LIPIDS AND ATHEROSCLEROSIS ANNUAL 2003

EDITED BY

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Preface

It is a remarkable testament to the vigour of lipid and atherosclerosis research that only 12 months after the publication of our first annual we have been able to assemble an entirely new set of reviews focusing on the most challenging areas in this rapidly evolving field of research and clinical practice. This is even more remarkable when we consider that not only have we had enough new subject matter to justify the publication of a new volume, but we have had to make difficult choices in what to leave out. We are confident, however, that the topics brought together here, and reviewed by acknowledged experts, will make this edition of the *Annual* as successful as the first.

We have included a variety of reviews that cover a broad spectrum—from the complexities of basic science to the sometimes even greater complexities of practical patient management. The realization that statins have anti-inflammatory properties has developed from the publication of a large portfolio of cell, animal and human experiments and these data are reviewed in detail. Similarly, other potentially beneficial effects of lipid-lowering drugs beyond their direct effects on the lipid profile are considered when we examine the impact of these drugs on rheology. In addition, we examine in detail the effect of statin therapy on HDL and consider its clinical implications, and review the current status of diagnosis and management of patients with familial hypercholesterolaemia. The discovery and development of new strategies for lipid management are reviewed in three separate chapters looking at the PPAR agonists, functional foods, and the use of novel drug combinations. In clinical practice our concerns over the use of lipid-lowering drugs are addressed in an important review of drug safety, while reviews on the use of these drugs in the management of the acute coronary syndrome, the issue of patient compliance, and the current status of treatment guidelines complete the volume.

Lipid and Atherosclerosis Annual is a team effort and we cannot close without thanking our contributors. As in the last annual, our co-authors are a team of the most eminent and prolific workers in the field: scientists and clinicians who individually have contributed exciting new data to the literature. It is with pleasure that we welcome them into the *Annual's* family of authors.

Lastly, we must not forget to acknowledge the patient, yet persistent, encouragement of our publisher, Pete Stevenson, who has driven this edition forward. Although a cliché, we can clearly state that without him this book would not be in your hands now.

Allan Gaw and Jim Shepherd
Glasgow, 2003

Conversion factors

Cholesterol mg/dl=mmol/l×38.67
Triglyceride mg/dl=mmol/l×88.57

1
Statins and inflammation

Naveed Sattar and Allan Gaw

Introduction

A number of large, international, multicentre trials have clearly demonstrated the impact of statin therapy. For example, the Scandinavian Simvastatin Survival Study (4S),[1] the West of Scotland Coronary Prevention Study (WOSCOPS),[2] the Cholesterol and Recurrent Events (CARE) trial[3] and the Long-term Intervention with Pravastatin in Ischaemic Disease (LIPID) study[4] have all shown clearly and unequivocally that statin therapy is highly beneficial in reducing the risk of vascular disease. These studies provided firm new evidence on which to base current clinical practice.

The primary mechanism of action shared by all statins is up-regulation of the low-density lipoprotein (LDL)-receptor and enhanced clearance of LDL and other apolipoprotein B-containing lipoproteins from the plasma. However, close scrutiny of the trial results raises the issue of whether the unexpectedly rapid onset of such striking clinical benefits can be attributed to cholesterol reduction alone.[5] Laboratory and clinical evidence is certainly accumulating to the effect that individual statins may possess benefits beyond their cholesterol-lowering capability, particularly with regard to:

- Promotion of plaque stabilisation
- Restoration of endothelial function
- Immunosuppression
- Anti-inflammatory effects
- Protection against lipoprotein oxidation
- Effects of rheological factors and blood coagulation
- Prevention of development of glucose intolerance.

Statin therapy impacts on many of the above processes, helping to reduce the likelihood of atherosclerotic plaque rupture, or limiting thrombus formation should rupture occur. Comparative investigations suggest that the lipid-soluble statins are capable of modulating the growth of vascular smooth muscle cells, independently of their cholesterol-lowering capability.[6] Interestingly, the water-soluble statin, pravastatin, has no appreciable effect on vascular smooth muscle cells at the normal pharmacological doses used in humans; this may be explained on the basis of tissue penetration. The statins may also exert a direct suppressant effect on platelet activation, thereby limiting platelet thrombus formation.[7] In addition, they may result in a reduction in the number of inflammatory cells within the plaque[8] and a change in plaque composition and architecture, leading to the development of a stiffer, more stable lesion.[9,10]

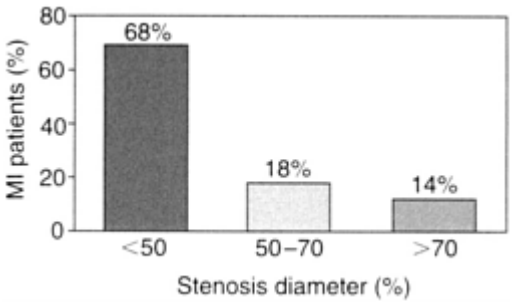

Figure 1.1

Relationship between occurrence of myocardial infarction (MI) and percentage diameter stenosis. Data from ref. 15.

This chapter focuses on the impact of the ancillary mechanisms of action of the statins, and, in particular, examines the evidence for, and the potential therapeutic consequences of, their anti-inflammatory mechanism of action. As a necessary preliminary we will first discuss the role of immune responses and inflammatory processes in the development of the atherosclerotic plaque

Role of inflammation in atherogenesis

Before the introduction of the statins, clinical trial experience with earlier lipid-lowering drugs, such as the bile acid sequestrant resins and the fibric acid derivatives, had suggested that an inevitable delay of two years or more was to be expected prior to the onset of benefit from cholesterol reduction.[11,12] Most pathologists linked this treatment gap to essential remodelling of the atherosclerotic lesion, which they speculated led to its shrinkage and, in consequence, an improvement in downstream tissue perfusion. Large, stenotic lesions were therefore considered to be the primary target for interventive strategies, consistent with the abundant experimental evidence that links the total vascular atherosclerosis burden to ultimate risk of cardiovascular death. The same argument directs the efforts of the vascular surgeon or interventional cardiologist to large occlusive lesions. However, despite the welcome provision of symptomatic relief, surgical intervention, in contrast to medical management of risk factors, does not consistently extend life or reduce coronary mortality overall. This suggests that the large coronary lesion is not necessarily the forerunner of the majority of life-threatening events.

In this regard, recent autopsy studies have redirected our attention to what seems to be a continuing, and in general asymptomatic, process of lesion rupture and healing, seen as commonly in non-occlusive as in occlusive plaques.[13,14] A meta-analysis of data from patients who had suffered a myocardial infarction (MI) and had undergone angiography shortly before the acute event showed that the smaller lesions—i.e. those causing<50% diameter occlusion—were much more commonly associated with subsequent infarction than lesions that caused>50% diameter occlusion (Figure 1.1).[15] Repetition of this cycle is frequently accompanied by intramural thrombosis, leading to substantial and sudden lesion growth. Occasionally, a thrombus may propagate into the vessel lumen, producing a catastrophic occlusive MI. So, plaque vulnerability to rupture is of greater clinical relevance than plaque size. Not surprisingly, therefore, those factors which govern the vulnerability of the plaque itself are under intensive investigation. Table 1.1 summarises what are thought to be the key features in this process.

Pathological studies of patients who have died suddenly of coronary ischaemia are consistent with this view. It appears that the typical causal lesion is often a previously unrecognised minimally occlusive plaque,

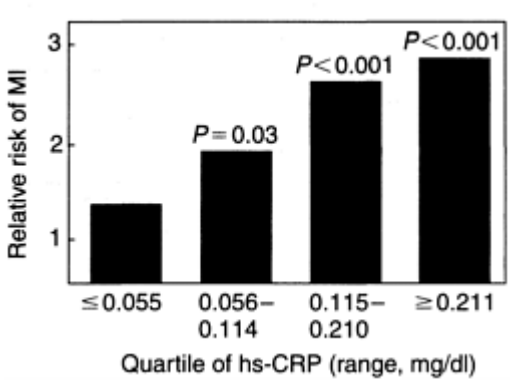

Figure 1.2

Relative risk of myocardial infarction (MI) stratified by quartile of baseline plasma CRP. Data from[21].

whose thin or fragmented fibrous cap (poorly supported by an underlying connective tissue skeleton) has ruptured, exposing the lumenal blood to the procoagulant effects of a lipid-rich core infiltrated with inflammatory cells.[15,16] Cytokines released by the latter have already attracted medial smooth muscle cells into the subintimal space. Both cell types trigger further matrix degeneration within the lesion by releasing metalloproteinases such as stromelysin and collagenase.[17] This degradative process is exacerbated by the secretion of interferon gamma (IFNγ), which suppresses collagen formation by intimal smooth muscle cells[18] and may lead to their apoptosis.[17]

Table 1.1 Characteristic features of the vulnerable atherosclerotic plaque

❑	Thin, fragmented fibrous cap
❑	Underdeveloped connective tissue skeleton
❑	Lipid enrichment
❑	Inflammatory cell infiltration
❑	Evidence of proteolytic enzyme release
❑	Apoptosis of smooth muscle cells

Inflammatory markers and coronary heart disease

In parallel with the realisation of the role of inflammatory cells as mediators in plaque evolution, systemic levels of acute phase markers such as white cell count, serum amyloid A (SAA) and C-reactive protein (CRP) have been shown to predict the risk of coronary heart disease (CHD) events independently in men and women (Figure 1.2).[19–23] Thesame phenomenon is observed regardless of whether the individuals studied are apparently healthy—with or without classical cardiovascular risk factors—or have established vascular disease at the outset. Much of the evidence for this association comes from studies measuring CRP, and the term ‘low grade chronic inflammation’ is now used, as even modestly elevated CRP concentrations, within the traditionally accepted ‘normal’ range, predict MI and ischaemic stroke in prospective studies.[19–23] Thestrength of the evidence is so consistent that some researchers have proposed CHD prediction algorithms that combine CRP with classical risk factors. In addition, several automated

methods for determination of CRP at low levels—so called 'sensitive' or 'ultra-sensitive' assays—are now available.[24,25]

The exact mechanisms behind this association remain unclear. One the total plaque load in blood vessels since there are an abundance of school of thought is that circulating markers of inflammation simply reflect inflammatory cells and molecules in plaques, particularly vulnerable plaques, and thus leakage into the circulation may occur. However, CRP concentrations correlate poorly with the extent of blood vessel occlusion as measured by angiography.[26] Rather, factors such as age, smoking and, in particular, adiposity appear to be important determinants of risk,[19] with the latter explaining as much as 30% of the systemic inflammatory burden in population studies. Moreover, CRP levels are elevated in obese children, many years before a risk for CHD manifests.[27] Thus, the pro-inflammatory state, rather than presence of a specific marker, may be the contributory causative condition.

The question then arises as to how circulating cytokines enhance CHD risk. The answer is likely to lie in the dual functions of cytokines for, in addition to their role in regulating immune responses, cytokines mediate numerous metabolic effects. One consequence of this functional pleiotropy is that the intensity of the metabolic adaptations parallels other cytokine effects. Cytokine-induced metabolic effects, which include transient alterations in lipids and peripheral insulin resistance, are favourable in the short term and function as part of the host response to infection and acute inflammation to target specific metabolic fuels to and from essential organs.[28] However, chronic systemic elevation in cytokine levels, even if modest (as in the case of obese individuals), is deleterious and may promote accelerated atherogenesis via aggravation of several risk factor pathways, including lipoprotein metabolism, insulin resistance and endothelial function. Indeed, CRP concentrations in population studies correlate with levels of many classical and novel CHD risk factors.[29]

Interestingly, there is an increasing perception that a heightened inflammatory state in some populations, such as individuals of South Asian origin, may have evolved to help fight infection in early and mid-life. In other words, a pro-inflammatory state could be a survival trait in situations where life-threatening infections are prevalent; i.e. in underdeveloped countries. When such individuals move to a more westernised environment, such a pro-inflammatory phenotype carries with it a risk of chronic illness, particularly CHD and diabetes.

Anti-inflammatory effects of statin

The influence of cholesterol-lowering on the passivation and dispersal of inflammatory cells in the atherosclerotic lesion has already been discussed above.[8] By their cholesterol-lowering action statins should confer this benefit, but are they also capable of suppressing the inflammatory response by some more direct means?

