Chapter 2

<table>
<thead>
<tr>
<th>Review of Literature</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1. Pathophysiology of atherosclerosis</td>
</tr>
<tr>
<td>2.2. Traditional risk factors</td>
</tr>
<tr>
<td>2.3. Nontraditional risk markers</td>
</tr>
<tr>
<td>2.4. Ideal characteristics of a serum risk marker and high-sensitivity C-reactive protein</td>
</tr>
<tr>
<td>2.5. Synthesis and Metabolism of C-reactive protein</td>
</tr>
<tr>
<td>2.6. Measurement of C-reactive protein and Normal Values</td>
</tr>
<tr>
<td>2.7. Population Distribution</td>
</tr>
<tr>
<td>2.7.1. Gender</td>
</tr>
<tr>
<td>2.7.2. Age</td>
</tr>
<tr>
<td>2.7.3. Race/ethnicity</td>
</tr>
<tr>
<td>2.8. Some conditions with elevated levels of C-reactive protein</td>
</tr>
<tr>
<td>2.9. Structure</td>
</tr>
<tr>
<td>2.10. Function</td>
</tr>
<tr>
<td>2.11. Effects of high sensitivity C-reactive protein on atherosclerosis</td>
</tr>
<tr>
<td>2.11.1. Complement activation</td>
</tr>
<tr>
<td>2.11.2. Interaction with cell surface receptors</td>
</tr>
<tr>
<td>2.11.3. Thrombosis</td>
</tr>
<tr>
<td>2.11.4. Cellular modulation, recruitment and activation</td>
</tr>
<tr>
<td>2.11.5. Expression of inflammatory mediators: cytokines, chemokines and adhesive molecules</td>
</tr>
<tr>
<td>2.11.6. Nitric oxide expression</td>
</tr>
<tr>
<td>2.11.7. Apoptosis</td>
</tr>
<tr>
<td>2.11.8. Lipids</td>
</tr>
<tr>
<td>2.11.9. Neuronal effect of C-reactive protein</td>
</tr>
<tr>
<td>2.12. Major prospective studies investigating high-sensitivity C-reactive protein as a marker of future cardiovascular risk among apparently health individuals</td>
</tr>
<tr>
<td>2.13. Major studies of the association between high-sensitivity C-reactive protein and stroke</td>
</tr>
</tbody>
</table>
2.14. Therapeutic Interventions

2.14.1. Behavioral interventions

2.14.1.1. Weight loss

2.14.1.2. Physical activity

2.14.1.3. Smoking cessation

2.14.1.4. Dietary factors

2.14.1.4. a. ω-3 fatty acids

2.14.1.4. b. Low-fat, low-cholesterol diet

2.14.1.4. c. Low glycemic load

2.14.1.4. d. Vitamins

2.14.1.4. e. Alcohol use

2.15. Pharmacologic interventions

2.15.1. Lipid-modulating agents

2.15.2. Statins

2.15.3. Fibrates

2.15.4. Niacin

2.15.5. Aspirin and other antiplatelet agents

2.15.6. Antihyperglycemic agents

2.16. Pleiotropic effects of Statins

2.16.1. Statins and cholesterol

2.16.2. Statins and isoprenylated proteins

2.16.3. Statins and endothelial function

2.16.4. Statins and anti-oxidant effects

2.16.5. Statins and endothelial progenitor cells

2.16.6. Statins and smooth muscle proliferation

2.16.7. Statins and platelet function

2.16.8. Statins and plaque stability

2.16.9. Statins and vascular inflammation

2.16.10. Statins and its effects on the myocardium

2.16.11. Statins and ischemic stroke

2.16.12. Statins and dementia

2.16.13. Statins and nephropathy

2.16.14. Statins and autoimmune disease

2.16.15. Statins and sepsis

2.16.16. Statins and gastrointestinal diseases

2.16.17. Statins bone remodeling osteoporosis

2.16.18. Statins and macular degeneration

2.16.19. Statins and non-cardiac vascular surgery

2.17. The benefits of Statin therapy

2.17.1. Secondary prevention trials

2.17.2. Primary prevention trials
CHAPTER 2 REVIEW OF LITERATURE

This section provides a selective review of pathophysiology of atherosclerosis, risk factors, hsCRP and statins. Atherosclerotic vascular disease (AVD) is the cause of the majority of chronic arterial diseases such as peripheral arterial disease (PAD), CHD and stroke leading to reduction in vascular reserve. Advanced atherosclerosis is directly linked to cause thrombotic complications such as acute myocardial infarction (AMI), unstable angina and stroke (Libby and Ridker, 2004).

2.1. Pathophysiology of atherosclerosis

Atherosclerosis and related vascular complications (Fig. 2.1) result in more than 19 million deaths annually, with CHD as the leading cause of death. The complex process of atherosclerosis involves several pathogenic cellular mechanisms, such as endothelial dysfunction, vascular smooth muscle cell activation and apoptosis, vascular remodelling, inflammation, accumulation of oxidatively modified lipids and matrix components in the arterial vessel wall, thus leading to plaque formation and progression (Libby, 2002).

This complex process can be triggered and promoted by several risk factors, such as hypertension, diabetes mellitus, hypercholesterolemia, obesity and cigarette smoking. Clinical studies and animal experiments show that dyslipidemia causes atheroma formation. During early atherogenesis, accumulation of excess oxidatively modified low-density lipoprotein (oxLDL) in atheroma due to hypercholesterolemia can trigger endothelial cell (EC) dysfunction and leukocyte invasion (monocytes, neutrophils, T cells) into the arterial wall (Steinberg et al., 1989). Atherosclerotic plaque formation starts with activation of arterial ECs and over expression of leukocyte adhesion proteins such as vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1), enhancing leukocyte attachment (e.g. monocytes) (Libby, 2002). Increased expression of monocyte chemoattractant protein-1 and -3 (MCP-1 and MCP-3) promotes migration of monocytes into the intima of the vessel wall, where differentiation of monocytes into macrophages is mediated by colony stimulating factors.
Late stage vascular complications

Risk Factors: hypercholesterolemia, hypertension, diabetes, age, obesity, smoking

Atherosclerosis

Healthy artery
- Endothelial dysfunction
- Inflammation
- Vascular remodelling

Plaque formation
- Rupture-prone plaque
- Ruptured plaque and thrombosis

PAD
- Intermittent claudication

CAD
- Acute myocardial infarction
- Stable angina pectoris
- Unstable angina pectoris

CVD
- TIA
- Stroke

Fig. 2.1 Progression of atherosclerosis and possible late stage vascular complications

Early atherosclerotic lesions are initiated by endothelial dysfunction and increased adhesion and invasion of circulating inflammatory cells. Subsequently, these events trigger plaque formation over a time period of months and years. At late stages plaque rupture can lead to vascular occlusive thrombosis formation and platelet embolism, being crucial events for severe vascular late stage complications in the peripheral, coronary or cerebral arterial system. Several risk factors can further accelerate formation and progression of atherosclerotic lesions and its resulting complications (PAD, peripheral artery disease; CAD, coronary artery disease; CVD, cerebrovascular disease).

(Kerstin and Ivo, 2005)
These atheroma located macrophages express scavenger receptors to engulf and accumulate lipoproteins (e.g. oxLDL), thereby transforming into foam cells, a characteristic feature during early atherosclerosis. The latter secrete various pro-atherogenic factors, such as tumour necrosis factor -α (TNF-α), interleukin-1β (IL-1β), macrophage colony-stimulating factor (M-CSF), matrixmetalloprotease-2 and -9 (MMP-2 and MMP-9) and release reactive oxygen species (ROS), including superoxide anion, hydrogen peroxide and peroxynitrite, all contributing to lipid accumulation in atherosclerotic lesions. Other important sources of ROS are vascular smooth muscle cells (VSMCs), ECs and fibroblasts. The two best-characterized effects of ROS include oxidation of LDL cholesterol and scavenging of endothelial produced nitric oxide (NO), the most potent vasodilator, besides DNA damage and increased production of inflammatory transcription factors, such as NF-kB.

Oxidative stress further induces expression of VCAM-1 (possibly via NF-kB expression) and MCP-1 and MCP-3 on ECs and decreased endothelial NO-synthase (eNOS) expression. These are effects, which subsequently favour smooth muscle cell proliferation and migration into the neointima, remodeling of the extracellular matrix (ECM) and monocyte invasion. ECs play an important role by secretion of autocrine and paracrine factors beside NO, such as prostaglandins, endothelin, angiotensin II and MMPs. The latter contributes to the degradation of ECM structures, thereby reducing plaque stability and promoting plaque rupture by weakening of the thin fibrous cap, a late complication of atherosclerosis. Activated VSMCs are mainly responsible for production of large amounts of ECM proteins including proteoglycans, collagen, elastin, fibronectin, laminin, vitronectin and thrombospondin.

Once the plaque enlarges to >40% of the vessel area, the artery ceases enlargement (outward remodelling during early stages) and the lumen narrows as the plaque is growing. When lesions have developed, endothelial dysfunction seems to favour progression of clinical processes, such as production of growth factors, cytokines and proteases that decrease plaque stability and render plaques susceptible to rupture. These vulnerable plaques are characterized by a large lipid core, a thin fibrous cap and infiltrated macrophages at the cap surface and accumulation of apoptotic VSMCs,
driven into apoptosis by Fas/FasL mediated pathway with FasL released by macrophages (Boyle, 2005) and over expression of growth factors such as connective tissue growth factor (CTGF) (Cicha et al., 2005). Inflammatory mediators found in atheroma include IL-1β, TNFa and CD154, augmenting MMP expression. Degradation of the cap is mediated by MMPs, which are produced by activated smooth muscle cells, ECs and macrophages, culminating in plaque rupture, thereby releasing the lipid core including tissue factor and further attracting and activating circulating platelets, which adhere rapidly to the vessel wall through platelet glycoproteins.

Activated platelets also secrete platelet-derived growth factor (PDGF) and transforming growth factor-β (TGF-β), of which latter is a potent stimulus for VSMC-mediated collagen synthesis, promoting plaque growth (Fig. 2.1). Plaque rupture favours occlusive thrombosis formation (e.g. acute cardiovascular events) (Davies, 1996) and arterio-arterial platelet embolism. The latter is thought to be one of the primary mechanisms for TIA and stroke in patients with significant stenosis of the posterior cerebral artery and carotids interna (Pessin et al., 1977). Patients affected by intracranial atherosclerosis have an elevated risk for recurrent major vascular events such as TIA and stroke.

2.2. Traditional risk factors

Although longstanding research has identified variables such as hypertension and hypercholesterolemia as traditional CVD risk factors, many have reported their absence in a substantial portion of individuals experiencing clinical vascular events. In fact, up to half of those having their first coronary event does not have traditional CVD risk factors (Braunwald, 1997). Although these findings may not apply to all populations, researchers from the FHS report that 50% of those with CHD had levels of TC ≤240 mg/dl, and 20% had TC <200 mg/dl (Kannel, 1995). Recent data from the Women’s Health Study (WHS) showed those three quarters of CVD events occurred in 27,939 women without a high level of LDL cholesterol (<160 mg/dl), and 45% occurred in women with normal LDL cholesterol (<130 mg/dl) (Ridker et al., 2002). When multiple large studies of CVD were recently reviewed, as one would expect, most
subjects had one or more traditional risk factors (Khot et al., 2003). However, one fifth had none of the traditional risk factors. Furthermore, among cohort participants who did not develop CHD, the rates of traditional cardiovascular risk factors were also quite high, supporting the idea that there must be other factors influencing the development of CVD end points (Greenland et al., 2003). Given these findings, recent research has focused on ways of augmenting our capacity to predict CVD.

2.3. Nontraditional risk markers

The epidemiological and basic science search for greater understanding of the etiology of CVD has produced multiple serum markers as candidates for indicating "nontraditional" risk. Several are part of the process of inflammation - a process, now understood to be central to atherosclerotic disease (Libby et al., 2002). Candidates have included homocysteine, coagulation markers such as fibrinogen, plasminogen activator inhibitor-1 (PAI-1), D-dimer, and thrombin/antithrombin III complex; and various inflammatory markers such as serum amyloid A (SAA), interleukin (IL), matrix metalloprotease (MMP), adhesion molecule, and CRP. Although many of these markers show promise, most are not used clinically, and the predictive power of many has not been confirmed.

2.4. Ideal characteristics of a serum risk marker and high-sensitivity C-reactive protein

To make the leap from research to clinical practice, an ideal marker of CVD risk must meet certain criteria, as outlined in Table 2.1. CRP has many of these qualifying characteristics. CRP levels detected by a hsCRP have been associated with cardiovascular risk in prospective epidemiological studies. HsCRP also adds to predictive models of CVD, such as the Framingham Risk Score, and there are standardized assays for its measurement (Kimberly et al., 2003). Finally, interventions that decrease cardiovascular risk also decrease hsCRP levels, and prospective trials are underway to assess interventions that specifically target hsCRP with the purpose of lowering cardiovascular risk.
### Table 2.1 Serum cardiovascular disease risk marker characteristics

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<th>Sl. No.</th>
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<tr>
<td>1.</td>
<td>Demonstrates biological plausibility</td>
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<td>2.</td>
<td>Predicts CVD across populations according to various research</td>
</tr>
<tr>
<td></td>
<td>methodologies (epidemiological, prospective, and population)</td>
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<tr>
<td>3.</td>
<td>Acts independently of other risk factors</td>
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<td>4.</td>
<td>Increases the ability to predict disease above the Framingham Risk Score</td>
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<tr>
<td>5.</td>
<td>Exhibits relative biological stability over time</td>
</tr>
<tr>
<td>6.</td>
<td>Has widely available assays that are reproducible and standardized</td>
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<tr>
<td>7.</td>
<td>Modifiable by relatively available interventions</td>
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Several other inflammatory makers have features that might make them attractive for use as serum risk markers; however, to date, none has come as close to meeting criteria for a useful indicator of CVD risk as hsCRP. PAI-1 and fibrinogen relate closely to thrombosis—the process that likely precipitates clinical CVD events. Interleukin-6 (IL-6) is an early stimulator of the inflammatory process, which is another potential risk marker. However, fibrinogen and IL-6 exhibit diurnal changes, and plasma PAI-1 measurement may be affected by PAI-1 release from platelets collected as part of routine blood sampling. Furthermore, for most of these markers, such as fibrinogen and IL-6, assays are not standardized.

2.5. Synthesis and Metabolism of C-reactive protein

The discovery and naming of CRP was done by Tillett and Francis, (1930) and Abernethy and Avery, (1941) who described that reactivity of CRP was calcium-dependent binding to C-polysaccharide of the cell wall of Streptococcus pneumonia. CRP is a cyclic, homogeneous molecule, composed of five identical noncovalently bound subunits (Osmand et al., 1977; Woo et al., 1985) having a molecular weight of 118,000 D (Volanakis et al., 1978). CRP is synthesized and secreted mainly by hepatocytes (Hurhimann et al., 1966) in response to cytokines such as IL-6. Induction of CRP in some models requires IL-6 and either interleukin-1 (IL-1) or TNF-α (Mackiewicz et al., 1991). CRP is primarily derived via IL-6 dependent hepatic biosynthesis. Glucocorticoids enhance the stimulatory effects of cytokines on the production of acute phase proteins (Baumann et al., 1987). Insulin, on the other hand, decreases their effects on the production of some acute phase proteins (Campos et al., 1994). Efficiency of secretion of CRP is greatly increased during acute-phase response (Yue et al., 1996). During an acute phase response the rate of secretion into the plasma may be relatively constant and the concentration achieved is dependent upon the duration of stimulation and resulting response by the liver (Yen-Watson and Kushner, 1974). Newly synthesized CRP is rapidly secreted by liver cells and hence difficult to show within the cytoplasm. Colchicine blocks secretion but not synthesis and would increase the accumulation of CRP intracellularly (Kushner and Feldmann, 1978). Both production and clearance of CRP is very rapid. Endotoxin treatment doubles the levels of CRP within 6–8 hours and
half disappearance time is 11-15 hours (Yen-Watson and Kushner, 1974). In man, the levels of CRP rise is as much as 1000-fold from the reference range within 1 to 2 days after active tissue damage processes and fall rapidly with disappearance of the stimuli (Claus et al., 1976). The average plasma half-life of CRP is 19 hours (Yeh et al., 2001). CRP response is not apparently affected by any of the commonly used anti-inflammatory or immunosuppressive drugs including steroids, unless these affect the activity of the underlying disease process.

