves over 30 epidemiological studies with a total of about 37,000 cases of MI or coronary death and about 120,000 controls. The objective is to conduct pooled analyses of individual participant data from these studies to examine the association between relevant CRP genetic variants and CHD risk under various circumstances, thereby helping to assess causality.

**CRP and risk stratification in acute coronary syndromes**

Several scores have been proposed to assess the short-term risk for recurrent events and adverse prognosis in patients presenting with acute coronary syndromes, such as the thrombolysis in myocardial infarction (TIMI) risk score, which is based on clinical and electrocardiogram variables [157]. As noted above, several studies have reported on associations between CRP levels and recurrent cardiovascular events in patients presenting with acute coronary syndrome [7, 8, 158, 159]. In 2003, the American Heart Association/Centres for Disease Control (AHA/CDC) recommended CRP measurement as an additional marker of prognosis in patients with acute coronary syndromes [16] on top of characteristics in the TIMI risk score [160, 161]. Further studies have proposed the addition of troponin T and N-terminal pro-brain natriuretic peptide to the TIMI risk score plus CRP [162]. Interpretation of this evidence would be enhanced by pooled analyses of individual data from each of the available studies of acute coronary syndromes (which is currently outside the scope of the ERFC) and by consideration of how addition of CRP (and other markers) to risk algorithms might lead to any change in the management of such patients.

**CRP and the evaluation of long-term risk of coronary disease**

Several risk algorithms have been proposed to help stratify risk of cardiovascular disease in general Western populations, such as Framingham, PROCAM, SCORE, Reynolds and QRISK [163–167]. Each of these algorithms involves a core set of the same established risk factors (e.g. smoking, blood pressure, total cholesterol) but differ in their inclusion of various other characteristics, such as HDL-C (in Framingham), triglycerides (in PROCAM only), CRP (in Reynolds only) and body mass index or

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**Table 1** Cardiovascular risk factor distribution according to the CRP/ +1444C>T polymorphism

<table>
<thead>
<tr>
<th>Variable</th>
<th>Subjects (studies)</th>
<th>Weighted mean difference [TT – C-carriers] (95%CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>6358 (7)</td>
<td>0.17 (–0.19, 0.52)</td>
<td>0.35</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>6356 (7)</td>
<td>–0.31 (–1.78, 1.16)</td>
<td>0.68</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>6356 (7)</td>
<td>–0.70 (–1.56, 0.16)</td>
<td>0.11</td>
</tr>
<tr>
<td>Body mass index (kg m(^{-2}))</td>
<td>6359 (7)</td>
<td>0.34 (0.06, 0.62)</td>
<td>0.02</td>
</tr>
<tr>
<td>Total cholesterol (mmol L(^{-1}))</td>
<td>6115 (6)</td>
<td>–0.01 (–0.08, 0.06)</td>
<td>0.87</td>
</tr>
<tr>
<td>HDL cholesterol (mmol L(^{-1}))</td>
<td>4714 (5)</td>
<td>–0.01 (–0.04, 0.01)</td>
<td>0.32</td>
</tr>
<tr>
<td>Triglyceride (mmol L(^{-1}))</td>
<td>6041 (5)</td>
<td>–0.03 (–0.11, 0.06)</td>
<td>0.51</td>
</tr>
<tr>
<td>Fibrinogen (g L(^{-1}))</td>
<td>5656 (4)</td>
<td>0.03 (–0.02, 0.08)</td>
<td>0.20</td>
</tr>
<tr>
<td>Glucose (mmol L(^{-1}))</td>
<td>1913 (3)</td>
<td>–0.01 (–0.13, 0.12)</td>
<td>0.90</td>
</tr>
<tr>
<td>Alcohol intake (U per week)</td>
<td>4549 (4)</td>
<td>0.01 (–1.38, 1.39)</td>
<td>0.99</td>
</tr>
<tr>
<td>Current smoking*</td>
<td>6132 (6)</td>
<td>1.00 (0.84, 1.20)</td>
<td>0.98</td>
</tr>
<tr>
<td>C-reactive protein (mg L(^{-1}))</td>
<td>4659 (6)</td>
<td>0.68 (0.31, 1.10)</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

CRP, C-reactive protein.
*For this variable instead of the WMD, the value reported is the weighted odds ratio.
Data source: Table taken from Casas et al., 2006 [154].
Comparisons are made between TT homozygotes and C-allele carriers.
markers of socio-economic status (in QRISK only). As CRP levels display relatively limited biological variability (outside acute phase fluctuations) and can be accurately assessed using assays with internationally agreed standards, their measurement for risk prediction has been regarded as particularly practicable by some workers (although such advantages also apply to the measurement of some other nonspecific inflammatory markers that may be measured more cost effectively than CRP such as the leucocyte count and serum albumin). In 2003, a report of the AHA/CDC recommended that ‘CRP may be used at the discretion of the physician as part of a global coronary risk assessment in adults without known cardiovascular disease’, concluding that a CRP value above a cut point of 3 mg L\(^{-1}\) was indicative of high risk [16]. The report acknowledged, however, the need for further research to address several uncertainties in the evidence. By contrast, European authorities have not recommended measurement of CRP to enhance cardiovascular risk assessment [17].