Statins and immunosuppression

In the late 1980s, experiments demonstrated an obligatory requirement for mevalonic acid (but not cholesterol) in order to facilitate the cytolytic activity characteristic of natural killer T-cells.[30] Statins (in pharmacological doses) were then shown to inhibit lymphocyte proliferation *in vitro* and to block their cytolytic actions.[31] These findings were, however, largely forgotten until they were rediscovered by Kobashigawa and his colleagues (Figure 1.3).[32] In a prospective randomised trial of pravastatin therapy administered to recipients of heart transplant, they assessed whether transplant vasculopathy, associated with raised plasma lipid levels, could be avoided. Serendipitously, they discovered that episodes of acute

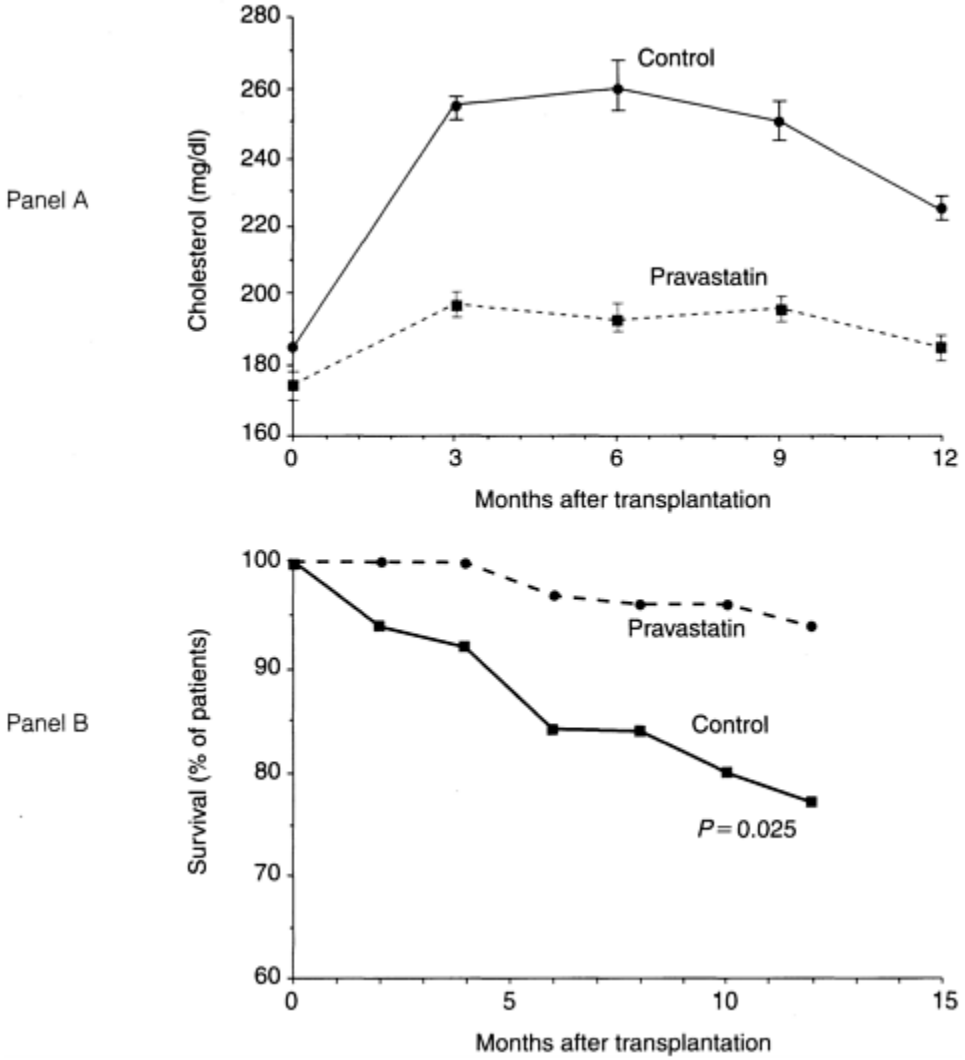

Figure 1.3

Cardiac transplant rejection and the effects of pravastatin therapy. Panel A: Mean (+/−SE) cholesterol levels during the first year after cardiac transplantation (Pravastatin group n=47, Control n=50). Panel B: Survival during the first year after cardiac transplantation (Pravastatin group n=47, Control n=50). Data from ref.[32]

graft rejection were reduced and, in consequence, graft survival prolonged. They pursued this line of investigation with a second study, which demonstrated prolongation of kidney graft survival following treatment with pravastatin.

They proposed several possible explanations for their intriguing findings, including reduction in natural killer T-cell cytotoxicity, enhancement of immunosuppression (due to synergism between pravastatin and the immunosuppressant drug, cyclosporin) and simple lowering of plasma lipid levels. They did point out,

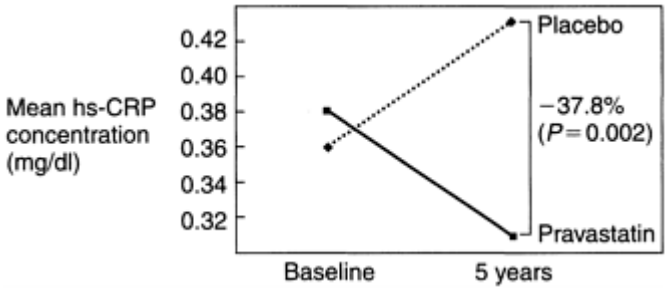

Figure 1.4

Effect of pravastatin on plasma CRP levels in the CARE study. Data from ref.[34].

in partial dismissal of the latter, that the immunosuppressive action is apparently independent of the degree of cholesterol-lowering achieved.

Statins, CRP and risk for CHD

The above data stimulated researchers to ask whether the potential anti-inflammatory effects of statins may also play an important role in the cardioprotective action of these drugs. As noted above, histological examinations of atherosclerotic lesions reveal the presence of inflammatory cells, together with both newly formed and disintegrating fibrous tissue.[19] If statins had innate anti-inflammatory properties, they would then be able to impact directly on the stability of the plaques.

In support of the above, an initial *ex vivo* observation demonstrated the ability of pravastatin to reduce lipopolysaccharide (LPS)-induced release of interleukin (IL)-6 and tumour necrosis factor alpha (TNF-α) from macrophages.[33] Subsequently, several *in vivo* studies have shown that all statin drugs reduce circulating CRP concentrations by approximately equivalent amounts.[34–36] Indeed, analysis of the long-term results of the CARE study showed that the CRP levels of all placebo-treated patients increased markedly during the five-year observation period, whereas in the pravastatin group there was a significant reduction in CRP levels of approximately 40% (Figure 1.4).[34] Interestingly, all such studies suggest no correlation between the extent of LDL-cholesterol reduction and decline in CRP. Additionally, administration of pravastatin in CARE was associated with a minor risk reduction in patients in whom CRP levels were low at baseline, but a marked reduction of risk in those with elevated CRP levels, even though the degree of reduction in LDL-cholesterol was directly comparable in both groups (Figure 1.5).[34] In WOSCOPS, individuals with high levels of CRP at baseline were also at greatest risk of major cardiovascular events.[37] Likewise, in the Air Force/Texas Coronary Atherosclerosis Prevention Study, individuals with low LDL cholesterol at baseline but high CRP were not only at high risk for future vascular events but also benefited greatly from statin therapy, whereas those with combined low CRP and LDL cholesterol had negligible benefit.[38] These observations clearly emphasise the importance of the inflammatory status of patients in terms of assessing their likely outcome with therapy. These results suggest not only that those patients who had a high CRP (and therefore a pro-inflammatory status at the beginning of the study) were more likely to benefit from statin therapy, but also that statin therapy reduces the levels of CRP.

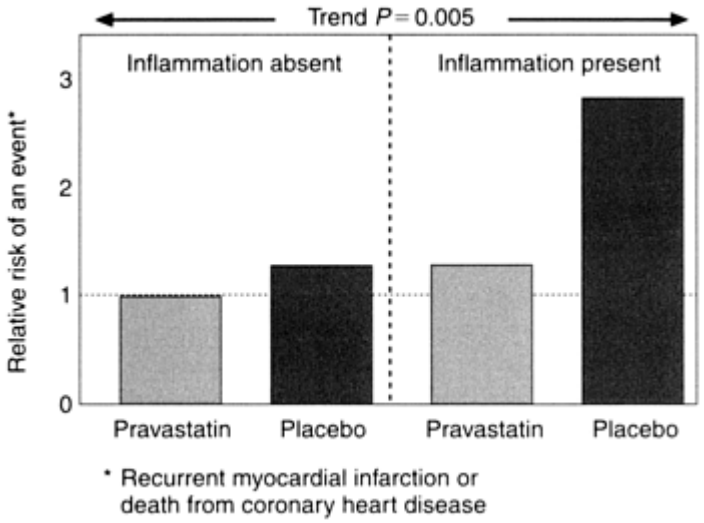

Figure 1.5

Inflammation, pravastatin, and relative risk of recurrent coronary events: subgroup analysis from Cholesterol and Recurrent Events trial. Data from ref.[34].

Statins, endothelial function and plaque stability

The ability of the statins to stabilise atherosclerotic plaques *in vivo* was investigated in two studies. The first was in primates, fed on a diet high in saturated fat that promoted the development of atherosclerotic lesions.[39] The animals were then divided into two groups. One group received pravastatin, while the other group had their diet adjusted so that plasma cholesterol levels would be approximately the same as in the treated group. This manoeuvre was designed to remove plasma cholesterol from the risk equation. The animals that received pravastatin maintained a normal healthy vasodilator response to acetylcholine infusion, but the control group, which were not receiving statin therapy, exhibited a small, paradoxical vasoconstrictor response to acetylcholine infusion. This mimicked the clinical observation in patients with endothelial dysfunction. It also showed that, despite the fact that both groups of animals had atherosclerotic lesions, the administration of a statin was able to preserve or restore endothelial function. Biopsies of the common carotid arteries from these animals were used to investigate three indicators of plaque stability: the numbers of macrophages, the amount of plaque calcification and the degree of plaque neovascularisation. Other studies have shown all three parameters to compromise plaque stability. Biopsies from animals that received statin therapy were found to contain fewer macrophages; their lesions remained uncalcified and were free of neovascularisation. The architecture of the plaques appeared to have been altered in a way that caused them to become more stable and, therefore, less likely to result in an acute coronary syndrome, even though there was no evidence of plaque shrinkage, and despite the fact that the haemodynamic influence of the plaques remained unaltered.

A complementary study was undertaken in Scandinavia in patients who were waiting to undergo carotid endarterectomy.[40] Eleven of these patients were given pravastatin 40 mg/day for three months before surgery and 13 patients received no treatment. Histological examination showed that lesions in the treated patients contained a significantly lower concentration of lipids (−66%), less oxidised LDL (−40%), and lower macrophage and T-cell counts (−41% and −54%, respectively) than did arteries from untreated control

individuals. These findings provide additional evidence to suggest that statin treatment alters plaque architecture and composition in human arteries. They also provide an explanation for the apparently conflicting observations from angiographic studies that show that statin therapy does not result in extensive plaque regression, even though the larger intervention trials indicated that the plaques of treated patients carried a lower risk for acute coronary events.[1–4]

Interestingly, a recent study of patients with type 2 diabetes demonstrated that an improvement in endothelial function with atorvastatin therapy did not correlate with percent change in LDL cholesterol or triglyceride, but did correlate significantly with the percent change in CRP.[41]

Potential mechanisms for the anti-inflammatory effects of statins

Elaborating the mechanisms whereby statins impact on inflammatory pathways and in particular determining whether such effects are independent of their lipid-lowering actions has assumed considerable importance. A number of recent observations have begun to unravel the mechanisms responsible.

3-Hydroxy-3-methyl-glutaryl coenzyme A (HMG-CoA) reductase catalyses the conversion of HMG-CoA to mevalonic acid (MVA) during cholesterol synthesis. Downstream metabolites—including geranylgeranyl pyrophosphate (GGPP) and farnesyl pyrophosphate (FPP)—regulate prenylation in several critical signalling pathways,[42] and one indirect effect of GGPP modulation by statins is activation of peroxisome proliferator-activated receptor-alpha (PPARα).[43] Thislatter effect may not only explain the HDL-cholesterol-raising property of statins[1–4] but also their anti-inflammatory effects since PPARα activation leads to inhibition of inflammatory pathways. Indeed, statin-induced changes in serum CRP concentrations have been inversely associated with changes in HDL cholesterol but not, as noted before, with LDL cholesterol.[44] Activation of PPARα leads to inhibition of a range of inflammatory response genes by interfering negatively with NFkβ and Apoprotein-1 signalling pathways; atorvastatin has been shown to reduce NFKβ activation in vascular smooth muscle cells and mononuclear cells.[45] Nevertheless, the above mechanisms appear to explain previous observations that statins reduce the production of IL-1β, IL-6, TNF-α, cyclo-oxygenase-2 and monocyte chemoattractant protein 1 (MCP-1) by a variety of cell types.[46–48]

Statins have been shown recently to inhibit IFNγ-inducible macrophage major histocompatibility complex class II (MHC-II) expression via class II transactivator (CIITA) suppression.[49] This recent and novel observation is relevant as MHC-II molecules are directly involved in the activation of T-lymphocytes and in the control of the immune response. Additionally, some statins (lovastatin, simvastatin) may modulate T-cell co-stimulation via direct effects on lymphocyte function associated intracellular adhesion molecule (LFA-1/ICAM-1) interactions, dependent upon recognition of a novel statin binding site on β2 integrins.[50] These properties indicate that statins may modulate functional maturation of T-lymphocytes.