CRP at low ionic strength undergoes calcium-dependent binding to polyanions such as heparin, nucleic acids, and dextran sulphate (Gotschlich and Edelman, 1967). CRP also binds to polycations such as L-lysine, myelin basic protein, leukocyte cationic proteins, and histones: but this binding is noncalcium-dependent (Siegel et al., 1975). CRP binds with lipoproteins of very low-density (VLDL) and LDL cholesterol fraction in the serum, which affects the proteins precipitability and electrophoretic mobility.

2.6. Measurement of C-reactive protein and Normal Values

There are various methods for detection of CRP including precipitation by C-polysaccharide of Pneumococcus (Tillett and Francis, 1930), crystallization (McCarty, 1947), tube precipitation using anti-CRP (MacLeod and Avery, 1941), complement fixation (Rapport and Graf, 1956), Ouchterlony (Nilsson, 1968), latex agglutination (Singer et al., 1957), radioimmunoassay (Claus et al., 1976), radial immunodiffusion (Nilsson, 1968) and fluorescence polarization (Claus et al., 1976). CRP is usually measured in clinical laboratories by either immunonephelometric or immunoturbidimetric assay. The current methods are generally reproducible, fully automated and capable of measuring CRP with detection limit of 3-5 mg/L. Such traditional assays do not have high sensitivity. Investigators and manufacturers have continued to use immunochemical techniques to measure hsCRP. Various approaches including the labeling of anti-CRP, antibodies with either an enzyme (ELISA) or a fluorescent compound, and attaching the antibodies, either monoclonal or polyclonal, to polystyrene heads have been made to measure CRP (Kapyaho et al., 1989; Ledue et al., 1998; Eda et al., 1999; Borque et al., 2000).
Concentrations of hsCRP as low as 0.15 mg/L can be measured. It has been reported by Roberts et al., (2000) that not all hsCRP assays possess a similar sensitivity or lower limit of quantification. Because of this variation in sensitivity and lower limit of quantification, a single CRP assay method should be used that is capable of measuring low and high concentrations: hsCRP is a very novel biochemical marker for the prediction of first and recurrent coronary events (Rifai and Ridker, 2001). Rifai et al., (1999) have shown that the latex method is as efficacious as the ELISA method in classifying patients into cutoff.

Points established by prospective studies for risk stratification for coronary and cerebrovascular disease. It has been reported that hsCRP has a degree of stability measurement that is similar to that of TC (Ockene et al., 2001). There is no diurnal variation of hsCRP concentrations (Meier-Ewert et al., 2001), and hence collection of blood for hsCRP can be performed without concern for time of collection. HsCRP can be assayed in plasma or serum. Plasma should be separated by low speed (2600 g) centrifugation for 4 minutes at 4° C and quickly frozen to - 70° C till assay.

Most normal subjects have plasma CRP concentrations of 2 mg/L or less (Gabay and Kushner, 1999). However, the normal values reported by various investigators vary. Median levels were 0.26 mg/dL with a range of 0.10 to 0.61 mg/dL in control subjects (Pradhan et al., 2001). Ridker et al., (1997) reported a mean value of plasma CRP of 1.1 mg/L with a median value of 1.13 mg/L in apparently healthy men. Women free of cardiovascular events had a median value of CRP of 0.28 mg/dL with interquartile range of 0.11 to 0.55 mg/dL (Ridker et al., 2000). When two methods of measurement of hsCRP in control subjects were compared, it was observed that the values for hsCRP with ELISA and Latex methods were 0.99 mg/L and 1.2 mg/L respectively (Rifai et al., 1999).

Elevated levels of CRP (>3 mg/L) are found in <10% of normal subjects, in <20% of patients with chronic stable or variant angina, but in >65% of patients with unstable angina and in >90% of patients with acute infarction proceeded by unstable angina (Liuzzo et al., 1994; Biasucci et al., 2001; Fleck and Myers, 1985). Using population-based
quantiles, the relative risk/risk ratio (RR) of suffering a future cardiovascular event increased 26% for men and 33% for women (Ridker, 2001). The median value of hsCRP was 0.16 mg/L and ranges of hsCRP for those with lowest (quintile 1) to highest (quintile 5) vascular risk were 0.01 to 0.069, 0.07 to 0.11, 0.12 to 0.19, 0.20 to 0.38 and >0.38 mg/dL respectively. Clinically these quintiles can be considered to represent individuals with low, mild, moderate, high, and highest RRs respectively. Use of the hsCRP is essential when CRP is used for risk assessment. Low risk is defined as hsCRP <1 mg/L; average risk 1.0–3.0 mg/L and high risk as 3.0–10 mg/L. If hsCRP is >10 mg/L, the test should be repeated and patient examined for sources of infection or inflammation (Fleck and Myers, 1985).

2.7. Population distribution

2.7.1. Gender

As shown in Table 2.2, data from several large United States and European cohorts indicate that the distribution of circulating hsCRP concentrations, measured by high-sensitivity assays, appears comparable among men and women not using postmenopausal hormone replacement therapy (HRT), (Rifai and Ridker, 2003; Imhof et al., 2003) with the 50th percentile for both sexes being approximately 1.5 mg/L. HsCRP levels are higher in women who use oral HRT than in women who do not, (Ridker et al., 1999; Cushman et al., 1999; Ridker et al., 2002; Pradhan et al., 2002; Albert et al., 2003). This suggests that elevations in hsCRP may be at least partly responsible for the increased risk of thrombotic events associated with oral HRT use observed in randomized trials such as the Women’s Health Initiative (Manson et al., 2003; Wasertheil-Smoller et al., 2003).

2.7.2. Age

Most studies report only a modest relation between age (range, 18-88 years) and serum hsCRP concentrations (Ledeu and Rifai, 2003; Rifai and Ridker, 2003; Imhof et al., 2003). In the WHS, for example, median hsCRP concentrations for individuals aged 45 to 54, 55 to 64, 65 to 74, and 75 years or older were 1.31, 1.89, 1.99, and 1.52 mg/L, respectively (Rifai and Ridker, 2003).
Table 2.2 Gender-specific population distributions of high-sensitivity C-reactive protein

<table>
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<td>5th</td>
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<tr>
<td>American women †</td>
<td>0.2</td>
</tr>
<tr>
<td>American men</td>
<td>0.3</td>
</tr>
<tr>
<td>European women †</td>
<td>0.3</td>
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<tr>
<td>European men</td>
<td>0.3</td>
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† Only women not using hormone replacement therapy

(Rifai and Ridker, 2003; Imhof et al., 2003)
2.7.3. Race/ethnicity

Information on the distribution of hsCRP concentrations in nonwhite populations is sparse. In the nationally representative National Health and Nutrition Examination Survey (NHANES) dataset, there were no significant differences in the distributions of hsCRP concentrations among European-American, African-American, and Mexican-American men (Ford et al., 2003). Moreover, a comparable hsCRP distribution was seen in Japanese men (Yamada et al., 2001). In contrast, the NHANES data suggest that hsCRP concentrations are higher in Mexican-American women than in European-American women (Albert et al., 2004). In the WHS, African-American women had higher hsCRP levels and Asian-American women had lower hsCRP levels than their white and Hispanic-American counterparts (Ford et al., 2004). Although additional studies on the distribution and prognostic ability of hsCRP in other than white populations are clearly needed, existing data are insufficient to support the exclusion of any racial or ethnic group from current guidelines for hsCRP testing.

2.8. Some conditions with elevated levels of C-reactive protein

Elevated levels of serum CRP have been widely considered to be nonspecific but sensitive markers of acute inflammatory response. A number of epidemiological studies show that CRP is an important risk factor for atherosclerosis and CHD (Shah, 2000). CRP is elevated in both acute and chronic inflammatory disease and conditions. Acute phase response is a generalized reaction of the body to inflammation (Fleck and Myers, 1985). CRP is very sensitive, especially to bacterial infections such as Pneumococcal pneumonia, Staphylococcal osteomyelitis, acute rheumatic fever and bacterial endocarditis (Tillett and Francis, 1930; Pepys and Baltz, 1983). Plasma concentrations increase within 10 hours of the onset of acute inflammation and normalize rapidly, usually within one week (Pepys and Baltz, 1983). The rapid rise in the circulating CRP from normal levels of less than 1 µg/ml by as much as 3,000-fold in response to acute tissue injury, inflammation, or infection is very dramatic and falls rapidly if injury or inflammation is terminated (Anderson and McCarty, 1950). CRP concentration rises rapidly in
incremental range at over 100 µg/ml from normal of 0.58 µg/ml (median) 24-48 hours after elective surgery (Claus et al., 1976).

CRP levels rose precipitously within hours of post cholecystectomy and subsided after a few days in the absence of inflammation or necrosis (Gewurz, 1983). It is elevated in AMI (de Beer et al., 1982; Pietila et al., 1993) and ischemia (Berk et al., 1990). There was an excellent correlation between peak levels of CRP and CK-MB in MI (de Beer et al., 1982). In some chronic inflammatory diseases, plasma CRP levels are elevated from moderate to high levels when active. CRP levels are elevated in systemic lupus erythematosus (Becker et al., 1980), rheumatoid arthritis (Amos et al., 1977), juvenile chronic arthritis (Still's disease, especially with polyarthritis and systemic presentation) (Pepys, 1981), ankylosing spondylitis (Cowling et al., 1980), Reiter's syndrome, psoriatic arthropathy, and arthritis following jejuno-ileal bypass (Pepys, 1981).

Patients with polyarteritis nodosa, disseminated systemic vasculitis, cutaneous vasculitis (Parish, 1976), Wegener's granulomatosis, polymyalgia rheumatica (Pepys, 1981) and Behcet's syndrome (Lehner and Adinol, 1980), likely have high CRP levels. It is also elevated in Crohn's disease (Pepys, 1982), ulcerative colitis (Pepys, 1982), scleroderma (Pepys, 1981), dermatomyositis (Pepys, 1982) osteoarthritis (Spector et al., 1997), and neoplastic disease (Weinstein et al., 1984). High levels of hsCRP have been reported in smokers (Tracy et al., 1997), obesity (Yudkin et al., 1999), and diabetes (Pradhan et al., 2001).

2.9. Structure

CRP belongs to the pentraxin family of calcium dependent ligand-binding plasma proteins, the other member of which in humans is serum amyloid P (SAP). The human CRP (hCRP) molecule (Mr 115,135) is composed of five identical nonglycosylated polypeptide subunits (Mr 23,027), each containing 206 amino acid residues. The protomers are noncovalently associated in an annular configuration with cyclic pentameric symmetry as shown in Fig. 2.2 (Thompson et al., 1999). Each protomer has the characteristic "lectin fold," composed of a two-layered β-sheet with flattened jellyroll topology.
Fig. 2.2 Molecular structure and morphology of human C-reactive protein

(a) Negatively stained electron micrograph showing the typical pentameric disc-like structure face-on and side-on (arrows).
(b) Ribbon diagram of the crystal structure, showing the lectin fold and the two calcium atoms (spheres) in the ligand-binding site of each protomer.
(c) Space-filling model of the CRP molecule, showing a single phosphocholine molecule located in the ligand-binding site of each protomer.

(Thompson et al., 1999)
The ligand binding site, composed of loops with two calcium ions bound 4 Å apart by protein side-chains, is located on the concave face. The other face carries a single helix (Fig. 2.2). The pentraxin family, named for its electron micrographic appearance from the Greek *penta* (five) *ragos* (berries), is highly conserved in evolution, with homologous proteins throughout the vertebrates and even in the phylogenetically distant arachnid, *Limulus polyphemus*, the horseshoe crab. SAP, named for its universal presence in amyloid deposits, is a constitutive, non-acute-phase plasma glycoprotein in humans and all other species studied, except the mouse, in which it is the major acute-phase protein. In contrast, mouse CRP is a trace protein whose concentration increases only modestly, to a maximum of about 2 mg/l, during the acute-phase response. No mouse CRP knockout, to our knowledge, has yet been made, and *in vivo* work on CRP function has largely been confined to passive administration of exogenous, heterologous CRP or to mice transgenic for rabbit or hCRP.

These artifactual heterologous systems may not provide physiologically relevant information. Despite the evolutionary conservation of sequence, subunit organization, and protein fold, there are considerable variations between CRPs of different species with respect to fine ligand-binding specificity, presence and nature of glycosylation, protomer assembly, capacity to precipitate and aggregate ligands, base-line circulating concentrations, behavior as acute-phase proteins, and capacity to activate autologous complement (Oliveira et al., 1980; de Beer et al., 1982; Baltz et al., 1982). Indeed, only hCRP has been rigorously shown to activate complement in isologous serum. These differences command extreme caution in extrapolating from animal models to humans.

2.10. Function

The main biologic function of CRP is determined by its ability to recognize pathogens and damaged cells of the host and to mediate their elimination by recruiting the complement system and phagocytic cells. These are summarized in the Table 2.3. Phosphocholine (Pch), the principal CRP ligand, is widely distributed in teichoic acids, capsular carbohydrates, and lipopolysaccharides of bacteria and other microorganisms.
Table 2.3 Biological properties and functions of high-sensitivity C-reactive protein

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<th>Biological property</th>
<th>Action</th>
<th>Function</th>
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<tr>
<td>Calcium-dependent binding of phosphocholin and other phosphate esters</td>
<td>Activation classical complement via C1q Binding to Fcg receptors Opsonophagocytosis</td>
<td>Innate host defense against microorganisms (bacteria, fungi)</td>
</tr>
<tr>
<td>Calcium-dependent binding of histones, snRNP, chromatin</td>
<td>Activation classical complement via C1q Binding to FcgRI/FcgRIIa receptors Opsonophagocytosis</td>
<td>Non-inflammatory clearance / processing of nuclear host material</td>
</tr>
<tr>
<td>Calcium-dependent binding of cellular host material</td>
<td>Activation classical complement via C1q Binding to FcgRI/FcgRIIa receptors Opsonophagocytosis</td>
<td>Non-inflammatory clearance / processing apoptotic cells</td>
</tr>
<tr>
<td>Binding of polycations</td>
<td>Modulatory effect on neutrophils</td>
<td>Modulatory effect on the inflammatory process</td>
</tr>
<tr>
<td>Expression of ICAM-1, VCAM-1 and E-selectin on endothelial cells by mCRP</td>
<td>Enhancing of adhesion and recruitment of monocytes and lymphocytes</td>
<td>Enhancing vascular wall inflammation</td>
</tr>
<tr>
<td>Binding of CRP/mCRP to FcgRIIib receptors of neutrophils</td>
<td>Shedding L-selectin on neutrophils</td>
<td>Prevention of adhesion of neutrophils to endothelial cell</td>
</tr>
</tbody>
</table>

ICAM-1, intercellular adhesion molecule-1; VCAM-1, vascular cell adhesion molecule-1; mCRP, modified CRP
Its presence has been reported in *Streptococcus pneumoniae* (Brundish and Baddiley, 1968), *Haemophilus influenzae* (Weiser et al., 1997), *Pseudomonas aeruginosa*, *Neisseria meningitides* and *Neisseria gonorrhoeae* (Serino and Virji, 2000), *Proteus morganii* (Potter, 1971), and *Aspergillus fumigatus* (Longbottom and Pepys, 1971). The calcium-dependent binding of CRP to Pch results in a CRP-Ca-PCh complex. This ligand complexed CRP is recognized by C1q and leads to the formation of C3 convertase and, thus, to activation of the classical pathway of the human complement (Agrawal et al., 2001; Wolbink et al., 1996). The activation of the classical pathway leads to opsonization and phagocytosis of phosphocholine-containing microorganisms via the terminal membrane attack complex (MAC). The processing and clearance of necrotic host cell material is done via the same route: calcium-dependent binding of nuclear material or other cell material to CRP leads to the activation of the classical pathway of complement and, thus, to opsonophagocytosis. However, for apoptotic host cell material, the last route, the terminal MAC, is not activated. In this way, the apoptotic host cell material is elegantly cleared without further inflammatory damage (Gershov et al., 2000). Another important biological property is the ability of ligand-complexed CRP to bind to the FcgRI and FcgRIIa receptors (Fc receptors for IgG molecules). This binding elicits a response of phagocytic cells and thus enhances the phagocytosis of microorganisms or damaged/dead host cell material (Gewurz et al., 1995). Some of the pentameric CRP molecules undergo processes of proteolysis or denaturization. The first process results in dissociation of pentameric CRP into monomeric subunits or smaller peptides. Conformational changes of the molecule due to denaturization lead to modified CRP molecules (mCRP). The final result of both processes is the expression of new epitopes, called neoepitopes, which normally are 'hidden' in the native molecule. Different functions are attributed to the distinct binding properties of the neoepitopes from native CRP (Zouki et al., 2001). For example, a third binding facility of mCRP is to the low-affinity IgG receptor FcgRIIIb on the neutrophil. This binding results in shedding of L-selectin and, thus, inhibition of adhesion of the neutrophil to the EC (Zouki et al., 1997). This anti-inflammatory effect of mCRP may play a role in the fact that neutrophils are absent in atherosclerotic lesions.
2.11. Effects of high-sensitivity C-reactive protein on atherosclerosis

Inflammatory mechanisms play a central role in all phases of atherosclerosis, from the initial recruitment of circulating leukocytes to the arterial wall to the rupture of unstable plaques, which results in the clinical manifestations of the disease. HsCRP may be involved in each of these stages by direct influencing processes like complement activation, apoptosis, vascular cell activation, monocyte recruitment, lipid accumulation and thrombosis (Fig. 2.3).