Part of the uncertainty stems from the fact that although many published prospective studies have commented on the potential value of CRP in risk prediction, they have often reported only measures of association (e.g. odds ratios, hazard ratios), which do not directly address the issue of the utility of a marker in prediction or stratification. Furthermore, even studies that have involved statistics relevant to the assessment of risk prediction have involved different metrics, including measures of discrimination (e.g. the C index [168, 169] and the measure D [170], with the former related to the area under the receiver operating characteristic curve) and reclassification methods that aim to summarize the potential of a marker to re-assign individuals into more appropriate risk groups [171]. Each of these approaches may impart somewhat different information. As recommended by the US National Heart Lung and Blood Institute 2006 workshop report on CRP, further work is needed to compare and contrast the strengths and limitations of each of these approaches [172] to enable their use in the multiple study situation (as in the ERFC database) and to incorporate health economic analyses to help judge the value of any such measurements (http://www.nhlbi.nih.gov/meetings/workshops/crp/report.htm).

CRP in acute ischaemic tissue damage

There is always a substantial CRP acute phase response following acute MI [173, 174]. An association between sustained high values of CRP following acute MI and adverse outcomes was first reported in 1982 [175] and subsequent large studies have shown that increased peak and postinfarct CRP concentrations are significantly associated with increased incidence of cardiac complications including heart failure and cardiac death, apparently independently of other predictors [14, 159, 176–181]. Acute MI is invariably associated with acute inflammation around the lesion [1] and complement makes a substantial contribution to the size of experimental acute myocardial infarcts produced by coronary artery ligation in mammals [182], including old world primates [183]. All human acute MI lesions contain CRP and activated complement co-deposited in and around the infarct [2, 184]. The rat provides an excellent model for testing the possible pathogenicity of human CRP in this situation because although rats have abundant CRP, their protein does not activate rat complement whereas human CRP potently activates both rat and human complement [185]. Effects of human CRP injected into rats can therefore reflect what human CRP may do in humans. Indeed, administration of authentic pure human CRP to rats after they have undergone coronary artery ligation or ischaemia-reperfusion injury causes significant worsening of cardiac function and increased mortality, which reflect increased infarct size [18]. The effect is specific for human CRP and absolutely complement-dependent, and human CRP is co-deposited with rat complement in the infarct [18]. The same pathogenic effect of human CRP is seen in the rat model of cerebral infarction produced by middle cerebral artery occlusion [186]. The adverse effect of human CRP in the acute MI situation is completely abrogated by administration of the novel small molecule specific CRP inhibitor drug, 1, 6 (bis)-phosphocholine hexane [120] (Fig. 5). Importantly, this drug has
no effect at all in rats subjected to coronary artery ligation which do not receive human CRP and is thus not cardioprotective *per se* but only by virtue of its inhibition of CRP [120].

The likely mechanism of CRP pathogenicity is therefore binding of abundant CRP to the ligands exposed in dead and damaged cells, triggering substantial complement activation with release of chemotactic factors and opsonization of cells in and around the lesion, leading to enhanced infiltration by inflammatory cells and consequent bystander damage. The terminal complement sequence may also directly kill cells which would otherwise survive and the end result is death of more myocardial tissue than would be killed by ischaemia alone. Development is now in progress towards clinical testing of CRP inhibition in patients with acute MI.

**Conclusion**

Until specific CRP inhibitor drugs suitable for long-term administration become available, the question of whether low level chronically increased CRP concentration is a risk factor for CHD, may be addressed by pooled analyses of data from prospective studies of circulating CRP levels and from studies of CRP genetic variants. Uncertainties related to the separate issue of risk prediction in the general population can also be addressed in the near future by collaborative approaches involving large combined datasets. Meanwhile, it is very important to distinguish clearly between (i) the potential pathogenicity of high circulating CRP concentrations in individuals with a substantial volume of damaged tissue in whom CRP binding to exposed ligands and consequent major complement activation can exacerbate injury and (ii) the possible actions of baseline or rather modestly increased CRP concentrations which together with complement may assist in clearance and resolution of minor tissue injury and thus be beneficial. The experimental evidence from animals indicates that human CRP can definitely be pathogenic after ischaemic infarction, but any relevance to the slow process of atherogenesis remains unproved.

**Conflict of interest statement**

Pentraxin Therapeutics Ltd is a UCL spin out company of which Professor Pepys is the founding director and which owns his proprietary knowledge and patents on CRP as a therapeutic target and bis-phosphocholine compounds as CRP inhibitor drugs.

**Acknowledgements**

The work of Professor Pepys reported here is supported by the Medical Research Council (Programme Grant PG 7900510) and the National Heart Lung and...
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