Relevance of the anti-inflammatory effects of statins to other diseases?

Type 2 diabetes

It was demonstrated recently that raised CRP is, in addition to a risk predictor for CHD, a predictor of the development of diabetes in middle-aged men, independent of established risk factors such as fasting plasma triglyceride, body mass index (BMI) and glucose.[51] Indeed, in multivariate analysis, a change in plasma CRP concentration of one SD was associated with a similar hazard ratio to each of these factors, each of which is known to predict diabetes. In addition, men in the top quintile of CRP (>4.18 mg/l) had more than a three-fold greater risk of developing diabetes than those in the lowest quintile (<0.66 mg/l) after

adjustment for all other variables. The ability of inflammatory markers to predict diabetes independently has been confirmed by numerous other investigators;[52–54] thus, low-grade inflammation may be a key factor in the pathogenesis of type 2 diabetes. Potential mechanisms linking inflammation to type 2 diabetes also exist. For example, cytokines such as TNF-α may produce insulin resistance by influencing the function of the insulin receptor[55] or by stimulating adipocyte lipolysis.[56,57] Alternatively, endothelial dysfunction may link inflammation to insulin resistance.[58–60]

Because of this close association between inflammatory mechanisms and the development of diabetes, and evidence that statins are anti-inflammatory, a recent investigation tested whether pravastatin therapy had a significant impact on the participants' risk of developing diabetes throughout the five-year WOSCOPS study.[61] The results, albeit generated as part of a post-hoc analysis, demonstrated that assignment to pravastatin therapy resulted in a 30% reduction (P=0.042) in the risk of developing diabetes. Interestingly, ACE inhibitors, which also have anti-inflammatory properties, may also lessen the risk for diabetes.[62,63]

Further work is clearly needed to examine the potential impact of statins on the progression of insulin resistance and the development of metabolic syndrome and diabetes. The outcome will have enormous impact on the overall cost-effectiveness of these drugs, and may point the way towards different preventive strategies for diabetes in the future.

Rheumatoid arthritis

All the *in vivo* evidence demonstrating anti-inflammatory effects of statins has, to date, focused on pathophysiologies—CHD and diabetes—associated with low-grade chronic inflammation. Whether statins are also able to modulate conditions linked to far greater levels of systemic inflammation, for example, rheumatoid arthritis (RA), remains to be examined. Recent animal data, however, are intriguing in this respect. Sparrow *et al.* noted that pre-treatment with simvastatin prevented carrageenan-induced foot pad oedema in mice, the classical model of acute inflammation.[64] Preliminary data from another laboratory have shown that simvastatin can also suppress the development of chronic inflammation *in vivo*.[65] This group noted that simvastatin markedly inhibited both developing and established murine collagen-induced arthritis, a surrogate model for human rheumatoid arthritis, despite no change in cholesterol concentration. *Ex vivo* analysis demonstrated significant suppression of collagen-specific Th1 humoral and cellular immune responses. Simvastatin also suppressed pro-inflammatory cytokine production *in vitro* by T-cell activated-macrophages and by fibroblast-like synoviocytes derived from patients with RA, suggesting that such observations may have direct clinical relevance. These data indicate that statins may find therapeutic utility in a variety of autoimmune conditions. Clearly, statin studies in patients with rheumatoid arthritis are urgently warranted, and the same group are performing one such study that will report in mid-2003.

Interestingly, numerous studies show that patients with RA are at markedly elevated risk of CHD, perhaps largely due to effects of systemic inflammation on several risk factor pathways.[66] It would therefore be of interest to examine whether statin therapy has an even greater benefit in terms of reducing CHD risk in patients with RA. Again, future clinical trials are required to address this important issue.

Table 1.2 Summary of evidence for the anti-inflammatory effects of statins

Source of data	Findings
Clinical	• Reduction in incidence of organ transplant rejection • Modification of plaque composition—reduced inflammatory cells, reduced LDL oxidation

Source of data	Findings
	• Reduction in CRP concentrations unrelated to LDL-cholesterol reduction
	• Benefit from statin therapy related to baseline CRP concentration
	• Improvement in endothelial function correlated to CRP reduction
Animal data	• Prevention of carrageenan-induced foot pad oedema
	• Prevention or delay in collagen-induced arthritis
	• Modification of plaque composition—reduced inflammatory cells, reduced calcification and neovascularisation
Ex vivo/In vitro data	• Reduction in LPS-induced macrophage release of pro-inflammatory mediators such as IL1β, IL-6, TNF-α, MCP-1
	• Similar data for endothelial cells
	• Stimulation of eNOS release from endothelial cells
Mechanistic	• Modulation of critical signalling pathways via effects on GGPP and FPP
	• PPARα activation
	• NFKβ inhibition via lkβ activation
	• Inhibition of IFNγ-inducible MHC class II expression
	• Binding to (β2 integrins to prevent T-cell co-stimulation through direct effects on LFA-1/ ICAM-1 interactions

Conclusion

It is now clear that statin therapy is a cornerstone of modern clinical practice for the prevention of cardiovascular disease. However, focus on the lipid-regulating actions of statins has shifted in recent years to encompass ancillary mechanisms of action, including, in particular, anti-inflammatory effects (Table 1.2). Our greater understanding of the process of atherogenesis and of the importance of immunological and inflammatory processes has helped explain the early and apparently lipid-independent benefits seen with statin therapy. In addition, the recognition of these important mechanisms of action has also allowed us to contemplate using statins in more imaginative ways. Perhaps any disease process with a vascular component to its aetiology and inflammatory processes at its core will benefit from statin therapy. Currently, investigations of the potential benefits of statin therapy in diseases as diverse as RA and diabetes mellitus are underway. While the benefits already accrued from statin therapy are great, we may expect even more from this fascinating class of drugs in the future, especially if novel statins, with enhanced ancillary mechanisms, and with even greater anti-inflammatory potential, are developed.

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2
Lipid-lowering drugs and rheology

Robert S Rosenson

Introduction

Modification of cardiovascular risk factors emphasizes biochemical approaches to the prevention of atherosclerosis, but current biochemical theories of atherosclerosis do not account for mechanical injury to the vessel wall or for impaired microcirculatory flow caused by certain risk factors. The discipline of fluid mechanics that pertains to the flow properties of blood under mechanical stress is (hemo)rheology,[1–3] and this chapter reviews fundamental hemorheological principles in relation to atherothrombosis. It will also focus on the relationships between lipoproteins, lipid-lowering therapies and blood rheology, and will integrate these hemorheological concepts in a discussion of the clinical relevance of lipoprotein-mediated viscosity changes on ischemic heart disease.

Rheological principles

The flow behavior of plasma and serum (Newtonian fluids) under conditions of laminar flow can be depicted as cylindrical layers sliding against each other (Figure 2.1). The flow velocity profile is parabolic, with the highest velocities occurring in the axis of the lumen and the lowest along the inner layer of the vessel wall. The velocity gradient (dV/dx) and shear stress are lowest toward the center of the vessel and highest at the endothelial or stationary surface. Shear stress is the tangential pressure applied against the vessel wall by laminar flow. In human coronary arteries, low vessel wall shear stress has been shown to correlate with progression of atherosclerosis,[4] and high vessel wall shear stress may induce rupture of an unstable plaque or cause a superficial erosion.

Under conditions of laminar flow, the flow of a simple or Newtonian fluid in a fixed-diameter cylindrical tube can be described by the Hagen-Poiseuille equation:

$$Q = \frac{[(\Delta P \cdot \pi \cdot r^4)/8L]}{\eta} \quad \text{or} \quad Q = \frac{\Delta P \pi D^4}{128 \eta L}$$

where Q=blood flow, ΔP=pressure gradient, r=radius of the vessel,D=diameter of the vessel, L=length of the vessel, and η=blood viscosity. Through application of Ohm's law to the circulatory system, bloodflow (Q) is inversely related to total vascular resistance (R):

$$Q = \Delta P/R \quad \text{or} \quad R = \Delta P/Q$$

Combining these two equations restates vascular resistance in hemorheological terms:

$$R = [(8 \cdot L)/(\pi \cdot r^4)]\eta$$

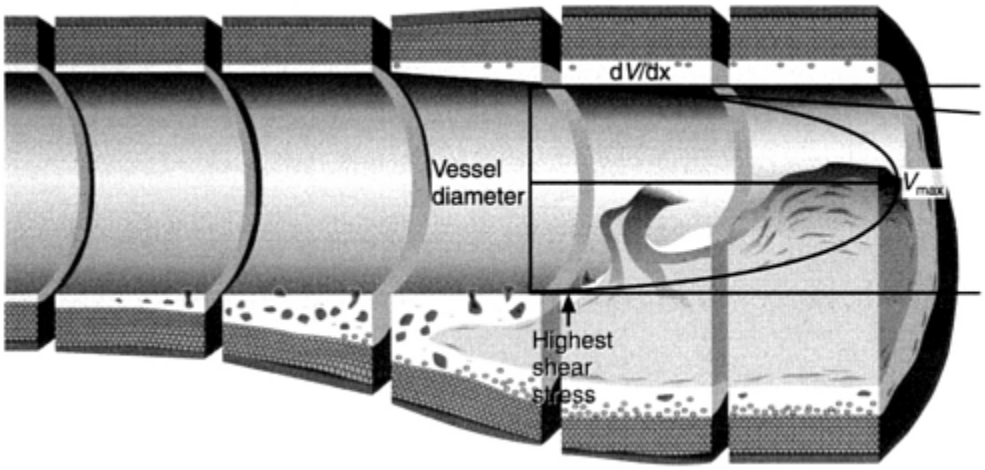

Figure 2.1

Laminar flow velocity. v_{max} is the maximal velocity at the vessel axis; dv/dx illustrates the greater rate change along the outer fluid layer along the endothelial surface.

Total peripheral vascular resistance is the product of both vascular and viscous components. Small changes in vessel wall diameter have marked effects on tissue blood flow or perfusion, as flow rate is directly proportional to the vessel diameter raised to the fourth power. Thus, minimal changes in blood viscosity can reduce microcirculatory perfusion and elevate vascular resistance,[5] particularly in vessels with a diameter <30 μm.[6,7]

The limitations of applying this formula to the human circulation include the complicated network of vessels in the circulation that occur both in parallel and in series, with intervening branch points, curves, and change in caliber; and dynamic changes in the vessel induced by pulsatile flow that changes with each cardiac cycle. In addition, the flow behavior of blood is not a simple function of shear rate—it exhibits non-Newtonian flow properties. At a constant hematocrit, the viscosity of blood increases at low shear rates and decreases at high shear rates. Nevertheless, the conceptual application of this equation is valid. For a given perfusion pressure and vascular diameter, blood flow is inversely related to blood viscosity.

Determinants of blood rheology

The major determinants of blood viscosity are:

- Concentration of erythrocytes
- Viscoelastic properties of erythrocytes
- Interactions of the cellular components of blood (erythrocytes, leukocytes and platelets) with each other and the vessel wall
- Plasma viscosity.