2.11.1. Complement activation

Activation of the classical pathway of the complement system is a well-known and direct biological function of hsCRP (Pepys and Hirschfield, 2003). Via this action, hsCRP directly amplifies and facilitates innate immunity (Torzewski et al., 1998; Volanakis and Kaplan, 1974), a process that has already been associated with initiation and progression of CVD for a long time. In situ hybridization showed intense mRNA signals for hsCRP and complement component C4 in VSMCs and macrophages present in the thickened intima of the lesion. HsCRP also co-localizes with C5–C9, the MAC, of complement (Yasojima et al., 2001). Activation of this MAC is initiated by the direct binding of hsCRP to Clq, also present in the atherosclerotic lesion (Volanakis and Kaplan, 1974), and characterized by elevated levels of component C5a (Szalai et al., 2000). C5a itself exerts potent chemotactic and pro-inflammatory effects and its plasma levels have been associated with increased cardiovascular risk in patients with advanced atherosclerosis (Speidl et al., 2005). HsCRP is also involved in the inhibition of complement activation through interaction with fH, which is also present in injured areas. The hsCRP-fH complex interferes with the activity of C3b (Giannakis et al., 2001), and thus will prevent formation of the MAC. Through the interaction with complement factors, hsCRP exerts a direct effect on arterial ECs, by increasing the expression of complement inhibitory factors on the ECs. This suggests that hsCRP-mediated complement activation is a system set to regulate the inflammatory reaction, because it will result in promoting the removal of debris from tissues and the deleterious effects of complement activation in patients with CVD (Szalai et al., 1999).
Fig. 2.3 Mechanisms relating high-sensitivity C-reactive protein to the development and progression of atherothrombosis

eNOS, endothelial nitric oxide synthase; ET-1, endothelin 1; LDL, low-density lipoprotein; MCP-1, monocyte chemoattractant protein 1; PAI-1, plasminogen activator inhibitor-1.

(Ridker, 2003)
However, since complement activation also leads to the production of a variety of pro-inflammatory molecules, this mechanism of hsCRP-mediated complement regulation might also aggravate the inflammatory status in the entire body as well as in the atherosclerotic plaque. Therefore, the direct interaction between hsCRP and complement can both activate and inhibit inflammation in atherosclerotic lesions.

2.11.2. Interaction with cell surface receptors

The close proximity of hsCRP to monocytic cells (Reynolds and Vance, 1987; Torzewski et al., 2000) in the arterial intima attenuates its possibilities for a direct contribution to the progression of atherosclerosis. The observation that hsCRP is localized between monocytes underlines the possibility of a direct interaction of hsCRP with these cells and with monocyte-derived macrophages via binding to a specific receptor. HsCRP binds to several receptors on human monocytes; to FcRgIIa (CD32) with high affinity and to FcRgI (CD64) with lower affinity (Crowell et al., 1991) increasing phagocytosis and the release of inflammatory cytokines (Marnell et al., 2005). The Fc receptors have been described to mediate the effect of hsCRP on human aortic endothelial cells (HAEC) (Devaraj et al., 2005). FcRgIIa is known as the putative hsCRP receptor for leukocytes (Bharadwaj et al., 1999) and also has been found on bovine aortic ECs (Escribano-Burgos et al., 2005). HsCRP also binds to the inhibitory receptor, FcgammaRIIb, blocking activating signals (Marnell et al., 2005).

The binding of hsCRP to a receptor suggests its capacity to induce a specific biological effect (Devaraj et al., 2005), such as direct involvement in cell-mediation and opsonization. Addition of hsCRP with and without anti-CD32- antibody to ECs demonstrated the partial mediation of hsCRP in regulation of cell surface protein expression, such as the endothelial protein C receptor, by CD32 (Nan et al., 2005). However, the downstream effects of hsCRP binding have not yet been elucidated. The interaction of hsCRP with CD36, a scavenger receptor which is expressed by macrophages and is involved in uptake of LDL particles, demonstrates a direct role of hsCRP through its interference with the binding of LDL cholesterol to CD36 (Zwaka et al., 2001).
2.11.3. Thrombosis

Thrombosis contributes to the progression of the atherosclerotic lesion and to the precipitation of the cardiovascular event. Direct actions of hsCRP, which contribute to the induction of a prothrombotic state, may be the enhancement of the procoagulant activity (Penn and Topol, 2001; Libby and Simon, 2001) or the reduction of fibrinolysis (Juhan-Vague et al., 1996; Cushman et al., 1999). HsCRP has been suggested to induce a prothrombotic state via induction of tissue factor expression in human monocytes (Whisler et al., 1986; Nakagomi et al., 2000), but only in the presence of and through direct interaction with other blood cells as T-lymphocytes, B-lymphocytes and natural killer cells (Paffen et al., 2004).

In transgenic mice expressing hCRP, the injury-induced occlusion of the femoral artery (75% after 28 days) was enhanced compared to the amount of occlusion observed in wild-type mice (17% after 28 days) (Danenberg et al., 2003) indicating a prothrombotic effect of hCRP. A direct effect of CRP on hemostasis was shown in a recent study, where recombinant hCRP was infused into human volunteers, which resulted in the stimulation of both hemostasis and inflammation (Bisoendial et al., 2005).

HsCRP may also inhibit fibrinolysis by increasing the expression and activity of the main inhibitor of fibrinolysis, PAI-1 in HAEC (Lip et al., 2002). Since PAI-1 promotes atherothrombosis and progression of ACS, this effect of hsCRP may also affect CVD (Devaraj et al., 2003). Also, besides this effect on PAI-1, recently hsCRP has been demonstrated to directly decrease antigen levels and the activity of tissue plasminogen activator (tPA) in HAEC. TPa is the substance normally inhibited by PAI-1. In this study, the direct and specific role of hsCRP is demonstrated by using hsCRP that was free from sodium azide and bacterial lipopolysaccharides (LPS) contamination (Singh et al., 2005).

2.11.4. Cellular modulation, recruitment and activation

HsCRP contributes to an arterial pro-inflammatory and proatherosclerotic phenotype by directly upregulating adhesion molecules and chemoattractant chemokines in ECs, VSMCs and monocytic cells. On the EC surface, expression of
adhesion molecules such as ICAM-1, VCAM-1, and E-selectin is upregulated by hsCRP (Pasceri et al., 2000). Via these processes, hsCRP induces platelet adhesion to ECs (Brill et al., 2005), hsCRP stimulates EC dysfunction and the recruitment of monocytes and T-lymphocytes towards the endothelial wall. These findings were reported by several groups, who also showed that hsCRP induced MCP-1 production.

This upregulation of adhesion molecules is partly mediated via the production of endothelin-1, a potent endothelium-derived vasoactive factor, and by the production of the inflammatory cytokines IL-6 and IL-8. As to the effects of hsCRP on MCP-1 expression, aortic ECs seem to be unresponsive whereas venous ECs (Pasceri et al., 2001) or monocytes (Thomassen et al., 1993) show increased expression of this chemoattractant. Since atherosclerosis mainly develops in the arteries, the clinical significance of the effect of hsCRP on venous cells is not clear.

HsCRP is also known to activate the NF-κB signaling pathway in saphenous vein ECs (Verma et al., 2003), which, in recent light of possible azide contamination (Liu et al., 2005), might be an artefact. Also in VSMCs, hsCRP has been indicated to activate NF-κB (Hattori et al., 2003). Therefore, hsCRP has been suggested to mediate proliferation and activation of VSMCs, causing the accumulation of these cells in the vascular intima, which is a key event in the development of arterial lesions. Another manner in which hsCRP directly affects the activation and proliferation of VSMCs, is via upregulation of mRNA and protein and increased cell surface expression of the angiotensin type 1 receptor (AT1-R). This was demonstrated in vitro in human VSMCs and in vivo in a rat carotid artery angioplasty model (Wang et al., 2003).

HsCRP also appears to be involved in the infiltration of monocytes into the vessel wall and their subsequent development into foam cells. The deposition of hsCRP in the arterial wall precedes monocyte infiltration and direct involvement of hsCRP in recruitment of blood monocytes has been demonstrated in vitro, suggesting hsCRP to be chemotactic for human blood monocytes (Torzewski et al., 2000). HsCRP also promotes MCP-1 mediated chemotaxis through upregulation of CC chemokine receptor 2 expression in human monocytes (Han et al., 2004).
The effect of hsCRP on T-lymphocytes is indirect. T-lymphocytes are recruited to the atherosclerotic lesion as a result of the ongoing inflammatory process. Through the stimulation of cytokine production and secretion by macrophages, hsCRP exerts an indirect effect on T-lymphocytes present in the atherosclerotic lesion. HsCRP induces macrophages to express interleukin-12 (IL-12), which contributes to the development of CD4+ T-helper cells (Yamashita et al., 2003). In turn, these cells express interferon-γ (INF-γ), which is synergistic with hsCRP in the execution of many functions contributing to the proatherosclerotic phenotype. In contrast, during hsCRP induced activation of complement and opsonization of apoptotic cells, the actively phagocytizing macrophages reduce expression of IL-12 and thereby suppress T-lymphocytes (Kim et al., 2003).

2.11.5. Expression of inflammatory mediators: cytokines, chemokines and adhesive molecules

HsCRP induces inflammatory cytokines in a dose-dependent way (Devaraj et al., 2005), which provides further support for the hypothesis that interaction with mononuclear phagocytes constitutes an important biological role for this acute phase protein. Quantitative analysis of the hsCRP-induced release of IL-6, IL-1 and TNF-α by freshly isolated normal human monocytes, revealed slight differences in time courses. All three cytokines were detected 4h after hsCRP addition in vitro, with maximal levels of TNF-α at 8h and of IL-1 and IL-6 at 16h (Ballou and Lozanski, 1992). IL-8, a member of the CXC chemokines promotes monocyte–EC adhesion and arrest and is abundant in atherosclerotic plaques. In HAEC in vitro, hsCRP increases IL-8 protein and mRNA expression in a time- and dose-dependent manner via specific upregulation of NF-nB activity (Devaraj et al., 2004). HsCRP induces production and secretion of MCP-1 in human umbilical vein ECs (Pasceri et al., 2001), but not in aortic ECs (Devaraj et al., 2004). MCP-1 present in the atherosclerotic lesion (Nelken et al., 1991) can also originate from monocytes.

HsCRP induces a 7-fold increase in the production of monocyte MCP-1 in purified peripheral monocytes (Paffen et al., 2004). In patients with ACS, baseline level of this chemoattractant was elevated. This elevated expression is associated with both
traditional risk factors for atherosclerosis as well as increased risk of MI, independent of baseline variables target (de Lemos and Marrow, 2003).

In atherosclerotic lesions, hsCRP directly upregulates mRNA expression of the macrophage markers CD11b and HLA-DR, as well as their protein products (Yasojima et al., 2001). Monocyte expression of CD11b increased significantly up to twofold when exposed to hsCRP, while no significant difference in CD32 expression was observed, whereas hsCRP exposure decreases CD31 expression. HsCRP can affect monocyte activation ex vivo and induce phenotypic changes that result in an altered recruitment to ECs (Woollard et al., 2002).

Another mechanism by which hsCRP influences the development and maintenance of the atherosclerotic lesion is its involvement in the CD40-CD40 Ligand (CD40L or CD154) interaction. CD40L, a 33-kDa activation-induced T lymphocyte surface glycoprotein, binds to CD40, a phosphorylated glycoprotein expressed on B-lymphocytes, vascular ECs, monocytes, macrophages and fibroblasts. Like hsCRP, the amount of soluble CD40 increases during inflammation and in the atherosclerotic lesion. Therefore CD40L has been suggested to be a marker for inflammation and involved in risk of cardiovascular events as well (Schonbeck and Libby, 2001). HsCRP upregulates the cell surface expression of CD40 and CD40L on human umbilical ECs in time (Lin et al., 2004). CD40L is shed into the vasculature. Elevated levels of this soluble CD40L (sCD40L) identify patients with ACS at increased risk of recurrent MI and death, independent of other variables as cardiac troponine T or hsCRP (Varo et al., 2003).

2.11.6. Nitric oxide expression

HsCRP has been described to decrease the expression and bioactivity of eNOS or NOS3 (Venugopal et al., 2002), which results in reduced bioavailability of NO and a subsequent effect of vasodilatation. It was demonstrated recently that this effect can be caused by sodium azide as well (Swafford et al., 2005); however, it is not clear whether there remains a role for hsCRP. It is therefore uncertain whether there is a causal role for hsCRP in the regulation of expression of NO and involvement in vascular reactivity.
Nevertheless, hsCRP has been suggested to exert a specific effect on endothelial eNOS expression through binding to the hsCRP receptor FcRgIIa (Escribano-Burgos et al., 2005). In HAECs and human coronary artery ECs (HCAECs), hsCRP contributes to a proatherogenic and prothrombotic state by decreasing the release of NO and of the vasodilator and inhibitor of platelet aggregation prostacyclin (PGI2), through directly increasing both superoxide and inducible NO synthase (Venugopal et al., 2003).

In VSMCs, hsCRP reduces expression of the inducible variant of NOS (Ikeda et al., 2003) and subsequent NO-synthesis as well. But again, this might be an artefact caused by sodium azide (Lafuente et al., 2005). In these VSMCs, hsCRP also has been described to induce activation of the iNOS promoter (Hattori et al., 2003), which, despite the fact that hsCRP seems to be no more than a weak inducer of NO-production, contradicts the study of Ikeda and coworkers. Nevertheless, both studies demonstrate that hsCRP is likely to be involved in the regulation of cellular NO-levels. Furthermore, interaction between hsCRP and INF-γ appears to enhance the effect of hsCRP on NO-regulation, which indicates that there may be a direct effect of hsCRP because contaminants will not be able to exert these specific interactions (Hattori et al., 2003).

2.11.7. Apoptosis

HsCRP is directly involved in the process of apoptosis (Blaschke et al., 2004). It binds to apoptotic cells in a Ca2+-dependent manner and augments the classical pathway of complement activation but protects the cells from assembling the terminal complement components (C5-C9). Furthermore, hsCRP enhances opsonization and phagocytosis of apoptotic cells by macrophages associated with the expression of the anti-inflammatory cytokine TGFβ. HsCRP and the classical complement components act in concert to promote non-inflammatory clearance of apoptotic cells (Gershov et al., 2000). The inhibitory effect of hsCRP on the NO expression of endothelial progenitor cells (EPCs) directly inhibits their mobilization and differentiation, survival and function, whereby it facilitates EC apoptosis and blocks the process of angiogenesis (Verma et al., 2004). Apoptosis of VSMCs also plays an important role in progression of atherosclerotic lesions and contributes to increased plaque vulnerability. Silencing the
hsCRP-regulated GADD153 gene in VSMCs indicated that hsCRP plays an essential role in induced apoptosis of VSMCs (Blaschke et al., 2004). HsCRP also binds to phosphatidylcholines, by which it participates directly in activation of macrophages and neutrophils in the clearance of apoptotic and necrotic cells (Du Clos, 2000; Chang et al., 2002). However, neutrophils are not present in the atherosclerotic plaque (Ross, 1999).