The most important influence on blood viscosity is hematocrit, as linear increases in hematocrit induce a logarithmic rise in blood viscosity.[8]

Erythrocyte aggregation increases blood viscosity at low shear rates. Erythrocyte aggregation is mainly determined by hematocrit and by the interaction of plasma proteins such as fibrinogen and lipoproteins.[9–12]

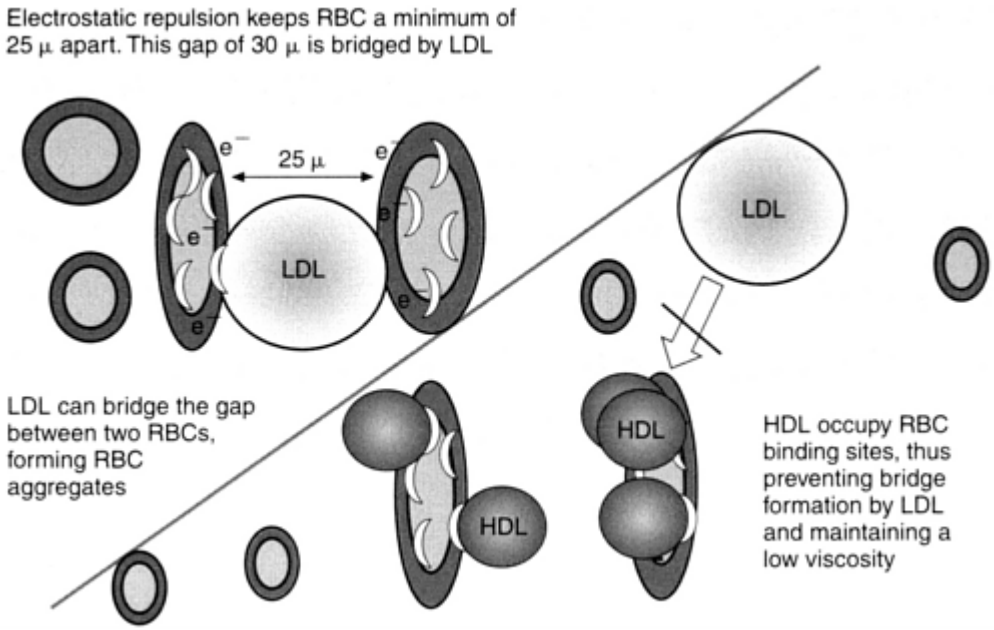

Figure 2.2

Lipoproteins and erythrocyte aggregation. LDL increases erythrocyte aggregation through the bridging of erythrocytes. HDL interferes with the formation of LDL-erythrocyte bridges through competitive inhibition of erythrocyte binding sites. (RBC=red blood cell)

An increase in the cholesterol/phospholipid ratio of the erythrocyte membrane increases erythrocyte aggregation (Figure 2.2).[12,13] Under condi

Table 2.1 Determinants of reduced erythrocyte deformability

- Small surface area to volume ratio
- Reduced membrane fluidity
  - –Large sterol ring of cholesterol reduces the flexing motion of phospholipids
  - –Oxidative modification of membranes and/or malonyldialdehyde accumulation results in crosslinks with membrane components

tions of low flow or shear, erythrocyte aggregates elevate blood viscosity and reduce perfusion. With increasing rates of flow, the erythrocyte aggregates dissociate.

The viscoelastic properties of deformability of erythrocytes influence blood viscosity at high shear rates. Cellular morphology (ratio of surface area to volume) and membrane fluidity of erythrocytes are the major features that determine the viscoelastic properties of the erythrocytes. Erythrocyte membrane fluidity decreases with increasing cholesterol content and oxidation of membrane lipids and phospholipids (Table 2.1).[14]

Phospholipids have considerable flexing motion in membranes, which is restricted by the large sterol ring of cholesterol.[15] As expected, increasing cholesterol content in the erythrocyte membrane reduces membrane fluidity and increases viscosity. Oxidative modification of membranes may result in

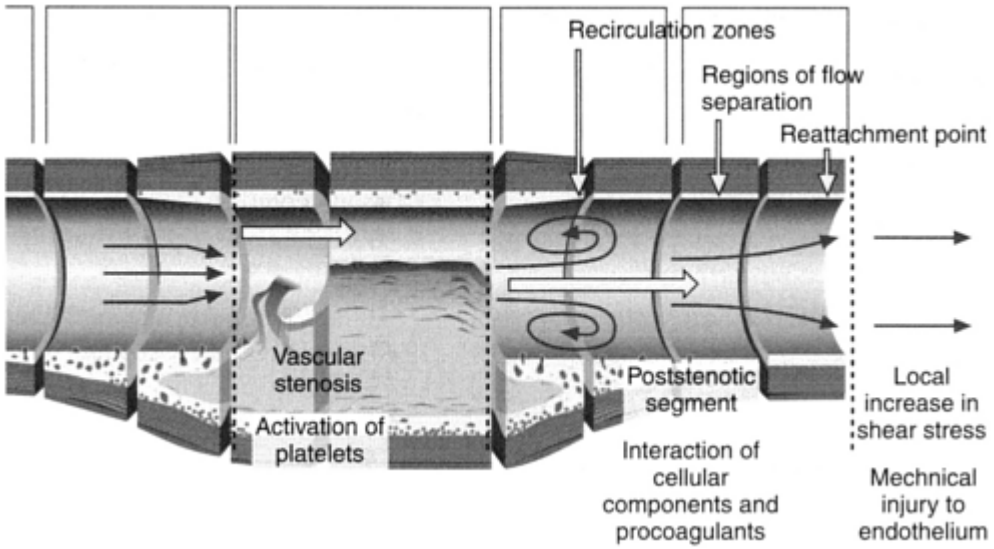

Figure 2.3

Hemodynamic changes around a stenotic arterial segment. Laminar blood flow is disrupted by the stenosis with subsequent acceleration of blood flow. In the immediate poststenotic region, recirculation zones and flow separation occur. After restoration of laminar flow, the shear stress along the endothelial surface is maximal. The arrows depict flow direction and velocity. The thicker arrows denote higher flow.

accumulation of malonyldialdehyde, which is an end product of lipid peroxidation. Malonyldialdehyde reduces erythrocyte deformability through the formation of crosslinks with membrane components.[14,16–18]

Plasma viscosity is determined by the content of water relative to the concentrations of high-molecular-weight plasma proteins such as fibrinogen, immunoglobulins and lipoproteins.[19–22] In addition, plasma viscosity increases the molecular size, rigidity and asymmetrical shape of these plasma proteins.[19]

Importance of rheology in atherosclerosis

Elevated blood and plasma viscosity may contribute to atherothrombosis through impaired microcirculatory flow, shear stress damage at the blood-endothelial interface, facilitation of plasma protein interaction with the endothelium in post-stenotic recirculation zones and increased propensity for thrombosis.[1–3]

The importance of hemodynamic forces in the initiation and progression of vascular stenoses is suggested by the selective distribution of atheromatous plaque at the orifice of arteries, along the outer walls of vessel bifurcations and on the inner walls of curvatures of arteries.[23–33] Atherosclerotic plaques are most often localized at sites of turbulence.

Turbulent blood flow occurs at arterial bifurcations or when blood passes an obstruction or rough surface caused by an erosion or rupture. The turbulence in low shear areas creates recirculation zones—that cause morphological and functional changes in endothelial cells and induce biochemical changes that lead to leukocyte adhesion and migration—and allows for increased interactions between cellular components, atherogenic lipoproteins, coagulation factors and the vessel wall. These factors promote cellular aggregation and adhesion, elevate local blood viscosity, and facilitate plaque growth and thrombus formation. Low

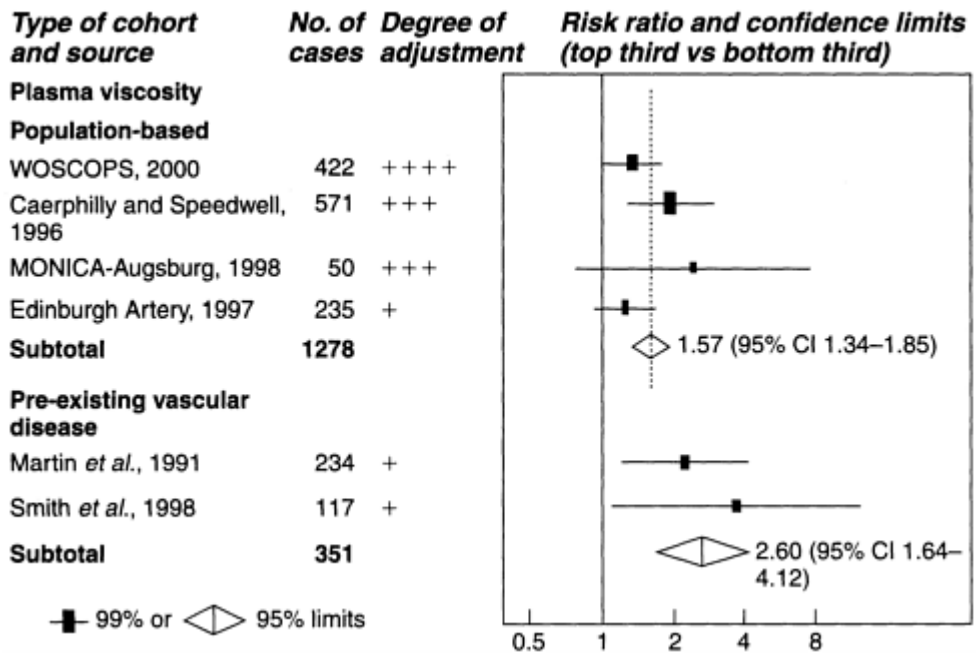

Figure 2.4 Prospective studies of coronary heart disease and viscosity or erythrocyte sedimentation rate. Adapted from ref. 40.

shear mechanical injury also occurs along the inner wall of a curved vessel or along the wall opposite a branch vessel. The resumption of laminar flow accentuates shear stresses along the endothelial surface and increases the potential for mechanical injury to the endothelium and subsequent plaque rupture. High shear stress (>15 dyn/cm^2) is observed at flow dividers of bifurcations and branches.

Hemodynamically significant stenoses change the blood flow profile and shear forces within a luminal narrowing and in the poststenotie arterial segment (Figure 2.3). Transient acceleration of blood in regions of vascular stenoses increase shear stress damage to the endothelium and induce platelet activation.[34] Poststenotic deceleration of blood creates regions of flow separation, recirculation zones and turbulent flow.[23] The acceleration and deceleration of blood flow caused by vascular stenoses is accentuated by pulsatile blood flow.

Changes in flow properties of blood are particularly important in atherosclerotic vascular disease, in which flow conditions are impeded by diminished perfusion pressure, reduced vessel diameter and impaired coronary vasodilatory reserve to the ischemic myocardium. Elevations in blood viscosity reduce coronary blood flow and oxygen delivery to the ischemic myocardium, particularly at the microcirculatory level.[35–37] The extent of postinfarction myocardial necrosis also may be influenced by microcirculatory perfusion and oxygen transport from surrounding tissues.[38,39]

Viscosity and ischemic heart disease

Elevated blood and plasma viscosities are independent predictors of subclinical atherosclerosis, initial and recurrent myocardial infarction (MI), coronary heart disease (CHD) death, stroke and all-cause mortality

(Figure 2.4).[40] In patients with CHD, elevated plasma viscosity predicts the development of acute MI in patients with unstable angina,[41] six-month to two-year mortality in acute patients,[42] stroke in claudicants[43] and recur rent stroke in survivors of an initial stroke.[44] In the Edinburgh Artery Study, blood viscosity was a strong predictor of carotid intima-media thickness.[45]

Several population studies have demonstrated that high blood and plasma viscosity predict incident cardiovascular events in apparently healthy adults.[40] The Caerphilly and Speedwell Collaborative Heart Disease Studies prospectively established that plasma viscosity was an independent predictor of CHD.[46] In this study, 4,641 men between the ages of 45 and 59 years were followed for an average of 5.1 years in Caerphilly and 3.2 years in Speedwell, and a total of 233 events occurred overall. Age-adjusted relative odds for major ischemic events (CHD death, clinical non-fatal MI or electrocardiographic evidence of MI) for men in the highest quintile (top 20%) compared to the lowest quintile (bottom 20%) was 4.5 (95% Cl, 2. 8–7.4) for plasma viscosity and 4.1 (95% Cl, 2.6–6.5) for fibrinogen. After adjustment for age, pre-existent CHD, smoking, diastolic blood pressure, body mass index (BMI) and total cholesterol, the odds ratios for plasma viscosity and fibrinogen were 3.2 (95% Cl, 1.8–5.6) and 2.6 (95% Cl, 1.6–4.3), respectively. At ten years, there were 544 incident events among the 4,463 men with a complete set of data for the study variables.[47] Age-adjusted relative odds of CHD were increased by 3.4 and 3.3, respectively, in the men with the highest levels of plasma viscosity and fibrinogen. After adjustment for major cardiovascular risk factors, the relative odds were 2.3 (95% Cl, 1.7–3.2) for plasma viscosity and 2.2 (95% Cl, 1.6–3.1) for fibrinogen.

The MONICA-Augsburg Cohort Study examined the association of plasma viscosity and the eight-year incidence of a first CHD event (CHD death, fatal and non-fatal MI and cardiac death) in 933 men aged 45–64 years.[48] The relative risk of CHD events for men in the highest versus lowest quintile of the plasma viscosity distribution was 3.31 (95% Cl, 1.19–9.25) after adjustment for age, total cholesterol, HDL cholesterol, smoking, blood pressure and BMI. At eight years, age-adjusted relative risk of death for a one standard deviation increase in plasma viscosity (0.070 mPa.s) was 1.45 (95% Cl, 1.19–1.76, P<0.001).[49] After additional adjustment for total cholesterol, smoking, BMI, blood pressure and education, the relative risk was 1.41 (95% Cl, 1.14–1.74, P=0.002). For men with plasma viscosity in the highest versus the lowest quintile, the relative risk of death was 2.68 (95% Cl, 1.63–4.42), as computed from the adjusted model.

The Edinburgh Artery Study was a prospective study that examined the relationships between blood viscosity and incident cardiovascular events (CHD, stroke) in 1,592 men and women aged 55–74 years.[50] There were 272 fatal and non-fatal cardiovascular events in five years. Subjects who experienced cardiovascular events had higher baseline levels of age-and sex-adjusted (mean±SEM) blood viscosity (3.70 ±0.04 vs 3.55±0.02 mPa.s, P=0.0003), hematocrit (46.2% ±0.21 vs 45.7%±0.10, P=0.015), hematocrit-adjusted blood viscosity (3.57±0.03 vs 3.48±0.01 mPa.s, P=0.0025), plasma viscosity (1.35±0.006 vs 1.33 ±0.003 mPa.s, P=0.0023) and fibrinogen (2.88±0.04 vs 2.67±0.02g/l, P=0.0001). After adjustment for conventional risk factors (smoking, LDL cholesterol, diastolic blood pressure), blood viscosity and hematocrit remained significant predictors of stroke, but not total events, whereas plasma viscosity and fibrinogen were significant predictors for total events and stroke.

The West of Scotland Coronary Prevention Study (WOSCOPS) examined whether rheological measures were predictive of non-fatal MI or definite CHD death in 6,595 men aged 45–65 years with mean LDL-cholesterol levels of 4.96±0.44 mmol/l (191.51±16.99 mg/dl).[51] Baseline levels of plasma viscosity, blood viscosity (calculated from the plasma viscosity and hematocrit), hematocrit, red cell count, white cell count and fibrinogen were significantly higher in men who subsequently developed incident CHD events. After multivariate adjustment for treatment group, age, CHD risk factors and baseline evidence of CHD, significant predictors of the primary endpoint were plasma (relative risk [RR]=1.14, 95% Cl, 1.02–1.28,

P=0.024) and blood viscosity [RR]=1.14, 95% Cl, 1.05–1.23, P=0.0025), hematocrit (RR=1.14, 95% Cl, 1.03–1.26, P=0.0093) and white cell count (RR=1.13, 95% Cl, 1.01–1.26, P=0.027).

The prospective population studies have shown that elevated blood and plasma viscosity consistently identifies subjects with high risk for cardiovascular events. These relationships between elevated blood and plasma viscosity and cardiovascular events remain significant after consideration of more established or conventional risk factors.

Effects of lipoproteins on rheology

Hyperlipoproteinemia is associated with high blood and plasma viscosities. The increase in viscosity is determined by the concentration of lipoprotein particles, the size of the lipoproteins, and interactions with other plasma proteins.[52] Isolated chylomicrons, very-low-density lipoprotein (VLDL), and LDL added to plasma or serum *in vitro* cause a dose-dependent and exponential rise in viscosity.[21,22] VLDL was accompanied by a greater viscosity change than LDL, thereby supporting theories regarding the influence of lipoprotein particle size on viscosity.[21] Subjects with type IV (VLDL) and IIb (VLDL+LDL) hyperlipoproteinemia have higher plasma and blood viscosity than type IIa (LDL) subjects or normolipidemic controls. In a cross-sectional study of 257 subjects, fasting triglycerides were correlated positively with hematocrit-adjusted blood viscosity values at high (r=0.35, p<0.00005) and low r=0.22 P<0.00005) shear rates.[53] In a stepwise regression analysis for uncor rected blood viscosity, hematocrit, total serum protein, plasma triglycerides, HDL cholesterol and fibrinogen were significant predictors of blood viscosity at 100 s^{-1}. Triglycerides remained significantly correlated with blood viscosity (P<0.0005) in the final equation, indicating a significant relationship after hematocrit, fibrinogen, total serum protein and HDL cholesterol were taken into consideration. In the same cohort, fasting triglycerides were correlated strongly with the natural logarithm of plasma viscosity (r=0.46, P<0.0005)[54] In a stepwise regression analysis with the natural logarithm of plasma viscosity as the dependent variable, total serum protein (r=0.19, P<0.0005) fibrinogen (r=0.43, P<0.0005), triglycerides (r=0.62, P<0.0005), LDL cholesterol (r=0.65, P=0.0001), age (r=0.67, P<0.0005), diabetes mellitus (r=0.68, P=0.003) female gender (r=0.69, P=0.0002) and HDL cholesterol (r=0.69, P=0.01) and chronic renal failure (r=0.70, P=0.02) were independent predictors.

Subjects with hypercholesterolemia also have increased blood and plasma viscosities. In a case-control series of 51 type II hyperlipoproteinemia subjects (34 type IIa and 17 type IIb), matched for age, gender and smoking-habit, these patients had elevated blood viscosity values that were 13% higher at a shear rate of 94 s^{-1} and 18% higher at a shear rate 0.94 s^{-1}.[55] The elevated blood viscosity was related to higher plasma viscosity (P<0.01) and fibrinogen (P<0.02) levels. Similar findings were reported in a case-control study of 20 heterozygous familial hypercholesterolemia patients and 20 age- and gender-matched controls.[10] Familial hypercholesterolemia patients had 1.2% higher blood viscosity at low shear (0.277s^{-1}), 1.1% higher plasma viscosity, 1.2% higher fibrinogen levels and more erythrocyte aggregation than control subjects.

In rare situations, severe hypercholesterolemia (>1,700 mg/dl) due to lipoprotein X (LP-X) has caused the hyperviscosity syndrome.[56] The correlation between total cholesterol and relative serum viscosity was 0.94 (95% Cl, 0.85–0.98). LP-X is a large (Stokes radii for the three subspecies 249–339 A) rigid particle with a high intrinsic viscosity.[57]

Isolated low HDL-cholesterol levels are associated with significant elevations in adjusted blood viscosity measured at shear rates of 100 and 20 s^{-1}.[58] These findings are consistent with an abnormality in red cell membrane mechanics. HDL protects against erythrocyte aggregation through inhibition of calcium ion (Ca^2

$^{+}$)-induced procoagulant activity on erythrocyte membranes[59] and competition with LDL-induced erythrocyte aggregation.

The viscoelastic properties of erythrocyte membranes are reduced in hypercholesterolemic subjects under conditions of weak shear stress,[10,11] with the erythrocyte deformability index correlating inversely with total cholesterol ($r=-0.84$, $P<0.01$) and LDL cholesterol ($r=-0.84$, $P<0.01$) concentrations. An increase in erythrocyte membrane cholesterol reduces the activity of Ca^{2+}/Mg^{2+} adenosine triphosphatase, increases intracellular Ca^{2+} concentration and may reduce deformability.[60] Erythro cyte deformability was reduced by 20% in hypercholesterolemic (total cholesterol≥6.48 mmol/l (≥250 mg/dl)) patients treated with pravastatin (10–20 mg/day) for one year.[61] Improvement in deformability index at 4.7dyn/cm^2 correlated with reductions in levels of total cholesterol ($r=0.60$, $P<0.01$), and LDL cholesterol ($r=0.57$, $P<0.01$).

Lipid-lowering therapy and rheology

The effects of pravastatin on one-year levels of plasma and blood viscosity were examined in men enrolled in WOSCOPS.[51] Plasma viscosity was reduced in the pravastatin group ($P<0.0001$) by one quarter of a standard deviation (mean change −0.19 mPa.s, 95% Cl, −0.022 to −0.016). Calculated blood viscosity was also reduced ($P<0.0001$) by one quarter of a standard deviation in the pravastatin group (mean change −0. 062 mPa.s, 95% Cl, −0.072 to −0.052). The change in plasma viscosity was correlated more strongly with the 25% reduction in LDL cholesterol ($r=0.19$) than with the 10% change in VLDL ($r=0.08$).

Smaller studies of statin therapy have reported inconsistent changes in viscosity. Lovastatin has been accompanied by an increase in whole blood viscosity,[62] while plasma viscosity is either reduced[63] or does not change.[62,64] Two studies with pravastatin reported a reduction in whole blood viscosity at shear rates ranging from 75–375 s^{-1}, corrected blood viscosity at 112.5 s^{-1} and 225 s^{-1}, and plasma viscosity.[10,65] In addition to the reduction in LDL cholesterol, fibrinogen levels were reduced by 7–9%. [10,65,66] In 18 subjects with hypercholesterolemia (seven familial and 11 primary polygenic) treated with simvastatin (10–40 mg/ day) for 12 weeks, there was no change in blood, plasma or serum viscosity, despite a reduction in total cholesterol from 9.51±1.53 mmol/l (367±59mg/dl) to 7.51±1.40 mmol/l (290±54mg/dl) ($P<0.001$) and LDL cholesterol from 7.46±1.48 mmol/l (288±57mg/dl) to 5.70±1.61 mmol/l (220±62mg/dl), respectively.[67]

Lipoprotein(a) (Lp(a)) levels may be an important determinant of viscosity changes in statin-treated patients. In 24 hypercholesterolemic patients treated with lovastatin (median dose 40 mg/day) for 12 weeks, blood viscosity tended to be reduced more in patients with low (≤25 mg/dl) levels of Lp(a) versus high (>25 mg/dl) levels of Lp(a).[63] The larger blood viscosity-lowering effect in patients with low Lp(a) levels may have resulted from the larger reduction in LDL cholesterol (−35% vs −28%, P=ns) and larger increase in HDL cholesterol (+15% vs +12%, P=0.89) than observed in patients with high Lp(a) levels.

LDL apheresis is accompanied by rapid improvement in endothelial function in acute coronary syndromes[68] and increased microcirculatory blood flow in patients with symptomatic peripheral arterial and ischemic heart diseases.[69,70] The clinical improvement during LDL apheresis has been ascribed to improved blood rheology.[71,72] In patients with familial hypercholesterolemia (two homozygous, ten heterozygous) undergoing regular LDL apheresis, adjunctive therapy with atorvastatin (80mg/day for eight weeks) reduced erythrocyte aggregation ($P<0.01$), whole blood viscosity at low (0.695s^{-1}), intermediate (2.37 s^{-1}), and high (94.5s^{-1}) shear rates, and plasma viscosity and platelet aggregation ($P<0.05$).[73]. Improvement in these rheological variables occurred despite a 5% ($P<0.01$) increase in plasma fibrinogen, indicating that the increase in fibrinogen was counterbalanced by the reduction in LDL cholesterol.

The importance of triglycerides in blood rheology was highlighted in a double-blind study of 17 heterozygous familial hypercholesterolemia patients randomized to treatment with pravastatin 20 mg twice

daily, cholestyramine 16–24 g/day or placebo.[10] Total cholesterol fell by 24.7% (P<0.01) with pravastatin and 21.5% (P<0.01) with cholestyramine. Cholestyramine was accompanied by a 42% increase in plasma triglycerides (1.28±0.55 mmol/l [113.28±48.68 mg/dl] to 1.82±0.81 mmol/l [161.07±71.69 mg/dl], P<0.05), whereas no significant change was observed with pravastatin (1.55±0.62 to 1.64±0.58, P=ns). Pravastatin reduced plasma viscosity (P<0.05), whereas no change was observed with cholestyramine.

Several studies have demonstrated that fibrates lower blood and plasma viscosities.[74–80] However, it was unclear as to whether the reduction in viscosity was related to triglyceride-lowering, as these studies utilized fibrates (clofibrate, bezafibrate, fenofibrate) that also lowered fibrinogen levels. In a study of 24 subjects with severe hypertriglyceridemia (≥5.67 mmol/l [≥501.80 mg/dl]) treated with gemfibrozil (1200 mg/day), plasma triglycerides decreased by 70% (P<0.001), fibrinogen did not change and plasma viscosity was lowered by 0.082 mPa.s (~1 standard deviation) (P=0.003).[73] Changes in triglycerides correlated with changes in plasma (rho=0.35, P=0.060) and serum viscosity (rho=0.49, P=0.042). This study provided the first evidence that triglyceride-lowering reduces plasma viscosity independent of changes in fibrinogen levels.