2.11.8. Lipids

The interaction between lipids and hsCRP is diverse. It has been suggested that hsCRP could be the factor that links lipoprotein-deposition and complement activation in atherosclerotic plaques. Binding of tissue-deposited hsCRP to enzymatically degraded LDL cholesterol enhances complement activation, which may be relevant to the development and progression of the atherosclerotic lesion, particularly at early stages of atherosclerosis when low concentrations of enzymatically degraded LDL cholesterol are present (Torzewski et al., 1998). And, although direct involvement of hsCRP has not been demonstrated, through this binding of hsCRP to enzymatically degraded LDL cholesterol, hsCRP may be involved in the massive release of MCP-1 from macrophages described to be caused by enzymatically degraded LDL cholesterol (Klouche et al., 1998).

Although the reports on interaction between hsCRP and ox-LDL are conflicting, complement activation as a result of this interaction is generally considered unlikely. Nevertheless, hsCRP has been described to directly induce lectin-like ox-LDL receptor-1 expression (LOX-1) in HAECs, because this could be reduced with antibodies against CD32/CD64, ET-1 or IL-6 (Li et al., 2004). Via LOX-1, hsCRP is suggested to regulate monocytes adhesion to ECs and uptake of ox-LDL by ECs.

The majority of sub-endothelial foam cells show positive staining for hsCRP. Zwaka et al., (2001) demonstrated that native LDL cholesterol that was co-incubated with hsCRP was taken up by macrophages via macropinocytosis. It was concluded that foam cell formation in human atherogenesis might be caused in part by uptake of hsCRP-opsonized native LDL cholesterol. High levels of HDL cholesterol are atheroprotective since HDL cholesterol is involved in transporting cholesterol from the periphery to the liver. HDL cholesterol might also protect the endothelium since the hsCRP-induced
upregulation of inflammatory adhesion molecules in HUVECs was completely blocked by HDL cholesterol. So, HDL cholesterol neutralizes hsCRP induced proinflammatory activity (Wadham et al., 2004). HDL cholesterol also inhibits atherosclerosis through prevention of oxidation of LDL cholesterol.

2.11.9. Neuronal effect of C-reactive protein

HsCRP has been shown to have direct neurotoxicity in vitro (Duong et al., 1998). A large number of immunohistochemical studies have repeatedly shown widespread immunoreactivity for hsCRP in the brains of patients with Alzheimer's disease (Duong et al., 1998; McGeer et al., 2000). As an important component of the innate immune system, hsCRP acts as an opsonin and activates the classic complement system. The hsCRP-mediated innate immune defence may be protective during an acute-phase response (such as in phagocytosis of pathogens). This defence mechanism may turn proinflammatory and thus destructive (such as in cell lysis and subsequent apoptosis) if inflammation persists.

In addition to the proinflammatory response that may cause direct neuronal damage, raised hsCRP concentrations, by acting as a cardiovascular risk factor and causing cerebral atherosclerosis, may result in cerebral macro-angiopathy (i.e., large observable stroke) or cerebral micro-angiopathy (i.e., leucoaraiosis). Both types of lesions disrupt the integrity of frontal-subcortical circuits and are responsible for the development of cognitive impairment, dementia, and depressive disorders (Kuo and Lipsitz, 2004; Stephens et al., 2004; Mast, 2004).

2.12. Major prospective studies investigating high-sensitivity C-reactive protein as a marker of future cardiovascular risk among apparently health individuals

There is now a wealth of evidence from large-scale prospective studies of apparently healthy individuals that baseline levels of hsCRP are an independent predictor of future cardiovascular events. These studies include both men and women, European and American cohorts, and both middle-aged and elderly individuals. In addition to predicting cardiovascular death and MI, CRP is also a robust predictor of
sudden cardiac death, stroke and the development of PVD (Albert et al., 2002; Rost et al., 2001; Ridker et al., 1998), indicating a role for hsCRP in the assessment of global vascular risk (Table 2.4). In support of this, analyses of population-based data from the WHS indicate that measurement of hsCRP in addition to lipid levels may improve identification of individuals at risk for cardiovascular events (Ridker et al., 2000), and that hsCRP may be an even stronger predictor than LDL cholesterol level and may provide additional prognostic information to that conveyed by the Framingham risk score (Ridker et al., 2002). The ability of hsCRP to add prognostic information on global cardiovascular risk after adjustment for all Framingham risk factors has been confirmed in 9 major prospective studies (Fig. 2.4).

2.13. Major studies of the association between high-sensitivity C-reactive protein and stroke

The epidemiological data that support the role of hsCRP as a predictor of vascular disease are consistent across different study populations. High concentrations of hsCRP have been shown to be associated with increased risk of developing cerebrovascular disease. Studies included were for an association between hsCRP and stroke (seven studies; Table 2.5). The possible explanations for the association between increased serum concentrations of hsCRP and stroke, cognitive disorders, and depression are summarised below and in (Fig. 2.5).

2.14. Therapeutic Interventions

Definitive evidence that lowering hsCRP levels will necessarily lead to a reduction in clinical cardiovascular events is not yet available. Nevertheless, many behavioral and pharmacologic interventions known to reduce the risk of clinical cardiovascular events have been linked to lower hsCRP levels. The goal of cardiovascular screening programs is the identification of high-risk individuals who can be targeted for weight loss, smoking cessation, increased physical activity, BP control, and, if necessary, pharmacologic therapy. A patient's compliance with recommended interventions depends at least in part on his or her perception of absolute disease risk.
### Table 2.4 Major prospective studies investigating high-sensitivity C-reactive protein as a marker of future cardiovascular risk among apparently health individuals

<table>
<thead>
<tr>
<th>Study</th>
<th>End Point</th>
<th>Relative risk (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ridker et al., 2002</td>
<td>Cardiovascular disease</td>
<td>3.6 (2.5-5.2)</td>
</tr>
<tr>
<td>Danesh et al., 2000</td>
<td>Coronary heart disease</td>
<td>2.13 (1.38-3.28)</td>
</tr>
<tr>
<td>Roivainen et al., 2000</td>
<td>Coronary heart disease</td>
<td>3.56 (1.93-6.67)</td>
</tr>
<tr>
<td>Tracy et al., 1997</td>
<td>Myocardial infarction</td>
<td>2.67 (1.04-6.81)</td>
</tr>
<tr>
<td>Ridker et al., 1997</td>
<td>Myocardial infarction</td>
<td>2.9 (1.8-4.6)</td>
</tr>
<tr>
<td>Albert et al., 2002</td>
<td>Sudden cardiac death</td>
<td>2.78 (1.35-5.72)</td>
</tr>
<tr>
<td>Lowe et al., 2001</td>
<td>Ischaemic heart disease</td>
<td>2.73 (2.73-4.67)</td>
</tr>
<tr>
<td>Ridker et al., 1998</td>
<td>Peripheral vascular disease</td>
<td>2.3 (1.1-4.8)</td>
</tr>
</tbody>
</table>

### Table 2.5 Major studies of the association between high-sensitivity C-reactive protein and stroke

<table>
<thead>
<tr>
<th>Study</th>
<th>Outcomes</th>
<th>Relative risk (RR) (95% CI)/ Odds ratio (OR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ridker et al., 1997</td>
<td>Ischaemic stroke</td>
<td>1.9 (1.1-3.3)</td>
</tr>
<tr>
<td>Ford and Giles, 2000</td>
<td>Stroke</td>
<td>OR 1.7 (1.1-2.6)</td>
</tr>
<tr>
<td>van Exel et al., 2002</td>
<td>Stroke</td>
<td>OR 2.1 (1.0-4.4)</td>
</tr>
<tr>
<td>Rost et al., 2001</td>
<td>Ischaemic stroke or TIA</td>
<td>1.6 (0.9-3.1) in men and 2.1 (1.2-3.8) in women</td>
</tr>
<tr>
<td>Curb et al., 2003</td>
<td>Ischaemic stroke</td>
<td>1.6 (1.1-2.4)</td>
</tr>
<tr>
<td>Cao et al., 2003</td>
<td>Ischaemic stroke</td>
<td>1.6 (1.2-2.1)</td>
</tr>
<tr>
<td>Cesari et al., 2003</td>
<td>Stroke</td>
<td>1.4 (0.7-2.7)</td>
</tr>
</tbody>
</table>
Fig. 2.4 High-sensitivity C-reactive protein was an independent predictor of cardiovascular risk in 9 large prospective studies across diverse populations.

ARIC = Atherosclerosis Risk in Communities; CHS = Cardiovascular Health Study; EPIC = European Prospective Investigation into Cancer and Nutrition; HPFS = Health Professionals Follow-up Study; MONICA = Monitoring Trends and Determinants in Cardiovascular Disease; NHS = Nurses’ Health Study; PHS = Physicians’ Health Study; WHS = Women’s Health Study.
Fig. 2.5 Involvement of proatherogenic high-sensitivity C-reactive protein in atherogenesis

(Hsu et al., 2005)
Because the addition of hsCRP to lipid evaluation improves the prediction tool, hsCRP screening may be useful for this reason alone.

2.14.1. Behavioral Interventions

2.14.1.1. Weight loss

Degree of adiposity is a major determinant of hsCRP levels in general populations (Visser et al., 1999; Ford, 1999; Ford, 2003; Rexrode et al., 2003). In the WHS, for example, women without CVD or cancer in the top body mass index (BMI) quartile (≥28.3 kg/m²) were 12 times more likely than women in the bottom quartile (<22.4 kg/m²) to have a top quartile hsCRP level, after adjustment for age, smoking, menopausal status, HRT use, alcohol use, physical activity, hypercholesterolemia, diabetes, and hypertension (Rexrode et al., 2003). The regional distribution of excess body weight is another important source of variation in hsCRP level; after additional adjustment for BMI, women in the top quartile of waist circumference (≥39 in) were twice as likely as women in the lowest quartile (<31 in) to have an elevated hsCRP.

In a recent randomized trial, obese premenopausal women assigned to a 2-year weight-loss program that emphasized a low-energy Mediterranean diet and moderate physical activity showed favorable changes in hsCRP levels (Esposito et al., 2003) as compared with women in the control group; reductions in proinflammatory cytokine concentrations and improvements in endothelial function were also noted (Ziccardi et al., 2002). Weight loss achieved by caloric restriction alone also has been shown to lower hsCRP levels in obese postmenopausal women (Tchernof et al., 2002). Weight loss after gastric surgery in morbidly obese patients resulted in significant declines in levels of hsCRP and IL-6 in association with amelioration of the insulin resistance syndrome (Kopp et al., 2003). Whether these effects translate into a reduced risk of subsequent cardiovascular events has not yet been determined.

2.14.1.2. Physical activity

Regular physical activity may favorably modulate inflammatory responses. In NHANES, after adjustment for BMI and other potential confounders, the RRs for
elevated hsCRP (i.e., ≥85th percentile of the sex-specific distribution) were 0.98, 0.85, and 0.53 for individuals who engaged in light, moderate, and vigorous leisure-time physical activity, respectively, during the previous month compared with individuals who did not engage in any leisure-time physical activity (Ford, 2002). Additional adjustment for the presence or absence of CHD, cancer, diabetes, asthma, or arthritis did not change these estimates. In a separate analysis limited to healthy participants without these conditions, frequency of physical activity was associated in a dose-dependent manner with hsCRP level (Abramson and Vaccarino, 2002). Compared with those engaging in leisure-time physical activity 3 or fewer times per month, the multivariate-adjusted RR of having an elevated hsCRP level (≥90th percentile of the sex-specific distribution) was decreased among those engaging in such activity 4 to 21 times per month and 22 or more times per month. In 3 large cohorts of elderly persons unselected for CVD—the Cardiovascular Health Study (CHS), (Geffken et al., 2001) the British Regional Heart Study (BRHS), (Wannamethee et al., 2002) and the MacArthur Studies of Successful Aging (MASSA) (Reuben et al., 2003) individuals who reported high levels of recreational or household-related physical activity were also significantly less likely to have elevated levels of hsCRP and other inflammatory and hemostatic markers than were their sedentary counterparts.

Data from the BRHS, which examined physical activity patterns over a 20-year period among men aged 40 to 59 years at baseline, suggest that exercise must be current to confer an anti-inflammatory effect (Wannamethee et al., 2002). Men who were active in midlife but became inactive in later life had hsCRP levels comparable to those of continuously inactive men, whereas men who took up even light activity in later life had hsCRP levels approaching those of continuously active men. In agreement with these findings, a population-based cross-sectional study of 1,856 Greek adults 18 years and older reported a significant inverse relation between current physical activity level and hsCRP but little association between recalled history of physical activity (i.e., number of years during which a participant engaged in exercise) and hsCRP (Pitsavos et al., 2003). Each of these studies relied on self-reported physical activity patterns as the predictor variable.
Cardiorespiratory fitness as assessed by maximal treadmill exercise test has also been shown to correlate inversely with hsCRP levels in women (LaMonte et al., 2002) and men (Church et al., 2002) without CVD. In support of observational findings, data from small nonrandomized intervention studies also suggest a beneficial effect of regular exercise on inflammation. For example, favorable changes in hsCRP levels after 9 months of endurance training (Mattusch et al., 2000) or 6 months of a supervised exercise program (2.5 hours of exercise per week) (Smith et al., 1999) have been documented by such studies. Large-scale randomized trials are required to verify these results. Whether or not the inverse association between physical activity and hsCRP is independent of the effect of adiposity is controversial.

Although the observational studies described above found significant relations between physical activity and hsCRP even after adjustment for BMI, which suggests that exercise influences hsCRP through other mechanisms in addition to regulation of body weight, associations between physical activity and hsCRP levels were not significant after control for BMI and leptin (a surrogate marker for fat mass) in a large sample of healthy women (Nurses' Health Study II - NHS II) and healthy men (Health Professionals' Follow-up Study - HPFS) (Pischon et al., 2003). Physical activity also was unrelated to hsCRP after adjustment for BMI in the Physicians' Health Study (PHS) (Rohde et al., 1999). Similarly, in several smaller studies, (Rawson et al., 2003; Manns et al., 2003) correlations between physical activity and hsCRP could be accounted for by a lower degree of body fat of the more active participants.

2.14.1.3. Smoking cessation

HsCRP and other markers of systemic vascular inflammation are higher in smokers than in nonsmokers. In NHANES, current smokers were approximately 70% more likely than those who never smoked to have an hsCRP level of 2.2 mg/L or above, whereas former smokers were only about 20% more likely to have this elevation (Bazzano et al., 2003). A similar gradient of increasing hsCRP levels across never, former, and current smokers was observed among participants in the WHS (Bermudez et al., 2002). These results suggest that smoking cessation can reduce hsCRP levels.
2.14.1.4. Dietary factors

Studies of dietary factors known or hypothesized to confer cardioprotection or harm and inflammatory markers have begun to provide information about their relations, but a clear picture has not emerged. This section provides a selective review of recent dietary findings.

2.14.1.4.a. ω-3 fatty acids

Observational and intervention studies suggest that fish intake reduces CHD incidence and mortality (Hu et al., 2002; Hu et al., 2003; Din et al., 2004). Such cardioprotection might be explained by an effect of long-chain ω-3 polyunsaturated fatty acids found in fish (and certain plant oils) on inflammation. Laboratory studies suggest that ω-3 fatty acids have anti-inflammatory effects and that ω-6 fatty acids antagonize these effects (James et al., 2000). A detailed investigation of dietary intake of polyunsaturated fatty acids and inflammatory biomarkers in the NHS II and the HPFS showed a weak inverse association between intake of ω-3 fatty acids, specifically the marine-derived eicosapentaenoic acid and docosahexaenoic acid but not the plant-derived α-linolenic acid, and hsCRP level (Pischon et al., 2003). ω-6 fatty acid intake had no correlation with hsCRP levels in main-effects analyses, but, counter to expectation, the inverse relation between ω-3 fatty acid intake and hsCRP level was slightly stronger in the presence of higher ω-6 fatty acid intake. In NHANES, however, intake of fish or of polyunsaturated fats was unrelated to hsCRP level (King et al., 2003).

Moreover, results from small-scale randomized trials of ω-3 fatty acid supplementation are inconsistent. For example, although 3 months of α-linolenic acid supplementation, relative to placebo, decreased hsCRP levels by 38% in male dyslipidemic patients following a typical Greek diet, (Rallidis et al., 2003) 12 weeks of ω-3 fatty acid supplementation did not affect hsCRP levels in healthy volunteers in the United States, (Madsen et al., 2003) and 6 weeks of treatment with eicosapentaenoic acid and docosahexaenoic acid did not decrease hsCRP levels among viscerally obese Australian men (Chan et al., 2002). In support of these results, a 1-year study of 58 healthy middle-aged Dutch monks found that fish oil supplementation had no effect
on mononuclear cell production of the proinflammatory cytokines TNF-\(\alpha\) and IL-1 (CRP was not examined) (Blok et al., 1997). However, the failure of supplementation to reduce hsCRP in healthy individuals with normal levels of this protein may represent a floor effect. Larger-scale trials in higher-risk populations are warranted.

2.14.1.4.b. Low-fat, low-cholesterol diet

In NHANES, although intakes of total fat and cholesterol were unrelated to hsCRP levels, a high intake of saturated fat was modestly associated with elevated hsCRP. After adjustment for BMI, total caloric intake, smoking, alcohol use, and exercise, persons in the third and fourth quartiles of saturated fat intake were more likely than persons in the lowest quartile of intake to have hsCRP levels exceeding 3 mg/L, with RRs of 1.58 and 1.44, respectively (King et al., 2003). A recent study suggests that individuals with high hsCRP levels may be more resistant to the benefits of a low-fat, low-cholesterol diet than those with low hsCRP levels (Erlinger et al., 2003).

In a sample of 100 healthy adults, most of whom were overweight African-American women with hypercholesterolemia, an elevated hsCRP level at baseline strongly predicted reduced lipid responsiveness to a low-fat, low-cholesterol diet relative to a diet high in carbohydrates (the Dietary Approaches to Stop Hypertension-Sodium (DASH) diet). In participants with hsCRP levels below the sample median of 2.37 mg/L, assignment to the DASH diet (27% of calories from total fat and 6% from saturated fat) rather than an equicaloric control diet (37% of calories from total fat and 16% from saturated fat) was associated with net reductions of 10% and 12% in total and LDL cholesterol, respectively, and an 18% increase in TG.

In contrast, in participants with hsCRP values above the median, reductions in total and LDL cholesterol were each 3%, and TG levels did not change. Thus, inflammation may indirectly increase heart disease risk by blunting lipid responses to dietary change. However, other factors, including obesity, have been shown to have similar effects (Grundy, 2003). Although the study is provocative, the findings require replication in larger investigations.
2.14.1.4.c. Low glycemic load

A high intake of rapidly digested and absorbed carbohydrates induces rapid postprandial glucose and insulin responses, which over time can lead to an insulin-resistant state characterized by hyperinsulinemia and dyslipidemia (Wolever, 1990). Exacerbation of proinflammatory processes may be another mechanism whereby such “high glycemic load” diets increase CHD risk. In the WHS, dietary glycemic load was associated in a positive dose-response fashion with hsCRP levels, especially among overweight individuals (Liu et al., 2002). Thus, lowering dietary glycemic load—by substituting whole grains for refined grains, for example—may be helpful in reducing plasma hsCRP levels in persons prone to insulin resistance. In support of these findings, NHANES respondents in the top quartile of fiber consumption were far less likely to have hsCRP levels exceeding 3 mg/L than were those in the bottom quartile (King et al., 2003). When fiber was modeled as a continuous variable, each additional gram of fiber consumed per day was associated with a 2% lower risk of elevated hsCRP.

2.14.1.4.d. Vitamins

In NHANES, elevated hsCRP (i.e., ≥85th percentile of the sample distribution) was inversely associated in a dose-response fashion with blood concentrations of retinol, serum retinyl esters, vitamin C, serum folate, carotenoids, and selenium, after adjustment for BMI and other covariates (Ford et al., 2003). The directionality of association is unclear. It is possible that inflammatory processes, through the production of ROS, can deplete the body’s antioxidant stores. Alternatively, low antioxidant intake may lead to inflammation. A third possibility is that the relation is bidirectional. Although fruit and vegetable consumption was unrelated to hsCRP status in multivariate analyses of the NHANES dataset and although large trials of antioxidant vitamin supplementation in persons at high cardiovascular risk on the basis of traditional factors such as hyperlipidemia have produced disappointing results (Bassuk et al., 2004), the possibility that increased intake of antioxidant or other vitamins offers cardioprotection in persons at risk of CHD by virtue of elevated hsCRP levels may be worthy of further study. In a post hoc analysis of a 6-month randomized trial of
multivitamin supplements in individuals with hyperhomocysteinemia, persons assigned to the active agent experienced a significant 14% decrease in hsCRP level as compared with those assigned to placebo (Church et al., 2003). The between-group difference was largest among patients with initially elevated hsCRP levels (≥1.0 mg/L). Small trials employing a “before-after” design have also reported that vitamin E supplementation, at high doses, may lower hsCRP levels in persons with type 2 diabetes (Devaraj and Jialal, 2000; Uritchard et al., 2000).

2.14.1.4.e. Alcohol use

In large prospective epidemiologic studies, light to moderate alcohol intake rather than abstention or heavy intake is associated with lower cardiovascular mortality (Charles Fuchs et al., 1995; Gaziano et al., 2000). Although potential explanations for the association have focused on lipoprotein and hemostatic factors, recent data suggest that alcohol may reduce cardiovascular risk in part through an anti-inflammatory mechanism. In a national survey of 1,776 West German adults, hsCRP and other markers of inflammation had strong U-shaped relations with alcohol intake, although these associations were largely confined to men (Imhof et al., 2001). Among 2,833 United States adults participating in the Pravastatin Inflammation/CRP Evaluation study, persons reporting moderate alcohol consumption had lower hsCRP concentrations than did persons reporting no or occasional intake, an effect that was independent of alcohol related effects on lipids. The association was observed in men and women not taking HRT, and in those with and without a history of CVD (Albert et al., 2003).

2.15. Pharmacologic interventions

Several pharmacologic agents with demonstrated cardioprotective ability appear to reduce hsCRP levels.

2.15.1. Lipid-modulating agents

Lipid-modulating medications reported to affect hsCRP levels favorably include HMG-CoA reductase inhibitors, fibrates, and niacin. Of these, the findings for statins are by far the most robust.
2.15.4. Niacin

Data from small trials also suggest that niacin may reduce hsCRP plasma levels. In a 1-year, open-label trial that tested escalating doses of a combination of niacin and lovastatin in dyslipidemic men and women, the 1000/20-mg dose reduced median hsCRP levels by 4% at week 8 and the 2000/40-mg dose reduced hsCRP by 24% at week 52 (Kashyap et al., 2002). In a 16-week, double-blind trial that randomized 148 men and women with type 2 diabetes to placebo or to niacin at doses of 1000 or 1500 mg/d, median hsCRP levels decreased by 2%, 11%, and 20% in the placebo, lower-dose niacin, and higher-dose niacin groups, respectively, during the course of the study (Grundy et al., 2002).

2.15.5. Aspirin and other antiplatelet agents

The efficacy of antiplatelet therapy in preventing future cardiovascular events appears to be modified by hsCRP level. In the PHS, a large primary prevention trial, the reduction in risk of future MI associated with assignment to aspirin (325 mg on alternate days) was 56% among those with baseline hsCRP levels in the highest quartile and declined proportionately with hsCRP levels such that there was a reduction of only 14% among those in the lowest quartile, suggesting that aspirin may prevent ischemic events through anti-inflammatory as well as antiplatelet effects (Ridker et al., 1997). In support of this finding, observational data indicate that the salutary effects of clopidogrel and abciximab may be strongest in patients with elevated hsCRP levels before percutaneous coronary intervention (PCI) (Chew et al., 2001; Lincoff et al., 2001). In contrast, ticlopidine was associated with a significant risk reduction in subsequent cardiovascular events among ischemic stroke patients with admission hsCRP levels in the bottom 2 tertiles of the sample distribution, whereas a non-significant excess risk was evident among those in the highest hsCRP tertile (Di Napoli and Papa, 2002). Despite the intriguing findings from the PHS, whether aspirin or other antiplatelet agents can lower hsCRP directly is uncertain. Although 1 small trial found that 6 weeks of aspirin therapy (300 mg/d) significantly reduced hsCRP levels in patients with long-term stable angina, (Ikonomidis et al., 1999) other trials, including our own unpublished study, observed no
short-term effect of aspirin in doses ranging from 32 to 325 mg/d on hsCRP levels in healthy individuals (Feng et al., 2000; Feldman et al., 2001).

2.15.6. Antihyperglycemic agents

Given the critical role hsCRP plays in the metabolic syndrome and in the development of diabetes, it is not surprising that efforts to improve glycemic control have been shown to lower hsCRP levels. Pharmacologic studies of metformin and the thiazolidinediones have been highly consistent in this regard. For example, in a 26-week trial among patients with type 2 diabetes, rosiglitazone therapy reduced hsCRP levels and other inflammatory markers (Haffner et al., 2002). Changes in hsCRP level were not correlated with changes in glycemic control, as measured by hemoglobin A1c and fasting glucose level, and were only minimally correlated with changes in insulin resistance. Similar data have been presented by other investigators (Staels et al., 1998). Whether the ability of such agents to lower hsCRP has clinical relevance beyond improvements in glycemic control is an area of active investigation.

2.16. Pleiotropic effects of Statins

2.16.1. Statins and cholesterol

Cholesterol is an essential component of cell membranes and is the immediate precursor of steroid hormones and bile acids (Goldstein and Brown, 1990). However, in excessive amounts, cholesterol becomes an important risk factor for CVD, as demonstrated in clinical trials from the FHS (Gordon and Kannel, 1971; Kannel et al., 1971) and the Multiple Risk Factor Intervention Trial (MRFIT) (Multiple risk factor intervention trial, 1982; Iso et al., 1989). Although dietary cholesterol can contribute to changes in serum cholesterol levels, more than two thirds of the body’s cholesterol is synthesized in the liver. Therefore, inhibition of hepatic cholesterol biosynthesis has emerged as the target of choice for reducing serum cholesterol levels (Panel NCEPE, 2002). The rate-limiting enzyme in cholesterol biosynthesis in the liver is HMG-CoA reductase (Goldstein and Brown, 1990), which catalyzes the conversion of HMG-CoA to mevalonic acid (Rodwell et al., 1976). Inhibitors of HMG-CoA reductase, or statins, were originally identified as secondary metabolites of fungi (Alberts, 1988). HMG-CoA
reductase catalyses the rate-limiting step of cholesterol biosynthesis, a four-electron reductive deacylation of HMG-CoA to CoA and mevalonate. One of the first natural inhibitors of HMG-CoA reductase was mevastatin (compactin, ML-236B), which was isolated from *Penicillium citrinium* by Endo et al., (1976). In its active form, mevastatin resembles the cholesterol precursor, HMG-CoA. When mevastatin was initially administered to rats, it inhibited cholesterol biosynthesis with a Ki of 1.4 nM. Unfortunately, it also caused unacceptable hepatocellular toxicity and further clinical development was discontinued. Subsequently, a more active fungal metabolite, mevinolin or lovastatin, was isolated from *Aspergillus terreus* by Hoffman and colleagues in 1979 (Alberts et al., 1980; Alberts, 1990).

Lovastatin differs from mevastatin in having a substituted methyl group. Compared to mevastatin, lovastatin was a more potent inhibitor of HMG-CoA reductase, with a Ki of 0.6 nM, but did not cause hepatocellular toxicity when given to rats. Lovastatin, therefore, became the first of this class of cholesterol-lowering agents to be approved for clinical use in humans. Since then, several new statins, both natural and chemically modified, have become commercially available, including pravastatin, simvastatin, fluvastatin, atorvastatin, cerivastatin, and most recently, pitavastatin and rosvastatin (Illingworth and Tobert, 2001). Indeed, statins have emerged as one of the most effective class of agents for reducing serum cholesterol levels.

Statins work by reversibly inhibiting HMG-CoA reductase through side chains that bind to the enzyme's active site and block the substrate-product transition state of the enzyme (Istvan and Deisenhofer, 2001). Thus, all statins share an HMG-like moiety and inhibit the reductase by similar mechanism. Recently, the structure of the catalytic portion of human HMG-CoA reductase complexed with different statins was determined (Istvan and Deisenhofer, 2001). The bulky, hydrophobic compounds of statins occupy the HMG binding pocket and block access of the substrate HMG. The tight binding of statins is due to the large number of van der Waals interactions between statins and HMG-CoA reductase. The structurally diverse, rigid, hydrophobic groups of the different statins are accommodated in a shallow nonpolar groove that is present only when COOH-terminal residues of HMG-CoA reductase are disordered. There are subtle
differences in the modes of binding between the various statins, with the synthetic compounds atorvastatin and rosuvastatin having the greatest number of bonding interactions with HMG-CoA reductase (Istvan and Deisenhofer, 2001). Statins bind to mammalian HMG-CoA reductase at nanomolar concentrations, leading to effective displacement of the natural substrate, HMG-CoA, which binds at micromolar concentrations (Moghadasian, 1999).

Oral administration of statins to rodents and dogs showed that these drugs are predominantly extracted by the liver and resulted in >30%-50% reduction in plasma TC levels and substantial decrease in urinary and plasma levels of mevalonic acid, the end product of the HMG-CoA reductase reaction. Similar reduction in cholesterol synthesis and decrease in circulating total and LDL cholesterol by these agents have been subsequently confirmed in humans. Because hepatic LDL cholesterol receptors are the major mechanism of LDL cholesterol clearance from the circulation, the substantial declines in serum cholesterol levels are accompanied by an increase in hepatic LDL cholesterol receptor activity. Statins, therefore, effectively reduce serum cholesterol levels by two separate mechanisms. They not only inhibit endogenous cholesterol biosynthesis via HMG-CoA reductase inhibition but also increase cholesterol clearance from the bloodstream via increases in LDL cholesterol receptor.

The rank order of potency for HMG-CoA reductase inhibition among the second-generation statins is simvastatin > pravastatin > lovastatin = mevastatin, with tissue IC₅₀ values of simvastatin and mevastatin being approximately 4 nM and 20 nM, respectively (Blum, 1994). The IC₅₀ values for these statins correspond to their relative potency for lowering serum cholesterol levels in vivo (i.e., simvastatin > lovastatin) (Dansette et al., 2000). The newer third-generation synthetic statins, which include fluvastatin, cerivastatin, the penta-substituted pyrrole atorvastatin, pitavastatin (NK-104), and rosuvastatin, are much more potent than the mevastatin derivatives (Fig. 2.6). These newer statins are active compounds, which share some physico-chemical properties with pravastatin, but have greater lipophilicity and half-life (McTaggart et al., 2001).
Fig. 2.6 Biological actions of isoprenoids. Diagram of cholesterol biosynthesis pathway showing the effects of inhibition of HMG-CoA reductase by statins

Decrease in isoprenylation of signaling molecules, such as Ras, Rho, and Rac, leads to modulation of various signaling pathways. BMP-2: bone morphogenetic protein-2; eNOS: endothelial nitric oxide synthase; t-PA: tissue-type plasminogen activator; ET-1: endothelin-1; PAI-1: plasminogen activator inhibitor-1.

(James and Ulrich, 2005)
Consequently, these statins, especially atorvastatin, pitavastatin, and rosuvastatin, appear to be quite effective in lowering serum cholesterol levels, perhaps, in part, owing to their ability to bind hepatic HMG-CoA reductase at higher affinity and inhibit the enzyme for a longer duration. Because statins differ in their tissue permeability and metabolism, they possess different potencies for extrahepatic HMG-CoA reductase inhibition. These differences in tissue permeability and metabolism may account for some of the observed differences in their peripheral side effects (Germershausen et al., 1989).

Lipophilic statins, such as simvastatin, are considered more likely to enter ECs by passive diffusion than hydrophilic statins, such as pravastatin and rosuvastatin, which are primarily targeted to the liver. However, lipophilicity does not entirely predict the ability of statins to exert extrahepatic effects in animal and human studies, and so other unidentified factors may play a role. It may be that there are specific mechanisms for hydrophilic statins to enter extrahapetic cells, such as ECs. Such a mechanism is present in the liver, where the organic anion transporter (OATP-C) enables hydrophilic statins to enter hepatocytes (Corsini et al., 1999).

Until recently, all cholesterol-independent or "pleiotropic" effects of statins were believed to be mediated by inhibition of mevalonate synthesis. However, statins can reportedly bind to a novel allosteric site within the β2 integrin, lymphocyte function-associated antigen-1 (LFA-1), independent of mevalonate production (Weitz-Schmidt et al., 2001). LFA-1 belongs to the integrin family and plays an important role in leukocyte trafficking and in T cell activation. Random screening of chemical libraries identified the HMG-CoA reductase inhibitor, lovastatin, as an inhibitor of the LFA-1/ICAM-1 interaction.