The effects of statin (atorvastatin 10 mg/day) versus fibrate (fenofibrate 200 mg/day) on several hemorheological parameters were examined in a randomized crossover trial of 13 subjects with type 2 diabetes mellitus (hemoglobin A_{1c} 7.3±1.1%), and mixed hyperlipoproteinemia, LDL cholesterol 4.25±0.98 mmol/l (164.0±37.8 mg/dl), triglycerides 2.93±1.21 mmol/l (259.7±107 mg/dl), HDL cholesterol 1.26±0.28 mmol/l (48.7±11.0 mg/dl).[81] Atorvastatin reduced LDL cholesterol by 29% and triglycerides by 4%, whereas fenofibrate lowered LDL cholesterol by 11% and triglycerides by 39%. Fibrinogen levels were unchanged by atorvastatin and lowered by fenofibrate (−15%, P<0.01). In addition, fenofibrate decreased plasma viscosity by 3% (P<0.01) and improved red cell aggregation by 15% (P<0.05), whereas there were no hemorheological changes with atorvastatin.

Clinical significance of lipoprotein-mediated hyperviscosity

Lipoprotein-mediated hyperviscosity may contribute to tissue ischemia endothelial dysfunction, as mediated by increased shear stress, post-prandial myocardial ischemia, increased plaque erosions and rupture and myocardial impedance through increased vascular resistance.

Viscosity has its greatest effect on reducing blood flow in small-caliber vessels. Reduced myocardial vasodilation has been reported in hypercholesterolemia and hypertriglyceridemia[82] subjects with anatomically normal coronary arteries. Impaired coronary flow reserve and myocardial perfusion abnormalities are often observed in the absence of an obstructive coronary stenosis in patients with type 2 diabetes mellitus,[83] and perfusion abnormalities caused by reduced myocardial vasodilation increase the risk of death or MI in these patients.

To further explore whether abnormal coronary blood flow reserve during hyperlipidemia is due to abnormal coronary vasomotion or increased blood viscosity, maximal hyperemia was induced with adenosine in nine dogs.[84] Incremental increases in serum triglycerides induced by intralipid infusions were highly correlated with blood viscosity (r=0.71, P<0.005). Myocardial vascular resistance increased (r=0.84, P<0.001) and hyperemic myocardial blood flow was reduced (r=−0.64, P<0.001). There was no change in myocardial blood volume, thus, in these fully dilated coronary arterioles, elevated blood viscosity increased capillary resistance and led to abnormal coronary blood flow. Future studies should test whether a therapeutic reduction in plasma viscosity affects endothelial dysfunction, tissue ischemia and the risk of cardiovascular events.

Triglyceride-mediated hyperviscosity may contribute to impaired vasomotor tone,[85] tissue ischemia and manifestations of the chylomicronemia syndrome, such as fatigue, blurred vision, dysesthesia and abdominal pain. In patients with severe hypertriglyceridemia (>11.3 mmol/l or >1,000 mg/dl) who report symptoms compatible with the chylomicronemia syndrome (fatigue, blurred vision, dysesthesia and abdominal pain), viscosity measurements may serve as a therapeutic guide for hydration and prompt initiation of triglyceride-lowering therapy.

Conclusion

Lipoproteins cause elevations in blood and plasma viscosity, and thus, increase the mechanical injury to the vessel wall and reduce tissue perfusion. Lipid-lowering therapies reduce the viscosity of blood and plasma, increase erythrocyte flexibility and reduce the tendency for erythrocytes to aggregate. These hemorheological changes may contribute to the clin-ical benefits of lipid-altering therapy in preventing progression of atherosclerosis lesions, improving symptoms associated with tissue ischemia and reducing cardiovascular events.

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3
PPAR agonists in the treatment of the metabolic syndrome and type 2 diabetes

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Introduction

Cardiovascular diseases (CVD), including acute myocardial infarction (MI), stroke and unstable angina pectoris, remain the leading cause of morbidity and mortality in Western societies. CVD develops as a consequence of vascular atherosclerosis, and large epidemiological studies have established high total and low-density lipoprotein (LDL) and low high-density lipoprotein (HDL) serum cholesterol concentrations as risk factors for this.[1] Primary[2] and secondary[3] prevention trials with cholesterol-lowering drugs of the statin class have provided evidence that a drastic reduction in LDL-cholesterol levels reduces the cardiovascular risk in hypercholesterolemic patients and in patients considered to have 'normal' LDL-cholesterol levels.[4] In addition, hypoalphalipoproteinaemia with or without hypertriglyceridaemia may be the cause of a substantial number of cases of coronary artery disease (CAD),[5] and correcting these lipid abnormalities with gemfibrozil, a drug belonging to the fibrate class, has been shown to result in a significant reduction in the incidence of cardiovascular disease.[6]

Patients with diabetes are considered to be at particularly high risk for cardiovascular disease.[7] Elevated serum concentrations of triglycerides and small-dense LDL and decreased HDL-cholesterol levels are particularly common in patients suffering from type 2 diabetes and the metabolic syndrome, which is characterized by insulin resistance and a clustering of metabolic abnormalities including dyslipidaemia.

Fibrates are a class of lipid-modifying agents chemically related to clofibrate, the first clinically used fibrate. These drugs are able to reduce plasma triglyceride-rich lipoproteins and increase HDL-cholesterol concentration. These actions may explain, at least in part, the clinical benefits of these compounds in reducing the incidence of CVD.[6,8–10] In addition, fibrates may also reduce the risk of CAD in patients with diabetes.[11] Part of the beneficial effects of fibrates on CVD risk may relate to factors other than their lipid-lowering properties, in particular, their anti-inflammatory properties.

Thiazolidinediones (TZDs) are a new class of drugs that are used for the treatment of type 2 diabetes, and act by improving insulin resistance. In addition, TZDs influence cholesterol homeostasis and exert direct anti-atherogenic effects. As such, they may improve the overall risk profile of patients with diabetes.

Both fibrates and TZDs exert their action via activation of transcription factors belonging to the peroxisome proliferator-activated receptor (PPAR) family of nuclear receptors.[12] This chapter presents a brief overview of the characteristics of this family of nuclear receptors, followed by a review of current knowledge regarding the molecular mechanism of action of PPAR agonists of the fibrate and TZD classes, with a focus on lipid metabolism.

PPARs

PPARs constitute a subfamily of the nuclear receptors,[12] which are ligand-activated transcription factors. Three distinct PPAR isotypes have been identified, termed a, δ (β) and γ. Each is encoded by a separate gene and shows a distinct pattern of tissue distribution.

PPARα is highly expressed in heart, skeletal muscle, kidney[13] and liver[14] tissue. The factors regulating PPARα gene expression have not been extensively documented. However, the recent identification and characterization of the human PPARα promoter[15] should allow studies of the molecular mechanism of regulation of this gene.

The human PPARδ(β) isotype is expressed ubiquitously, with the highest levels of expression in adipose tissue, skeletal muscle, kidney, liver and the small and large intestine.[13] PPARδ(β) is induced upon adipocyte, myoblast and macrophage differentiation[16,17] and appears to play important roles in each of these tissues.

Two PPARγ isoforms have been identified—PPARγ1 and PPARγ2– which differ in their amino-terminal end.[18] PPARγ, especially PPARγ2, is expressed abundantly in adipose tissue,[13,19,20] and recent observations identified a dominant role for PPARγ2, but not PPARγ1, in adipogenesis.[21] Moderate-to-low PPARγ expression is also detected in other tissues, including the liver, heart and muscle.[20,22]

All PPARs are expressed in vascular cell types, including endothelial cells, smooth muscle cells and macrophages.[14,17,23–30] The detection of PPARα[31] and PPARγ[26,29,31] in atherosclerotic lesions suggests a possible relationship between the PPAR receptors and the development of atherosclerosis. Furthermore, the observation that PPARs are expressed in liver, muscle and adipose tissue suggests a role for these receptors in the control of the metabolic syndrome.

Ligand-activated PPARs heterodimerize with another nuclear receptor, the 9-cis retinoic acid receptor RXR, and alter the transcription of target genes after binding to specific peroxisome proliferator response elements (PPREs) (Figure 3.1). PPREs consist of a direct repeat of the nuclear receptor hexameric AGGTCA DNA core recognition motif separated by one or two nucleotides (DR1 and DR2).[32] In addition, PPARs have also been shown to repress gene transcription by interfering with different transcription pathways, such as NF-$_K$B, STAT and AP-1, in a manner independent of DNA binding. These actions of PPARs probably form the basis of the recently discovered anti-inflammatory actions of fibrates and TZDs.[33]

PPAR agonists

As PPARs are ligand-activated transcription factors, they are attractive candidates for drugs development. Most nuclear receptor ligands are small lipophilic molecules. However, although it was demonstrated that all PPARs are activated by fatty acids, there is considerable debate regarding the exact nature of endogenous PPAR ligands. Nevertheless, a certain PPARs, such as leukotriene B4 (LTB4), 8(S) hydroxyeicosate-number of eicosanoids appear to be more selective natural activators of traenoic acid and 8 (S)hydroxyeicosapentaenoic acid for PPARα[34–36] and prostaglandin J2 derivatives, 9-hydroxyoctadecadienoic acid and 13–hydroxyoctadecadienoic acid for PPARγ.[37–39]

Fibrates and TZDs are specific PPARα and PPARγ synthetic agonists, respectively. Recently, a series of subtype-specific high-affinity PPAR agonists have been synthesized. These include the PPARα ligand GW7647,[40] the PPARγ activators GW1929 and GW7845[41,42] and the PPARδ(β) agonist GW501516.[43]

A new class of dual PPARα/PPARγ agonists—such as the phenyl propanoic acid analogue, ragaglitazar (NNC 61–0029, (-) DRF2725), which has completed phase II analysis—are attracting particular scientific attention, and have been shown to reduce glucose and lipid levels in animal models.[44] Recently, ragaglitazar

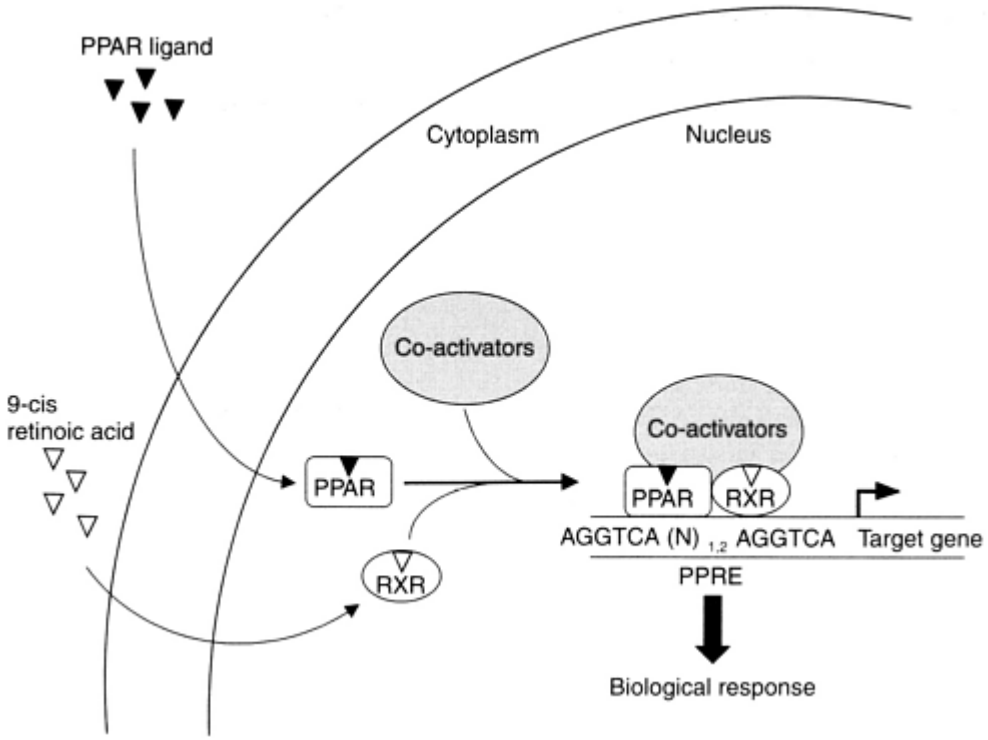

Figure 3.1

Mechanism of transcriptional regulation by PPARs. (▼) PPAR agonists. (▽) 9–cis retinoic acid (9–cis RA), a RXR agonist. PPRE, peroxisome proliferator response element.

demonstrated its capacity to improve the diabetic lipid profile while simultaneously reducing glucose levels in patients with type 2 diabetes.[45] Other non-TZD PPARα/PPARγ agonists include LY465608,[46] AZ-242[47] and NNC 61–4424.[48]

Upon binding, different PPAR ligands induce distinct conformation of the receptors, leading to differential coactivator recruitment[32] and consequently, differential biological responses[49,50] (Figure3.1). These observations form the basis of the 'selective PPAR modulator' or SPPARM concept.[51]

Clinical actions of PPARα agonists

Fibrates are a class of hypolipidaemic drugs that includes the widely used second-generation drugs beclofibrate, bezafibrate, ciprofibrate, etofibrate, fenofibrate and gemfibrozil. These molecules bind to and activate the PPARα isotype,[35,36] albeit with relatively low affinity, but selectivity for the PPARα form differs between the fibrates used currently.

Fibrates are firstline therapy in the treatment of primary hypertriglyceridaemia, and are useful in the treatment of hypoalphalipoproteinaemia, combined hyperlipidaemia, type III dyslipoproteinemia and the secondary lipid abnormalities observed in patients with type 2 diabetes, obesity and the metabolic syndrome.[52]

The most pronounced effect of fibrates is a decrease in plasma triglyceride-rich lipoproteins. Levels of LDL cholesterol tend to decrease in individuals with elevated baseline plasma concentrations and HDL cholesterol levels are usually increased when baseline plasma concentration is low.[53] Treatment of patients with an atherogenic LDL profile (increased small-dense and decreased large-light LDL particles) with fibrates results in a reduction of dense LDL particles, with an equivalent increase in the intermediate subfraction.

The increased HDL concentrations found after fibrate treatment are generally reflected by increased plasma levels of apolipoprotein A-I (apoA-I) and apoA-II. Indeed, fenofibrate increases the rate of apoA-I synthesis, and to a lesser extent its catabolic rate, in hypercholesterolaemic patients.[54]

Based on its apolipoprotein content, HDL can be separated into particles containing only apoA-I (LpA-I) or both apoA-I and apoA-II (LpA-I: A-II). Fibrates increase HDL levels, a change that is associated with an increase in LpA-I: A-II, and a decrease in LpA-I concentrations.[53]

The rationale behind the use of fibrates in certain types of dyslipidaemia has gained support from a subgroup analysis of the Helsinki Heart Study,[8] which showed a greater than 70% reduction in coronary heart disease (CHD) risk after gemfibrozil treatment of overweight patients presenting with an elevated LDL: HDL-cholesterol ratio and increased triglyceride levels.[55,56]

Results from two secondary prevention angiographic studies using bezafibrate and gemfibrozil revealed that fibrate treatment retards the progression of coronary atherosclerosis and decreases the number of coronary events.[9,10] Results from the Veterans Affairs-High Density Lipoprotein Cholesterol Intervention Trial (VA-HIT) with gemfibrozil[6] demonstrated that raising HDL cholesterol and lowering triglycerides, even in the absence of a significant lowering of LDL cholesterol, reduced both CAD mortality and non-fatal MI in men with documented CAD. Furthermore, 25% of patients included in this study were diabetic, and gemfibrozil reduced risk of myocardial infarction by 24% in these patients, thus suggesting the utility of fibrates in the treatment of diabetic dyslipidaemia and metabolic syndrome. In agreement with this hypothesis, micronized fenofibrate corrected lipid abnormalities and reduced the risk of CAD in patients with diabetes.[11] Furthermore, administration of bezafibrate to patients with hypertriglyceridaemia and characteristics of the metabolic syndrome improved both the lipid profile and insulin resistance,[57] and resulted in anti-inflammatory effects.

Effects of PPARα agonists on triglyceride metabolism

The decrease in plasma levels of triglyceride-rich lipoproteins caused by PPARα agonists is due to a decrease in their synthesis rates and to an acceleration of their intravascular catabolism (Figure 3.2).

Intracellular fatty acid (FA) concentrations are determined partly by a regulated import system that is controlled by FA transport proteins, such as FATP-1 and FAT/CD36. Coupling of FA uptake to their conversion in acyl-CoA esters by the activity of acyl-CoA synthetase (ACS) results in a positive import gradient. Fibrates, via activation of PPARα, induce an increase in FATP-1, FAT and ACS mRNA levels in the liver.[58,59]

Once activated, fatty acids are transported into the mitochondria. Expression of muscle- and liver-type carnitine palmitoyltransferase-I (CPT-I), a pivotal enzyme in the uptake of mitochondrial FA, is induced by fibrates via PPARα binding to a PPRE localized in their promoter regions.[60] Thus, PPARα regulates the entry of FAs into the mitochondria, which is a crucial step in their metabolism, especially in tissues like heart, skeletal muscle and brown adipose tissue, in which FAs are a major source of energy. Finally, PPARα activators regulate the expression of mitochondrial enzymes of the FA β-oxidation pathway. The observation that PPARα activators may also regulate the expression of uncoupling proteins (UCPs)[61] raises

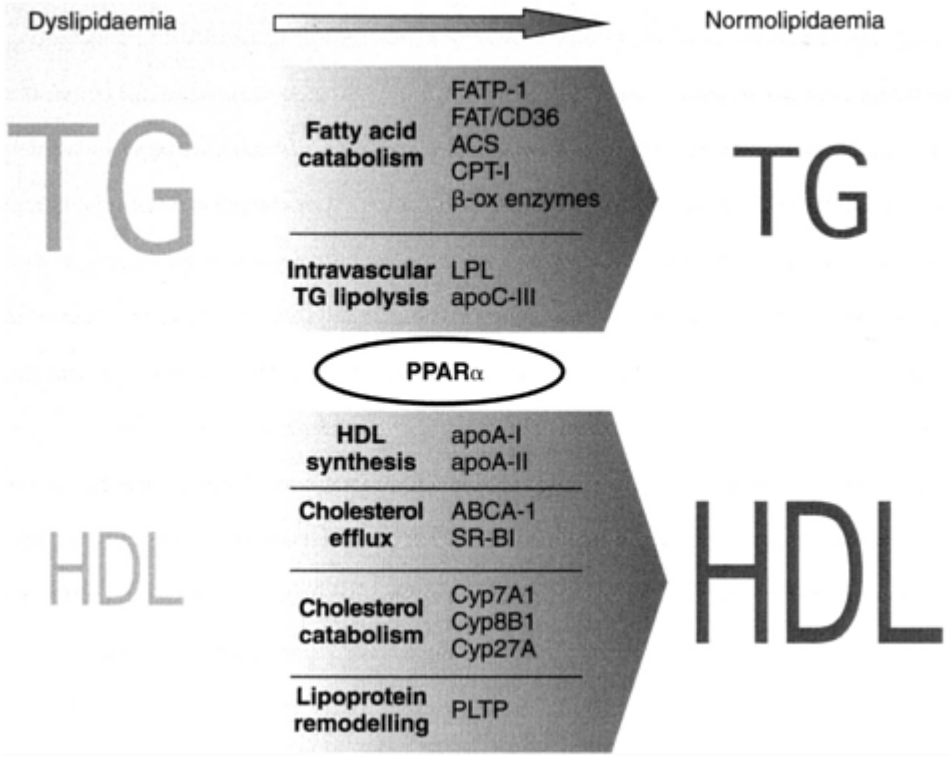

Figure 3.2

Role of PPARα in the control of triglyceride (TG) and HDL metabolism. In the liver, PPARα regulates the expression of genes involved in fatty acid (FA) uptake, cellular retention, activation to acyl coenzyme A esters, mitochondrial uptake, and catabolism (β-oxidation (β-ox) enzymes). PPARα favours lipolysis and clearance of TG by inducing the expression of lilpoprotein lipase (LPL) and by inhibiting the expression of apoC-III. On the other hand, activated PPARα induces HDL synthesis, regulates cholesterol catabolism and lipoprotein remodelling. Furthermore, in macrophages, PPARα induces cholesterol efflux.

the intriguing possibility that these drugs induce, under certain conditions, FA catabolism without ATP generation, an action that may be beneficial in obese patients.

Together, these data show that PPARα activators stimulate different steps in FA oxidative metabolism in different organs, particularly in the liver where they reduce the quantity of FA available for very-low-density lipoprotein (VLDL) synthesis and secretion, as has been observed with gemfibrozil and fenofibrate.[62,63]

Fibrates also enhance intravascular VLDL and chylomicron-triglyceride hydrolysis. At least two distinct mechanisms appear to be involved in the induction of intravascular lipolytic activity by fibrates. The first is the induction of LPL gene expression, which leads to a higher hydrolytic activity of the enzyme. The human LPL promoter has been shown to contain a PPRE that mediates its positive responsiveness to PPARα activators.[64] The second mechanism involves alterations in the composition of triglyceride-rich lipoproteins secreted by the liver following treatment with fibrates, producing particles with a higher susceptibility for lipolysis.

ApoC-III may delay the catabolism of triglyceride-rich particles through different mechanisms. It appears to inhibit the binding of triglyceride-rich lipoprotein to the endothelial surface and lipolysis by LPL, to interfere with apoE-mediated receptor clearance of remnant particles and to decrease VLDL glycosaminoglycan binding in an apoE-independent manner.[65–67] It was established recently that plasma concentrations of VLDL particles and apoC-III in VLDL and LDL correlate highly with CHD risk.[68] Fibrates repress hepatic expression of apoC-III, leading to lowered lipoprotein particles containing both apoC-III and apoB.[69] Increased plasma apoC-III may be an important determinant of the delayed remnant lipoprotein catabolism observed in individuals with metabolic syndrome, and fibrates may improve this phenomenon, thus decreasing CHD risk.

Fibrates lower apoC-III mRNA levels in a dose- and time-dependent manner in both *in vivo* rat livers and *in vitro* primary cultures of human and rat hepatocytes.[70,71] Anobligatoryrole for PPARα in the repression of apoC-III gene expression by fibrates was demonstrated in PPARα-deficient mice.[72] The regulation of apoC-III gene transcription is complex, being governed by different transcription factors. Whereas hepatocyte nuclear factor-4 (HNF-4), RXR and PPARα[73,74] can all activate apoC-III gene transcription, apoA-I regulatory protein 1 (ARP-1) and v-erb-a homolog/chicken ovalbumin upstream promoter transcription factor (EAR/COUP-TF) act as repressors.[75]

The transcriptional suppression of apoC-III gene expression by fibrates may be due to one or more of the following mechanisms. First, the suppression of apoC-III by PPARα activators may be due to a displacement of the strong transcriptional activator HNF-4 by the less active PPARα/RXR complex, resulting in lower apoC-III promoter activity.[74] Second, PPARα activators may decrease the expression of HNF-4, although this effect has not been observed with clinically used fibrates such as fenofibrate or ciprofibrate.[76] Third, PPARα activators may induce the expression of repressor proteins, such as ARP-1, Ear-COUP-TF or Rev erbα. Interestingly, fibrates induce Rev-erbα expression in liver cells via the interaction of PPARα with a PPRE in the Rev-erbα gene promoter,[76,77] and Rev-erbα reduces apoC-III gene expression.[78]

Effects of PPARα agonists on the reverse cholesterol transport pathway

The role of PPARα agonists in the control of HDL metabolism implicates different mechanisms, including stimulation of cholesterol efflux from lipid-laden cells, its transport back to the liver and cholesterol secretion into the bile (either directly or after conversion to bile acids). This process is termed the ‘reverse cholesterol transport (RCT) pathway’.

Cholesterol efflux from cells occurs via the ATP-binding cassette transporter A1 (ABCA1) in an apoA-I mediated mechanism,[79] to nascent HDL particles. Subsequently, cholesterol is esterified by the action of lecithincholesterol acyl transferase (LCAT). HDL cholesteryl esters are cleared from plasma either through selective uptake by the liver via the scavenger receptor-BI (SR-BI)/CLA-1 or after transfer to VLDL-LDL particles, which are cleared via classical receptor pathways. SR-BI/CLA-1 binds HDL with high affinity, and mediates the selective uptake of cholesteryl esters in liver and steroidogenic tissues.[80] In addition, studies demonstrating that the rate of cholesterol efflux mediated by HDL or serum is correlated with cellular SR-BI expression levels[81] suggest that SR-BI may promote the removal of cholesterol from peripheral cells, including macrophages.