2.16.2. Statins and isoprenylated proteins

By inhibiting L-mevalonic acid synthesis, statins also prevent the synthesis of other important isoprenoid intermediates of the cholesterol biosynthetic pathway, such as farnesylpyrophosphate (FPP) and geranylgeranylpyrophosphate (GGPP) (Goldstein and Brown, 1990). These intermediates serve as important lipid attachments for the
posttranslational modification of a variety of proteins, including the γ subunit of heterotrimeric G-proteins; Heme-a; nuclear lamins; and small guanosine triphosphate (GTP)-binding protein Ras; and Ras-like proteins, such as Rho, Rab, Rac, Ral, or Rap (Van Aelst and D’ Souza-Schorey, 1997). Thus, protein isoprenylation permits the covalent attachment, subcellular localization, and intracellular trafficking of membrane-associated proteins.

Members of the Ras and Rho GTPase family are major substrates for posttranslational modification by prenylation (Van Aelst and D’ Souza-Schorey, 1997; Hall, 1998). Both Ras and Rho are small GTP-binding proteins, which cycle between the inactive GDP-bound state and active GTP-bound state (Fig. 2.7). In ECs, Ras translocation from the cytoplasm to the plasma membrane is dependent on farnesylation, whereas Rho translocation is dependent on geranylgeranylation (Laufs et al., 1998; Laufs and Liao, 1998). Statins inhibit both Ras and Rho isoprenylation, leading to the accumulation of inactive Ras and Rho in the cytoplasm.

Because Rho is major target of geranylgeranylation, inhibition of Rho and its downstream target, Rho-kinase, is a likely mechanism mediating some of the pleiotropic effects of statins on the vascular wall (Laufs et al., 2000; Takemoto et al., 2002). Each member of the Rho GTPase family, which consists of RhoA, Rac, and Cdc42, serves specific functions in terms of cell shape, motility, secretion, and proliferation, although overlapping functions between the members could be observed in overexpressed systems.

The activation of RhoA in Swiss 3T3 fibroblasts by extracellular ligands, such as platelet-derived lysophosphatidic acid, leads to myosin light chain phosphorylation and formation of focal adhesion complexes (Hall, 1994; Van Aelst and D’ Souza-Schorey, 1997; Hall, 1998). Indeed, Rho-associated protein kinase increases the sensitivity of vascular smooth muscle to calcium in hypertension (Uehata et al., 1997) and coronary spasm (Katsumata et al., 1997). In contrast, activation of Rac1 leads to the formation of lamellipodia and membrane ruffles, whereas activation of Cdc42 induces actin-rich surface protrusions called filopodia.
Fig. 2.7 Regulation of Rho GTPase by isoprenylation

Rho proteins change between a cytosolic, inactive, GDP-bound state and an active, membrane, GTP-bound state. This cycle is controlled by several cofactors, including guanine nucleotide exchange factors (GEF), GTPase-activating proteins (GAP), and guanine nucleotide dissociation inhibitors (GDI). An important step in the activation of Rho GTPases is the posttranslational isoprenylation, which allows the translocation of Rho to the cell membrane and the subsequent activation.

(James and Ulrich, 2005)
These distinct but complementary functions of Rho family members also extend to their effects on cell signaling. When cells undergo reorganization of their actin cytoskeleton in response to extracellular signals, such as growth factors, or during cell movement and mitosis, they alter the three-dimensional colocalization of intracellular proteins (Van Aelst and D’ Souza-Schorey, 1997; Hall, 1998).

Thus, changes in Rho-induced actin cytoskeleton can affect intracellular transport, membrane trafficking, mRNA stability, and gene transcription. It is therefore not surprising to find that Rho-induced changes in the actin cytoskeleton and gene expression are related. Indeed, experimental evidence suggests that inhibition of Rho isoprenylation mediates many of the cholesterol-independent effects of statins not only in vascular wall cells (Laufs et al., 1998; Laufs et al., 1999), but also in leukocytes (Singh et al., 1999) and bone (Mundy et al., 1999).

### 2.16.3. Statins and endothelial function

The vascular endothelium serves as an important autocrine and paracrine organ that regulates vascular wall contractile state and cellular composition. Hypercholesterolemia impairs endothelial function, and endothelial dysfunction is one of the earliest manifestations of atherosclerosis, occurring even in the absence of angiographic evidence of disease (Liao et al., 1991; Libby, 1995). An important characteristic of endothelial dysfunction is the impaired synthesis, release, and activity of endothelial-derived NO. Endothelial NO has been shown to inhibit several components of the atherogenic process. For example, endothelium-derived NO mediates vascular relaxation (Ignarro et al., 1987) and inhibits platelet aggregation (Radomski et al., 1992), vascular smooth muscle proliferation (Garg and Hassid, 1989), and endothelial-leukocyte interactions (Kubes et al., 1991; Gautheir et al., 1995). Inactivation of NO by superoxide anion (O$_2^-$) limits the bioavailability of NO and leads to nitrate tolerance, vasoconstriction, and hypertension (Munzel et al., 1995; Harrison, 1997).

Acute plasma LDL cholesterol apheresis improves endothelium-dependent vasodilatation (Tamai et al., 1997), suggesting that statins could restore endothelial function, in part, by lowering serum cholesterol levels. However, in some studies with
statins, restoration of endothelial function occurs before significant reduction in serum cholesterol levels (Anderson et al., 1995; Treasure et al., 1995; O’Driscoll et al., 1997), suggesting that there are additional effects on endothelial function beyond that of cholesterol reduction. Indeed, statins increase endothelial NO production by stimulating and upregulating eNOS (Laufs et al., 1998; Kureishi et al., 2000). Furthermore, statins have been shown to restore eNOS activity in the presence of hypoxia (Laufs et al., 1997) and ox-LDL cholesterol (Laufs et al., 1998), conditions which lead to endothelial dysfunction. Statins also increase the expression of tissue-type t-PA (Essig et al., 1998) and inhibit the expression of endothelin-1, a potent vasoconstrictor and mitogen (Hernandez-Perera et al., 1998). Statins, therefore, exert many favorable effects on the endothelium and attenuate endothelial dysfunction in the presence of atherosclerotic risk factors.

Although the effects of statins on Ras and Rho isoprenylation are reversed in the presence of FPP and GGPP, respectively, the effects of statins on eNOS expression is only reversed by GGPP and not by FPP or LDL cholesterol (Laufs and Liao, 1998). Indeed, direct inhibition of geranylgeranyltransferase or RhoA leads to increases in eNOS expression (Laufs and Liao, 1998; Laufs et al., 2000). These findings are consistent with a noncholesterol-lowering effect of statins and suggest that inhibition of RhoA by statins mediates the increase in eNOS expression. Indeed, statins upregulate eNOS expression by prolonging eNOS mRNA half-life but not eNOS gene transcription (Laufs and Liao, 1998). Because hypoxia, ox-LDL cholesterol, and cytokines such as TNF-α decrease eNOS expression by reducing eNOS mRNA stability, the ability of statins to prolong eNOS half-life may make them effective agents in counteracting conditions that down regulate eNOS expression.

Additional important effects of statin treatment on eNOS function include inhibition of caveolin (Brouet et al., 2001; Plenz et al., 2004). Statins also increase eNOS activity via posttranslational activation of the phosphatidylinositol 3-kinase/protein kinase Akt (PI3K/Akt) pathway (Kureishi et al., 2000). Phosphorylation of Akt is an important event in several cellular activities. Indeed, production of NO by the endothelium can be regulated by phosphorylation and activation of eNOS by Akt, which
is promoted in the presence of statins (Fulton et al., 1999; Dimmeler et al., 1999). Caveolin-1 binds to eNOS in caveolae, thereby negatively regulating the enzyme (Dimmeler et al., 1999). Exposure of cultured ECs to hypercholesterolemic serum upregulates caveolin-1 abundance and promotes association of caveolin-1 and eNOS into inhibitory complexes, thereby decreasing NO production (Feron et al., 1999). Statins have been shown to reduce caveolin-1 abundance and decrease its inhibitory action on both basal and agonist-stimulated eNOS activity.

2.16.4. Statins and anti-oxidant effects

Another potential mechanism by which statins may improve endothelial function is through their antioxidant effects. For example, statins enhance endothelium dependent relaxation by inhibiting the production of ROS, such as superoxide and hydroxy radicals, from aortas of cholesterol-fed rabbits (Rikitake et al., 2001). Although lipid lowering by itself can lower vascular oxidative stress (Cai and Harrison, 2000), some of these antioxidant effects of statins appear to be cholesterol-independent.

For example, statins attenuate angiotensin II-induced free radical production in VSMCs by inhibiting Rac1-mediated NADH oxidase activity and downregulating AT1-R expression (Wassmann et al., 2001) (Fig. 2.8). Because NO is scavenged by ROS, these findings indicate that the antioxidant properties of statins may also contribute to their ability to improve endothelial function (Munzel et al., 1995; Harrison, 1997).

2.16.5. Statins and endothelial progenitor cells

Statins have also been found to increase the number of circulating EPCs (Llevadot et al., 2001). EPCs augment ischemia-induced neovascularization (Murohara et al., 2000), accelerate re-endothelialization after carotid balloon injury (Walter et al., 2002; Werner et al., 2002) and improve postischemic cardiac function (Kawamoto et al., 2001). Indeed, statins induce angiogenesis by promoting the proliferation, migration, and survival of circulating EPCs (Dimmeler et al., 2001). In patients with stable CHD, administration of statins for four weeks augmented the number of circulating EPCs and enhanced functional capacity in patients with stable CHD (Vasa, et al., 2001).
Fig. 2.8 Antioxidative mechanisms of statins

The core NAD(P)H oxidase comprises five components: p40phox (PHOX for phagocyte oxidase), p47phox, p67phox, p22phox, and gp91phox. In the resting cell (left), three of these five components, p40phox, p47phox, and p67phox, exist in the cytosol as a complex. The other two components, p22phox and gp91phox, are located in the membranes. When it is stimulated by angiotensin, the cytosolic component becomes heavily phosphorylated and the entire cytosolic complex migrates to the membrane. Activation requires the participation not only of the core subunits but also of two low-molecular-weight guanine nucleotide-binding proteins, Rac and Rap. During activation, Rac binds GTP and migrates to the membrane along with the core cytosolic complex. Treatment with statin downregulates AT1-receptor expression and inhibits Rac1 GTPase, a necessary component of the NAD(P)H oxidase complex.

(James and Ulrich, 2005)
These findings agree with earlier data showing that statins rapidly mobilize EPCs from the bone marrow and accelerate vascular structure formation via activation of PI3K/Akt and eNOS (Kureishi et al., 2000; Dimmeler et al., 2001; Aicher et al., 2003). These angiogenic effects were observed at lower concentrations of statins and were cholesterol-independent. At higher concentrations, statins appear to have an anti-angiogenic effect (Vincent et al., 2002; Park et al., 2002), suggesting a biphasic effect of statins on angiogenesis (Weis et al., 2002). However, this suggestion remains controversial because higher doses of statins have also been shown to be angiogenic (Sata et al., 2001).

2.16.6. Statins and smooth muscle proliferation

The proliferation of VSMCs is a central event in the pathogenesis of vascular lesions, including post-angioplasty restenosis, transplant arteriosclerosis, and veinous graft occlusion (Braun-Dullaeus et al., 1998). Recent studies have shown that statins attenuate vascular proliferative disease, such as transplant-associated arteriosclerosis (Braun-Dullaeus et al., 1998). In contrast to atherosclerosis, transplant-associated arteriosclerosis is more dependent on immunological mechanisms as opposed to lipid disorders, although hypercholesterolemia exacerbates the immunologic process (Jon Kobashigawa et al., 1995). Inhibition of isoprenoid but not cholesterol synthesis by statins decreased PDGF-induced DNA synthesis in VSMCs (Laufs et al., 1999; Yang et al., 2000). Treatment with statins decreased PDGF induced Rb hyperphosphorylation and cyclin-dependent kinases (cdk)-2, -4, and -6 activities. This correlated with increases in the level of Cdk inhibitor, p27Kip1, without concomitant changes in p16INK4, p21Waf1, or p53 levels. These findings indicate that statins inhibit VSMC proliferation by arresting cell cycle between the G1/S phase transition. It remains to be determined whether the upregulation of p27Kip1 is responsible for the cell cycle arrest and whether there are differences between different statins in terms of p27Kip1.

Because the small GTP-binding proteins, Ras and Rho, require posttranslational modification for membrane localization and activity and are implicated in cell cycle regulation, they are likely targets for the direct antiproliferative vascular effects of statins. Ras can promote cell cycle progression via activation of the MAP kinase
pathway (Hughes, 1995), whereas RhoA causes cellular proliferation through destabilizing p27Kip1 protein (Hengst and Reed, 1996). Interestingly, inhibition of VSMC proliferation by statins was reversed by GGPP, but not FPP or LDL cholesterol (Laufs et al., 1999). Indeed, direct inhibition of RhoA by Clostridium botulinum C3 transferase, which ADP-ribosylates and inactivates RhoA, or by a dominant-negative RhoA mutant increased p27Kip1 and inhibited Rb hyperphosphorylation and VSMC proliferation following PDGF stimulation. Taken together, these findings indicate that RhoA mediates PDGF-induced VSMC proliferation and that inhibition of RhoA by statins is the predominant mechanism by which statins inhibit VSMC proliferation.

2.16.7. Statins and platelet function

Platelets play a critical role in the development of ACS (Fitzgerald et al., 1986). Circulating platelets are associated with mural thrombus formation at the site of plaque rupture and vascular injury (Lacoste et al., 1995; Willerson et al., 1989). Hypercholesterolemia is associated with increases in platelet reactivity (Opper et al., 1995). These abnormalities are linked to increases in the cholesterol/phospholipid ratio in platelets. Other potential mechanisms include increases in thromboxane A2 (TXA2) biosynthesis (Notarbartolo et al., 1995), platelet a2-adrenergic receptor density (Baldassarre et al., 1997), and platelet cytosolic calcium (Le Quan Sang et al., 1995).

Statins have been shown to influence platelet function, although the precise mechanisms involved are not fully understood (Hale et al., 1998; Huhle et al., 1999). One of the well-characterized effects of endothelial NO is the inhibition of platelet aggregation (Radomski et al., 1992). Statin-mediated upregulation of eNOS has been shown to be associated with downregulation of markers of platelet reactivity (Laufs et al., 2000). Potential additional mechanisms include a reduction in the production of TXA2 and modifications in the cholesterol content of platelet membranes (Lijnen et al., 1996; Vaughan et al., 2000). The cholesterol content of platelet and erythrocyte membranes is reduced in patients taking statin therapy. This may lead to a decrease in the thrombogenic potential of these cells. Indeed, animal studies suggest statin therapy inhibits platelet deposition on damaged vessels and reduces platelet thrombus.
formation (Lacoste et al., 1995; Alfon et al., 1999). Furthermore, in vitro experiments have demonstrated that statins inhibit tissue factor expression by macrophages, thereby potentially reducing thrombotic events in the vascular wall (Aikawa et al., 2001).

2.16.8. Statins and plaque stability

Plaque rupture is a major cause of ACS (Fuster et al., 1990; Libby, 1995; Fuster, 1995). The atherosclerotic lesion contains highly thrombogenic materials in the lipid core that are separated from the bloodstream by a fibrous cap (Fernandez-Ortiz et al., 1994). Fissuring, erosion, and ulceration of the fibrous cap eventually lead to plaque rupture and ensuing thrombosis (Fuster et al., 1990). Collagen is the main component of fibrous caps and is responsible for their tensile strength. Because macrophages are capable of degrading the collagen-containing fibrous cap, they play an important role in the development and subsequent stability of atherosclerotic plaques (Moreno et al., 1994; Shah et al., 1995).

Indeed, degradation of the plaque matrix appears to be most active in macrophage-rich regions (Libby, 1995; Fuster, 1995). Secretion of proteolytic enzymes, such as metalloproteinases (MMPs), by activated macrophages may weaken the fibrous cap, particularly at the “vulnerable” shoulder region where the fibrous cap joins the arterial wall (Richardson et al., 1989; Henney et al., 1991). Weakened fibrous caps lead to plaque instability, rupture, and ensuing thrombosis, which ultimately present as ACS (Libby, 1995; Fuster et al., 1990; Davies, 1995).

Lipid lowering by statins may contribute to plaque stability by reducing plaque size or by modifying the physiochemical properties of the lipid core (Koh, 2000; Fukumoto et al., 2001). However, changes in plaque size by lipid lowering tend to occur over extended time and are quite minimal as assessed by angiography. Rather, the clinical benefits from lipid lowering are probably due to decreases in macrophage accumulation in atherosclerotic lesions and inhibition of MMP production by activated macrophages (Aikawa et al., 2001). Indeed, statins inhibit the expression of MMPs and tissue factor by cholesterol-dependent and -independent mechanisms (Aikawa et al., 2001; Fukumoto et al., 2001; Bourcier and Libby, 2000), with the cholesterol-independent
or direct macrophage effects occurring at a much earlier time point. The plaque-stabilizing properties of statins, therefore, are mediated through a combined reduction in lipids, macrophages, and MMPs (Crisby et al., 2001). These effects of statins may reduce the incidence of ACS by lessening the propensity for plaque to rupture and may explain the rapid time course of event reduction in patients at high risk for recurrent coronary ischemia in the Myocardial Ischemia Reduction with Aggressive Cholesterol Lowering (MIRACL) (Schwartz et al., 2001) and Pravastatin or Atorvastatin Evaluation and Infection trials (PROVE-IT) (Cannon et al., 2004).

2.16.9. Statins and vascular inflammation

Atherosclerosis is a complex inflammatory process that is characterized by the presence of monocytes or macrophages and T lymphocytes in the atheroma (Ross, 1993; Ross, 1999). Inflammatory cytokines secreted by these macrophages and T lymphocytes can modify endothelial function, VSMC proliferation, collagen degradation, and thrombosis (Libby, 1995). An early step in atherogenesis involves monocyte adhesion to the endothelium and penetration into the subendothelial space (Ross, 1999). Recent studies suggest that statins possess anti-inflammatory properties owing to their ability to reduce the number of inflammatory cells in atherosclerotic plaques (Vaughan et al., 2000). The mechanisms have yet to be fully elucidated but may involve inhibition of adhesion molecules, such as ICAM-1, which are involved in the recruitment of inflammatory cells (Niwa et al., 1996). Furthermore, statins attenuate P-selectin expression and leukocyte adhesion in normocholesterolemic animals by increasing endothelial NO production (Lefer et al., 1999; Scalia et al., 2001). This cholesterol-independent effect of statins was absent in eNOS-deficient mice, suggesting that eNOS mediated the vascular protective effects of statins (Stalker et al., 2001).

The activation of T-lymphocytes and the control of the immune response are mediated by the major histocompatibility complex class II (MHC-II) and CD40/CD40L. Under physiological conditions, antigen-presenting cells express MHC-II constitutively, whereas the induction of INF-γ leads to an increase of MHC-II expression in numerous cells, including human ECs and monocytes. An important regulator in this pathway is
the transactivator CIITA. Statins inhibit MHC-II expression on ECs and monocyte-macrophages via inhibition of the promoter IV of the transactivator CIITA and thereby repress MHC-II-mediated T cell activation (Kwak et al., 2000). In addition, statins have been shown to decrease CD40 expression and CD40-related activation of vascular cells (Mulhaupt et al., 2003).

A clinical marker of inflammation is hsCRP (Ridker et al., 2001). HsCRP is an acute phase reactant that is produced by the liver in response to proinflammatory cytokines, such as IL-6, and reflects low-grade systemic inflammation (Baumann and Gaudie, 1994). Statin therapy lowers hsCRP levels in hypercholesterolemic patients (Ridker et al., 2001; Musial et al., 2001; Ridker et al., 2001). In the Cholesterol and Recurrent Events (CARE) trial, statins significantly decreased plasma hsCRP levels over a five-year period in patients who did not experience recurrent coronary events (Ridker et al., 1998; Ridker et al., 1999). Similarly, an analysis of baseline and one-year follow-up from the Air Force/Texas Coronary Atherosclerosis Prevention Study (AFCAPS/TexCAPS) demonstrated that hsCRP levels were reduced in statin-treated patients who were free of acute major coronary events (Ridker et al., 2001).

Furthermore, preliminary data from the Pravastatin Inflammation/CRP Evaluation (PRINCE) study confirm that statin therapy can significantly reduce serum hsCRP levels in primary and secondary prevention populations (Albert et al., 2001). Following 24 weeks of therapy with a statin, the hsCRP level was reduced by approximately 13% in primary and secondary prevention populations, whereas placebo treatment of subjects in the primary prevention arm of the study had no effect. These studies, therefore, indicate that statins are effective in decreasing systemic and vascular inflammation. However, any potential clinical benefits conferred by the lowering hsCRP are difficult to separate from that of the lipid-lowering effects of statins without performing further clinical studies. The JUPITER trial is the first large-scale, multinational, double-blind, placebo-controlled clinical trial to investigate the effects of statins in the primary prevention of cardiovascular events in individuals with low levels of LDL cholesterol who may be at risk because of their elevated hsCRP levels. These studies indicate that the hsCRP level achieved after initiation of statin therapy may be as
important as the LDL cholesterol level achieved. All of these data raise the possibility that hsCRP could be used to target high-risk patients who may benefit from early statin use. Ongoing work will determine whether hsCRP reduction, independent of LDL cholesterol reduction, results in a net clinical benefit (Samia et al., 2006).

2.16.10. Statins and its effects on the myocardium

Cardiac hypertrophy is an adaptive response of the heart to pressure overload. In the myocardium, the small GTP-binding proteins, Ras, Rho, and Rac, and oxidative stress are involved in the hypertrophic response (Wang et al., 1997; Thorburn et al., 1997). Indeed, recent animal studies suggest that a phagocyte-type NADPH oxidase may be a relevant source of ROS in the myocardium (Aikawa et al., 2001; MacCarthy et al., 2001; Bendall et al., 2002).

NADPH oxidase-dependent ROS production appears to be involved in cardiac hypertrophy in response to pressure overload (MacCarthy et al., 2001; Li et al., 2002), stretch (Aikawa et al., 1999), angiotensin II-infusion (Bendall et al., 2002; Nakagami et al., 2003), and α-adrenergic stimulation (Xiao et al., 2002). In the cardiomyocytes, three of its five components, p40phox (PHOX for phagocyte oxidase), p47phox, and p67phox, exist in the cytosol, forming a complex. The other two components, p22phox and gp91phox, are bound to the membranes. Various stimuli lead to the phosphorylation of the cytosolic components, and the entire cytosolic complex then migrates to the membrane.

Importantly, not only the core subunits but also two low-molecular-weight guanine nucleotide-binding proteins, Rac1 and Rap, are required for activation. During activation, Rac1 binds GTP and migrates to the membrane with the core cytosolic complex. Therefore, Rac1 is critically involved in the activation of cardiovascular NADPH oxidase. Recent evidence both from animal and from human studies indicates that in failing myocardium, upregulation of Rac1 and p47phox membrane protein expression, as well as increased Rac1-GTPase activity, may resemble the underlying mechanisms for increased oxidase activity and may represent a novel therapeutic target for statin therapy.
Although the main impact of statin therapy in CVD appears to be predominantly vascular, recent animal and human studies suggest that statins may also have direct beneficial effects on the myocardium. Because Rac1 is required for NADPH oxidase activity and cardiac hypertrophy is mediated, in part, by myocardial oxidative stress, it is likely that statins could inhibit cardiac hypertrophy through an antioxidant mechanism involving inhibition of Rac1 geranylgeranylation. Indeed, statins inhibit angiotensin II-induced oxidative stress and cardiac hypertrophy in rodents (Takemoto et al., 2001). This has also been observed in clinical studies where statins inhibit cardiac hypertrophy in humans with hypercholesterolemia (Lee et al., 2002). NADPH-oxidase-mediated ROS are increased in left ventricular myocardium from patients with heart failure and correlate with an increased activity of Rac1 GTPase, and oral statin treatment is able to decrease Rac1 function in the human heart (Maack et al., 2003).

The development of congestive heart failure (CHF), a common sequela of decompensated cardiac hypertrophy, is a major cause of death and morbidity in the Western world. Several lines of evidence suggest that statins may emerge as a novel treatment option for patients with CHF. Retrospective analysis of the large statin trials, such as the Scandinavian Simvastatin Survival Study (4S), suggests that statins reduce the incidence and morbidity of heart failure (Kjekshus et al., 1997). Second, patients with heart failure are characterized by increased vascular tone and endothelial dysfunction (Drexler, 1998), which may be improved by statin therapy, irrespective of serum cholesterol levels. Third, statins have proven to preserve cardiac function in animal models of myocardial hypertrophy and heart failure, such as aortic banding, MI, and several transgenic models (Takemoto et al., 2001; Bauersachs et al., 2001; Dechend et al., 2001; Laufs et al., 2002).

In a recent prospective, double blind, placebo-controlled study, patients with symptomatic, nonischemic, dilated cardiomyopathy were randomly divided into two groups receiving statin or placebo for 14 weeks (Node et al., 2003). Although patients receiving statins exhibited a modest reduction in serum cholesterol level compared to patients receiving placebo, these patients demonstrated a significant improvement in exercise endurance, as exhibited by a lower New York Heart Association functional class.
Id to patients receiving placebo. This corresponded to improved left ventricular function in the statin group, but not in the placebo group. The improvements in their exercise endurance and heart function were in addition to the improvements already observed with two current treatments for heart failure, beta-blockers and angiotensin converting enzyme (ACE) inhibitors.

Furthermore, plasma concentrations of TNF-α, IL-6, and brain natriuretic peptide (BNP) were lower in the statin group compared to the placebo group. This study indicates that short-term statin therapy improves cardiac function, neurohormonal imbalance, and symptoms associated with idiopathic dilated cardiomyopathy. These observations were confirmed in a second study using cerivastatin (Laufs et al., 2004). These findings suggest that statins may have therapeutic benefits in patients with heart failure irrespective of serum cholesterol levels or atherosclerotic heart disease.

2.16.11. Statins and ischemic stroke

Although MI is closely associated with serum cholesterol levels, neither the FHS nor the MRFIT demonstrated significant correlation between ischemic stroke and serum cholesterol levels (Kannel et al., 1971). An intriguing result of large clinical trials with statins is the reduction in ischemic stroke (Crouse et al., 1998). For example, the recent Heart Protection Study (HPS) shows a 28% reduction in ischemic strokes in over 20,000 people with cerebrovascular disease or other high-risk conditions (Collins et al., 2004).

The proportional reductions in stroke were approximately one quarter in all subcategories studied, including those aged over 70 years at entry and those presenting with different levels of BP or lipids, even when the pretreatment LDL cholesterol was below 116 mg/dl. Thus, the findings of these large statin trials raise the interesting question of how a class of cholesterol-lowering agents can reduce ischemic stroke when ischemic stroke is not related to cholesterol levels. It appears likely that there are cholesterol-independent effects of statins, which are beneficial for ischemic stroke. Some of these beneficial effects may relate to the effects of statins on endothelial and platelet function.
Cerebrovascular tone and blood flow are regulated by endothelium-derived NO (Dalkara et al., 1994). Mutant mice lacking eNOS (eNOS/-) are relatively hypertensive and develop greater proliferative and inflammatory response to vascular injury (Huang et al., 1995). Indeed, eNOS/- mice develop larger cerebral infarcts following cerebrovascular occlusion (Huang et al., 1996). Thus, the beneficial effects of statins in ischemic stroke may, in part, be due to their ability to upregulate eNOS expression and activity (Laufs et al., 1998; Kureishi et al., 2000). For example, mice that were prophylactically treated with statins for up to two weeks have 25%-30% higher cerebral blood flow and 50% smaller cerebral infarct sizes following cerebrovascular occlusion (Endres et al., 1998). No increase in cerebral blood flow or neuroprotection was observed in eNOS/- mice treated with statins, indicating that the upregulation of eNOS accounts for most, if not all, of the neuroprotective effects of these agents. Interestingly, treatment with statins did not affect BP or heart rate before, during, or after cerebrovascular ischemia and did not alter serum cholesterol levels in mice, consistent with the cholesterol-independent, neuroprotective effects of statins.

In addition to increases in cerebral blood flow, other beneficial effects of statins are likely to occur that can impact on the severity of ischemic stroke. For example, statins attenuate P-selectin expression and leukocyte adhesion via increases in NO production in a model of cardiac ischemia and reperfusion (Scalia et al., 1999; Lefer et al., 2001). Others have reported that statins upregulate tissue-type t-PA and downregulate PAI-1 expression through a similar mechanism involving inhibition of Rho geranylgeranylation (Essig et al., 1998). Thus, the absence of neuroprotection in eNOS-deficient mice emphasizes the importance of endothelium-derived NO in not only augmenting cerebral blood flow but also, potentially, in limiting the impact of platelet and white blood cell accumulation on tissue viability following ischemia. In humans, atherosclerosis of precerebral arteries causes stroke through plaque disruption and artery-to-artery thromboembolism, and, in contrast to the mouse models, statins exert additional stroke-protective effects in humans through their anti-atherosclerotic and plaque stabilizing effects. Furthermore, the anti-inflammatory actions and mobilization of EPc of statins may also contribute to neuroprotection. It is therefore possible that
Statins have contributed to the decrease in the incidence of ischemic strokes in clinical trials, in part, by reducing cerebral infarct size to levels that were clinically unappreciated.

2.16.12. Statins and dementia

Recent epidemiological reports suggest that statins might be protective for Alzheimer’s disease, and for other types of dementia (Vaughan, 2003). Dementia is a syndrome of chronic or progressive nature with multiple disturbances of higher cortical functions. This syndrome occurs in Alzheimer’s disease, in cerebrovascular disease (i.e., multi-infarct dementia), and in other conditions primarily or secondarily affecting the brain. Alzheimer’s disease is related to the effects of β-amyloid, a peptide that accumulates in the brain, causing neurotoxicity and neurodegeneration. Experimental and clinical studies suggest that there is a pathophysiologic relation between β-amyloid and cholesterol levels. Elevated β-amyloid levels and the ε4 allele of the apolipoprotein E (Apo E4) are risk factors for Alzheimer’s disease (Corder et al., 1993). In addition, Apo E4 is correlated with increased risk for atherosclerosis and amyloid plaque formation (Bales et al., 1997; Hofman et al., 1997). Observational studies revealed that an elevated serum cholesterol level is a risk factor for Alzheimer’s disease (Jarvik et al., 1995). Statins, regardless of their brain availability, have been suggested to induce alterations in cellular cholesterol distribution in the brain. Such cholesterol-independent effects of statins might be mediated via NO or Apo E (Kirsch et al., 2003; Fassbender et al., 2001). Across-sectional analysis of three hospital databases by Wolozin and colleagues suggested that the prevalence of Alzheimer’s disease in patients taking statins is 60% lower in comparison to patients taking other medications used in the treatment of CVD (Wolozin et al., 2000).

A nested case control study based on the United Kingdom-based General Practice Research Database showed that among individuals 50 years and older with a statin therapy, the risk for developing dementia was significantly reduced, independent of their lipid status (Jick et al., 2000). Furthermore, other lipid-lowering agents had no influence on the risk of developing dementia in this population. The systemic vascular
protective effects of statin treatment are very likely to contribute to their beneficial effects, especially on vascular forms of the dementia syndrome. However, the precise underlying molecular mechanisms are poorly understood. Indeed, the results of the recent HPS and Prospective Study of Pravastatin in the Elderly at Risk (PROSPER) trials do not demonstrate the efficacy of statins in slowing cognitive decline and dementia (Shepherd et al., 2002).

Recent evidence has stimulated the discussion of statins as potential novel anti-inflammatory and vascular protective agents for the treatment of other cerebral diseases, such as multiple sclerosis and depression. Oral atorvastatin was shown to prevent chronic and relapsing experimental autoimmune encephalomyelitis, a CD4 (+) Th1-mediated central nervous system (CNS) demyelinating disease model of multiple sclerosis (Youssef et al., 2002). Statin induced STAT6 phosphorylation and secretion of Th2 cytokines and TGFβ. Conversely, STAT4 phosphorylation was inhibited and secretion of Th1 cytokines was suppressed. Statin promoted the differentiation of Th0 cells into Th2 cells. In adoptive transfer, these Th2 cells protected recipient mice from induction of autoimmune encephalomyelitis.

Statin reduced CNS infiltration and mMHC-II expression. Treatment of microglia inhibited IFN-γ -inducible transcription at multiple MHC-II transactivator (CIITA) promoters and suppressed MHC-II upregulation. Statin suppressed IFN-γ -inducible expression of CD40, CD80, and CD86 costimulatory molecules as well as antigen-specific T cell activation. Similarly, a second study shows that oral statin inhibited the development of actively induced chronic CD4+T cell-mediated experimental autoimmune encephalomyelitis in a preventive and therapeutic fashion and significantly reduced the inflammatory infiltration into the CNS (Aktas et al., 2003). This potentially therapeutic effect was associated with downregulation of Th1 immune response. In addition, similar to the effects of statins in VSMCs, statins inhibited the cell cycle of human antigen-specific T cells. Thus, statins exert pleiotropic immunomodulatory effects involving both antigen presenting cells (APC) and T cell compartments, and they may be beneficial for multiple sclerosis and other Th1-mediated autoimmune diseases.
A possible association between lipid-lowering drug therapy and psychological well-being has been an issue of debate. A recent nested case-control analysis of the United Kingdom General Practice Research Database revealed that the use of statins and other lipid-lowering drugs is not associated with an increased risk of depression or suicide (Yang et al., 2003). On the contrary, individuals with current statin use may have a lower risk of developing depression, an effect that could be explained by improved quality of life owing to decreased risk of cardiovascular events or more health consciousness in patients receiving long-term treatment. Furthermore, another comparison of patients who had continuous use of statins with the patients who did not use any cholesterol-lowering drugs showed that statin use was associated with lower risk of abnormal depression scores, anxiety, and hostility, after adjustment for the propensity for statin use and potential confounders. Interestingly, the beneficial psychological effects of the statins appeared to be independent of the drugs' cholesterol-lowering effects (Young-Xu et al., 2003).

2.16.13. Statins and nephropathy

The potential to ameliorate the impact of diabetes on renal function (Usui et al., 2003) and to slow the progression to end stage renal disease are among the most promising roles for statins in the field of nephrology. Diabetic nephropathy is a major cause of end stage renal disease. Its pathology includes the accumulation of ECM around the glomerulus and leukocyte infiltration into the glomerulus. These insults lead to glomerular hypertrophy and proteinuria. Using an experimental animal model of diabetic nephropathy, Usui et al., (2003) demonstrated a decrease in glomerular hypertrophy and albuminuria with cerivastatin treatment (Usui et al., 2003). One potential mechanism for this benefit was that cerivastatin inhibited leukocyte adhesion molecule expression on the glomerular ECs, hence decreasing macrophage migration into the glomeruli (Usui et al., 2003).

Statins may also benefit patients with chronic glomerulonephritis (CGN) such as immunoglobulin A (IgA) nephropathy. Podocytes, which are essential components of the structure and function of the glomerulus, are damaged in these conditions
(Nakamura et al., 2002), as well as in diabetes. Consequently, the appearance of podocytes and protein in the urine is used as markers of disease progression. In a placebo-controlled human study of 40 patients with CGN, Nakamura et al., (2002) found that cerivastatin-treated patients, compared with placebo, had significantly fewer numbers of podocytes in the urine and milder proteinuria. This reduction may be due to statins' inhibition of both monocyte infiltration into and platelet activation within the glomeruli (Nakamura et al., 2002).

Statins have also been reported to attenuate renal injury after an ischemic event. This has been shown in two animal models of renal ischemia-reperfusion injury (IRI) (Joyce et al., 2001; Yokota et al., 2003), which were designed to approximate renal injury and acute tubular necrosis in humans. Pre-treatment with pravastatin (Joyce et al., 2001) or cerivastatin (Yokota et al., 2003) decreased the effect of the ischemic insult when compared to placebo as evidenced by improved urine production and diminished protein leakage. The acute nature of the effects (<24 h) pointed toward effects that are independent of lipid lowering. Potential mechanisms for this benefit include both the anti-inflammatory properties of statins and improved endothelial function (Yokota et al., 2003; Joyce et al., 2001).

Furthermore, pre-medication with statins prior to the use of radiographic contrast agents may have protective effects on renal function. Contrast agents are thought to cause acute renal failure by direct toxic effects on the renal tubules and by inducing renal vasoconstriction. In a retrospective study, Attallah et al., (2004) compared statins versus placebo in 1,002 patients with chronic mild to moderate renal insufficiency undergoing cardiac catheterization. Patients pre-treated with statins had a lower incidence of acute renal failure and a lower post-catheterization serum creatinine compared to placebo. (Attallah et al., 2004).

2.16.14. Statins and autoimmune disease

Statins may have beneficial effects on autoimmune diseases such as rheumatoid arthritis, diabetes mellitus, psoriasis and inflammatory bowel disease (IBD) (Leung et al., 2003). Modulation of T lymphocyte proliferation, differentiation and activation appears
to be the primary mechanism. Macrophages are thought to express MHC-II when they are activated (Ross, 1999). This allows them to activate T cells that, in turn, contribute to the inflammatory process (Hansson et al., 1989). Statins inhibit the expression of MHC-II on human macrophages and ECs. They also inhibit the INF-γ induced up-regulation of MHC-II expression on smooth muscle cells (SMC) (Hansson et al., 1989; Kwak et al., 2000). This inhibition leads to a reduction of T cell proliferation and differentiation and subsequently to a reduction in the release of pro-inflammatory cytokines (Palinski and Tsimikas, 2002; Leung et al., 2003).

Statins also inhibit the activity of the β2 integrin, LFA-1 (Weitz-Schmidt et al., 2001), which is expressed on the surface of leukocytes and participates in the process of T cell activation. Lovastatin and simvastatin bind to an allosteric site within LFA-1 and interfere with its activation and consequent pro-inflammatory effect (Weitz-Schmidt et al., 2001). In a murine model of inflammatory arthritis, simvastatin suppressed both developing and clinically evident cases of the disease. Progression of synovial hyperplasia and erosion of cartilage and bone were inhibited by doses of statins that did not significantly alter cholesterol levels (Leung et al., 2003). The immunomodulatory effects of statins may also be beneficial for recipients of solid organ transplants.

This is especially true for heart transplant patients in whom statins have been shown to improve survival and to decrease certain types of rejection (Jon Kobashigawa et al., 1995; Mehra et al., 2002). Jon Kobashigawa et al., (1995) randomized 97 post-cardiac transplant patients to receive either pravastatin or placebo. Patients in the pravastatin arm had a significant decrease in the frequency of major cardiac transplant rejection that was defined as rejection accompanied by hemodynamic compromise. However, the study showed no difference in the incidence of mild and moderate cases of cardiac rejection. Furthermore, patients in the pravastatin arm had both significantly greater 1-year survival and less coronary vasculopathy as measured by intracoronary ultrasonography.

Interestingly, the incidence of major cardiac transplant rejection and the degree of coronary vasculopathy did not correlate with higher levels of serum cholesterol; thus,
suggesting an alternative mechanism for the benefit of statins (Jon Kobashigawa et al., 1995). However, the evidence for benefit in renal allograft transplant patients is controversial. A recent literature review (Lentine and Brennan, 2004) examined five randomized clinical trials that investigated the effect of statins on the rate of acute renal allograft rejection. Two studies, each with a relatively small sample size, showed significant decreases in rejection rates (Katznelson et al., 1996; Tuncer et al., 2000). Three more recent and larger studies did not find any such benefit (Kaisiske et al., 2001; Holdaas et al., 2001; Sahu et al., 2001).

2.16.15. Statins and sepsis

Statin use may decrease mortality in patients with bacterial-induced sepsis. Possible mechanisms include interference with leukocyte–endothelium interaction, prevention of toxin-induced cellular damage and modulation of endothelial function. Leukocyte–endothelial interaction is a critical event that precedes the trans-migration of leukocytes from the vasculature to tissue (Pruefer et al., 2002). Statins interfere with many of the steps involved in this process by increasing the level of endothelial NO, inhibiting the adhesion of monocytes to the endothelium and by inhibiting the release of polymorphonuclear (PMN) cell chemoattractants (Pruefer et al., 2002).

Statins also exert activity against the α toxin released by *Staphylococcus aureus* in septic patients. Alpha toxin causes the activation of various inflammatory mediators and may provoke cardiovascular collapse (Adamo et al., 1989; Sibelius et al., 2000; Burke et al., 2002). It is also thought to have profound negative inotropic effects and to induce coronary vasoconstriction. Alpha toxin activity is mediated by increases in the formation of thromboxane, a vasoconstrictor, and by up-regulation of the adhesion molecules P selectin and ICAM-1 that mediate PMN cell adherence to the vascular endothelium. Pruefer et al., (2002) demonstrated that simvastatin, given 18 hours before injection of a toxin, decreased PMN adherence and migration, decreased P selectin expression on ECs and increased endothelial NO availability in rats. In another study, statins significantly decreased overall mortality in bacteremic patients compared to controls (Liappis et al., 2001).
2.16.16. Statins and gastrointestinal diseases

There is some evidence that statins may decrease the incidence of colon cancer (Sacks et al., 1996; Pedersen et al., 1996) and potentially ameliorate some of the effects of IBD (Sasaki et al., 2003). Lovastatin, in combination with the chemotherapeutic agents 5-FU and cisplatin, was found to increase apoptosis and to decrease the proliferation of cells in colon cancer cell lines, thereby inhibiting the progression of the disease. Furthermore, the addition of statins to the therapeutic regimen allowed for the use of lower doses of chemotherapeutic drugs resulting in milder side effects (Agarwal et al., 1999). In an experimental murine IBD model, pravastatin reduced the degree of cachexia, hematochezia and intestinal epithelial permeability compared to control (Sasaki et al., 2003). Increased local production of NO with a subsequent reduction of both gut endothelial adhesion molecule (i.e., MAdCAM-1) expression and leukocyte infiltration into the gut wall has been postulated as the mechanism of benefit (Sasaki et al., 2003).

2.16.17. Statins bone remodeling osteoporosis

The evidence supporting a beneficial effect of statins in osteoporosis is equivocal (Bauer et al., 2004). Many in vivo and in vitro animal studies have suggested such benefits (Mundy et al., 1999; Song et al., 2003) and human observational studies have also indicated as such (Chan et al., 2000; Meier et al., 2000). However post-hoc analysis of prospective human studies has failed to show a statistically significant change in bone fractures with statin therapy. Consequently, prospective randomized controlled trials have been advocated (Bauer et al., 2004).

2.16.18. Statins and macular degeneration

Age-related macular degeneration (AMD) is a retinal disorder that is considered the leading cause of blindness in the Western world. There is conflicting evidence regarding the association of statin use with the prevalence, incidence and progression of AMD (Klein et al., 2003; Hall et al., 2001). However, recent evidence indicates that statin use decreases the incidence of choroidal neo-vascularization (CNV), which has been
associated with a large proportion of severe vision loss among patients with AMD (Wilson et al., 2004).

2.16.19. Statins and non-cardiac vascular surgery

Emerging evidence suggests a benefit for short-term statin administration perioperatively in patients undergoing non-cardiac vascular surgery. Durazzo et al., (2004) prospectively studied the effect of a short, 45-day course of atorvastatin in 100 patients undergoing various non-cardiac vascular surgeries. Compared to placebo, the statin arm of the study had an 18% absolute risk reduction, 26% to 8%, for the combined incidence of cardiac death, non-fatal MI, unstable angina and stroke after a 6-month follow-up period. Interestingly, although LDL cholesterol was decreased in the statin arm of the study, the short duration of therapy suggests that lipid-independent properties of statins may have contributed to the benefits observed.

2.17. The benefits of Statin therapy
2.17.1. Secondary prevention trials

Early statin trials, such as the Scandinavian Simvastatin Survival Study, (1994) revealed that statin therapy reduced coronary morbidity and mortality as well as all-cause mortality (Fig. 2.9). Although the CARE trial suggested a possible threshold of baseline LDL cholesterol below which no further benefit was observed (Sacks et al., 1996) the larger HPS suggested similar benefits in high-risk patients across baseline LDL cholesterol (Heart Protection Study collaborative Group, 2002). Recently completed open-label trials of atorvastatin addressed the benefit of intensive lipid lowering versus usual care. In patients with established CHD treatment with atorvastatin, targeted to meet NCEP LDL cholesterol treatment goals versus usual care, resulted in a 95 versus 3% goal attainment (Athyros et al., 2002). The number of coronary events and deaths in the usual-care group was twice that in the atorvastatin group (Athyros et al., 2002). Aggressive treatment with atorvastatin yielded a 15% reduction in LDL cholesterol levels, a 17% reduction in major cardiovascular events, and a 47% reduction in MI versus usual care in another secondary prevention trial (Koren and Hunninghake, 2004).
Fig. 2.9 Secondary prevention statin trials:
Placement of boxes against timeline represents study duration from its onset to publication of results.
N = number of subjects, ▼ = involves intensive lipid lowering, » = ongoing study.
ACS = acute coronary syndrome, CA = coronary atherosclerosis, CABG = coronary artery bypass graft, CAD = coronary artery disease, CHD = coronary heart disease, CV = cardiovascular, DYS = dyslipidemia, LDL-C = low-density lipoprotein cholesterol, MI = myocardial infarction, PCI = percutaneous coronary intervention, PTCA = percutaneous transluminal coronary angioplasty. (Antonio et al., 2005)
Although earlier trials examined treatment effects in patients with stable CHD, recent studies have examined the impact of early, aggressive statin therapy in patients with ACS. A complicated study of moderate versus high-dose simvastatin reported no greater benefit of the more intensive approach (de Lemos et al., 2004). In contrast, atorvastatin 80 mg/day versus placebo reduced recurrent ischemia in patients with ACS by 26% in 16 weeks of treatment (Schwartz et al., 2001). Over a longer term, robust lipid lowering with atorvastatin reduced deaths and major cardiovascular events by 16% versus moderate therapy with pravastatin in patients with ACS (Cannon et al., 2004). The recently completed Treating to New Targets (TNT) study returned to a patient population (n = 10,003) with stable CHD.

In TNT, treatment with 80 mg/day of atorvastatin reduced LDL cholesterol levels to 77 mg/dl and resulted in a 22% risk reduction for the primary endpoint (major cardiovascular events including CHD death, nonfatal MI, resuscitated cardiac arrest, or stroke) compared with 10 mg/day atorvastatin, which reduced LDL cholesterol to 101 mg/dl (LaRosa et al., 2005). Furthermore, even in the 10 mg/day group, the event rate was lower than rates reported with statin treatment in placebo-controlled secondary prevention trials of populations with a similar baseline risk. Data from these trials have consistently shown a positive relation between event rates and LDL cholesterol levels achieved, thus supporting the "lower LDL cholesterol is better" concept.

2.17.2. Primary prevention trials

Primary prevention trials have expanded the populations shown to benefit from statins to include patients without CHD (Fig. 2.10). Most recently, simvastatin 40 mg/day versus placebo reduced the incidence of a first major vascular event by 24% (Heart Protection Study Collaborative Group, 2002). The use of statins in primary prevention has been controversial because of issues concerning cost effectiveness and benefit-to-risk. Patent expiry and the resulting generic pricing may ameliorate economic issues. More pressing is the issue of safety. To date, primary prevention trials have not identified greater safety risks in treating patients with statins versus placebo; however, the selected cohorts may differ from the overall population.
Fig. 2.10 Primary prevention statin trials: Placement of boxes against timeline represents study duration from its onset to publication of results

FH = familial hypercholesterolemia, HDL-C = high-density lipoprotein cholesterol, HTN = hypertension, NIDDM = non-insulin-dependent diabetes mellitus, PMP = postmenopausal. Other abbreviations as in Fig 2.9.

(Antonio et al., 2005)
The first large primary prevention trial involving patients with hypertension studied pravastatin versus usual care and reported no difference (ALLHAT Officers and Coordinators for the ALLHAT Collaborative Research Group, 2002). This was likely because of statin use in one third of the usual-care patients, resulting in a nonsignificant LDL cholesterol difference between the pravastatin and usual care groups. In contrast, the lipid-lowering arm of the Anglo-Scandinavian Cardiac Outcomes Trial (ASCOT-LLA), involving hypertensive, normocholesterolemic patients, ended 2 years earlier than expected because of a significant 36% risk reduction in the primary endpoint of combined nonfatal MI and fatal CHD with atorvastatin versus placebo treatment (Sever et al., 2003).

As was observed in HPS, statin therapy provided similar CV benefit regardless of baseline LDL cholesterol levels. The consistent reduction in CVD with statin treatment, even in the absence of severe hypercholesterolemia, has led the NCEP ATP III to suggest more stringent lipid-lowering options for high-risk individuals in their 2004 revisions (Grundy et al., 2004). Ongoing and future statin studies are expected to continue to influence treatment thresholds and goals.