PPARα agonists regulate the RCT pathway (Figure 3.2). Indeed, the PPARα ligand Wy 14,643 was reported to induce the expression of ABCA1 in human macrophages, via an indirect mechanism involving stimulation of the nuclear receptor LXRα pathway.[82] Furthermore, PPARα agonists enhance the expression of SR-BI/CLA-1.[31]

Fibrates influence HDL cholesterol and apoA-I differently in humans and rodents. In rats, fibrate treatment results in a considerable lowering of plasma HDL concentrations[83,84] because of a marked PPARα-dependent decrease in the expression of apoA-I and apoA-II genes in the liver.[84,85] In humans, fibrates increase plasma HDL concentrations, at least in part, through induction of expression of the human apoA-I and apoA-II genes.[85–87] ApoA-Igene expression and production in vivo in humans and *in vitro* in both human hepatocytes and hepatoma cells[76,78] as well as in cynomolgus monkey hepatocytes[88] are enhanced. In human apoA-I transgenic mice, in which expression of the transgene is driven by its own human promoter,[85] treatment with fibrates increased HDL-cholesterol and human apoA-I plasma concentration concomitantly with an increase in hepatic human apoA-I mRNA levels.

Fibrates induce the transcriptional rate of human apoA-I gene via PPARα, interacting with a positive PPRE located in the A site of the human apoA-I gene promoter liver-specific enhancer.[86] The failure of fibrates to induce rat apoA-I gene expression is due to three nucleotide differences between the rat and the human apoA-I promoter A-site, rendering the PPRE in the human apoA-I promoter non-functional in rats.[76] In contrast, rat but not human apoA-I transcription is repressed by the nuclear receptor Rev-erbα, which binds to the rat but not to the human apoA-I promoter. Therefore, it appears that the opposite regulation of apoA-I expression in human versus rodents is linked to differences in ciselements in their respective promoters leading to repression by Rev-erbα of rat apoA-I and activation by PPARα of human apoA-I.

Using a transgenic rabbit model, which expresses the human apoA-I gene under control of its homologous regulatory regions, it was demonstrated that the action of fibrates on apoA-I metabolism occurs independently of peroxisome proliferation.[89] In these human apoA-I transgenic rabbits, administration of fenofibrate increased plasma human apoA-I concentration via increased expression of the human apoA-I gene in the liver, without increasing liver weight or acyl-CoA oxidase (ACO) activity. These data provide *in vivo* evidence that the beneficial increase in apoA-I levels can occur in a mechanistic way, dissociated from any deleterious effect on peroxisome proliferation or, possibly, hepato-carcinogenesis.

Fibrates also induce the expression of the second most abundant HDL apolipoprotein, apoA-II, in human hepatocytes,[87] and a PPRE was identified in the human apoA-II promoter. Thus, fibrates increase apoA-II plasma levels by stimulating transcription of this gene.

Administration of fenofibrate to mice induces expression and activity of the phospholipid transfer protein (PLTP), an enzyme that catalyzes the transfer of phospholipids from VLDL/LDL to HDL,[90] contributing to the enlargement of HDL. In humans, high plasma PLTP activity is associated with insulin resistance and altered triglyceride metabolism,[91] but the real contribution of fibrates in the regulation of this protein in humans has yet to be evaluated.

PPARα regulates bile acid synthesis and composition. Fibrates have been reported to repress the activity of the first (and rate-limiting step) enzyme in the 'classic' bile acid biosynthetic pathway, cholesterol 7α-hydroxylase (CYP7A1), in different species including humans.[92–95] The mechanistic basis of the regulation of CYP7A1 by PPARα activators is unclear. PPARα was shown to inhibit murine but to slightly induce human CYP7A1 promoter activity.[96,97] CYP7A1 mRNA levels are diminished in ciprofibrate-treated PPARα wild-type mice but not in PPARα-deficient mice,[98] indicating that the reported regulation *in vitro* may be relevant under physiological conditions, at least in mice. Furthermore, PPARα induces the expression of cholesterol 12α-hydroxylase (CYP8B1) and repression of sterol 27-hydroxylase (CYP27A1), thereby modifying the ratio of cholic acid to chenodeoxycholic acid (CA: CDCA) in bile.[98,99]

Thus, the regulation of the RCT pathway, resulting in an increase of HDL by fibrates, may improve the hypoalphalipoproteinaemia characteristic of the metabolic syndrome, and may result in a decreased risk for CHD.

Clinical actions of PPARγ agonists

TZDs are a new class of orally active drugs that are designed to enhance the actions of insulin. They include troglitazone (which is no longer commercially available), rosiglitazone and pioglitazone. These molecules bind to and activate the PPARγ isotype.[100,101]

Several studies testing the efficacy of TZD treatment in diabetic and insulin-resistant patients have been conducted. Treatment of patients with type 2 diabetes with TZDs lowers both fasting and postprandial glucose levels, as well as circulating insulin levels.[7,102] These changes in glucose homeostasis parameters are related to an improvement in insulin-stimulated glucose disposal after the drug treatment period. The effects of TZDs on basal hepatic glucose production (HGP) rates in patients with type 2 diabetes have been variable. Some studies have shown striking decreases in this HGP,[103] while others have shown little effect.[104] Additionally, certain TZDs have been shown to have beneficial effects on serum lipids,[105] blood pressure and cardiac function.[106] However, weight gain,[107] which may be due to the increased adipogenesis[108] and/or edema observed after TZD treatment, has also been described. Troglitazone has also been linked to a number of cases of liver failure,[109] which resulted in its withdrawal from the market for use as monotherapy in newly diagnosed type 2 diabetes. Neither rosiglitazone nor pioglitazone displays hepatoxicity, suggesting that these hepatic adverse events are not a class effect of PPARγ agonists.

PPARγ agonists in the control of diabetic dyslipidaemia

Patients with type 2 diabetes are at greatly increased risk for CVD.[110] Characteristic abnormalities of the lipid profile in patients with the metabolic syndrome and type 2 diabetes include elevated levels of triglyceride, decreased HDL cholesterol and increased small-dense LDL[7] A recent study in patients with type 2 diabetes demonstrated that pioglitazone is able to reduce triglycerides and increase HDL-cholesterol without inducing major changes in total or LDL cholesterol.[111] However, rosiglitazone treatment appears to have no major effects on lipid profile.[112] These observations suggest a potential utility of selected PPARγ agonists in diabetic dyslipidaemia.

All TZDs affect the circulating levels of free FAs, triglycerides and cholesterol in obese-animal models. TZDs decrease the circulating levels of triglycerides by inducing lipolysis (via activation of LPL expression in adipocytes) and clearance of triglyceride-rich lipoproteins (Figure 3.3).[113,114] Inrodents, simultaneous administration of PPARα and PPARγ activators results in a more efficient hypotriglyceridaemic activity, which is most likely due to combined action on liver apoC-III and adipose tissue LPL expression.[115] The induction of LPL by PPARγ promotes FA delivery, whereas induction of FATP and ACS results in enhanced FA uptake in adipose tissue.[116] These actions contribute to the enhanced triglyceride storage in adipose tissue observed after treatment with TZDs. Although the triglyceride-lowering activity of PPARγ agonists in animal models is well documented, substantial variability exists concerning their hypotriglyceridaemic activity in humans.

The activity of PPARγ agonists in stimulating the RCT pathway was demonstrated recently (Figure 3.3). The PPARγ agonist rosiglitazone induces the expression of ABCA1 in human and mouse macrophages, via an indirect mechanism involving the LXRα pathway, and consequently promotes cholesterol efflux.[82] The requirement of PPARγ for the induction of ABCA1 expression and cholesterol efflux by PPARγ agonists was demonstrated recently.[117] PPARγ also enhances the expression of SR-BI/CLA-1[31] and FAT/CD36.[30]

Activation of PPARγ does not stimulate foam cell formation, but induces the expression of genes involved in the first steps of the RCT pathway.

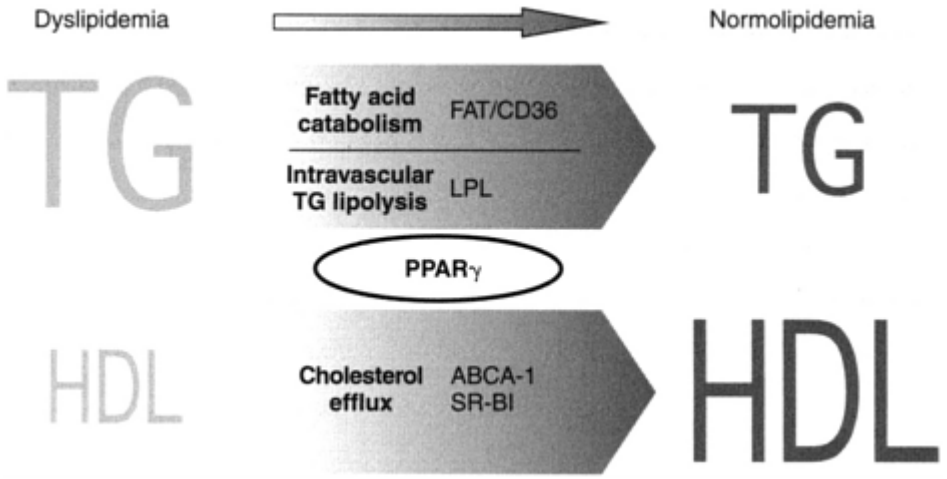

Figure 3.3

Role of PPARγ in the control of TG and HDL metabolism. PPARγ regulates cellular metabolism of FA by inducing FAT/CD36 expression. Furthermore, PPARγ favours lipolysis and clearance of TG by inducing the expression of LPL in adipose tissue. In macrophages, activated PPARγ induces cholesterol efflux.

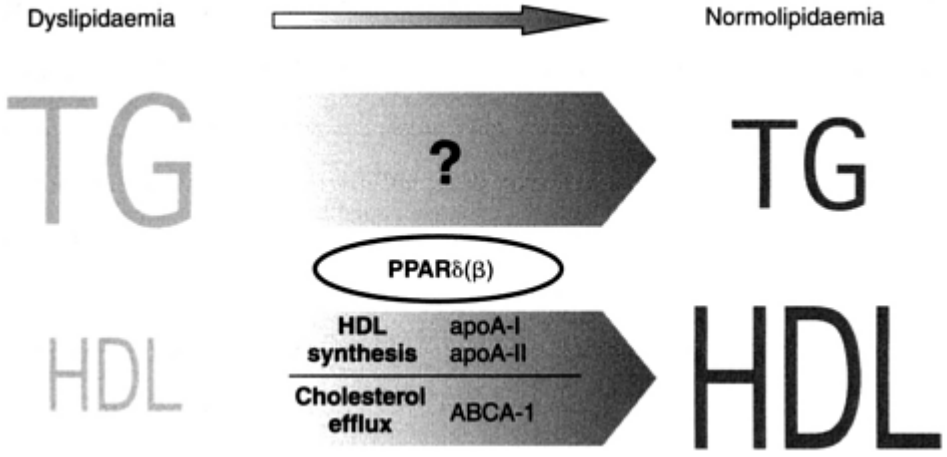

Figure 3.4 Role of PPARδ(β) in the control of TG and HDL metabolism. Activated PPARδ(β) regulates TG metabolism by a mechanism not yet clarified. On the other hand, activated PPARδ(β) induces HDL synthesis and cholesterol efflux.

PPARδ(β) agonists: new molecules in the control of lipid homeostasis?

Recent studies have established a possible link between PPARδ(β) and the metabolic syndrome. In a primate model displaying many of the features of human metabolic syndrome—including dyslipidaemia, insulin resistance and hyperinsulinaemia—treatment with the specific PPARδ(β) agonist GW501516 resulted in profound effects on the serum lipid profile, including a rise in HDL-cholesterol, a decrease in triglycerides and a reduction in the proportion of small-dense LDL, together with a partial correction of the hyperinsulinaemia. The rise in HDL cholesterol was accompanied by an increase in serum apoA-I and apoA-

II concentration.[43] In db/db mice, which develop obesity, hyperglycaemia and hypertriglyceridaemia, treatment with a less specific PPARδ/γ agonist L-165041 increased total plasma cholesterol, principally due to an increase in HDL-cholesterol, without affecting hyperglycaemia and hypertriglyceridaemia.[118] Furthermore, PPARδ(β) agonists also induce ABCA1 expression in human macrophages, and stimulate cholesterol efflux,[17,43] a process that is apparently independent of LXRα.[43] However, a recent study suggested that PPARδ(β) agonists also promote lipid accumulation in macrophages.[17] Thus, the overall effects of PPARδ(β) agonists on atherogenesis require further study (Figure 3.4).

Overall, emerging data suggest a role for PPARδ(β) in the control of lipid homeostasis. Further studies should demonstrate whether PPARδ(β) agonists may be useful in the treatment of cardiovascular disease associated with the metabolic syndrome.

Conclusion

Our knowledge of the physiological role of the PPAR family of transcription factors in the regulation of lipid and lipoprotein metabolism has evolved enormously over the past few years. It is becoming increasingly clear that PPAR activators, such as fibrates and TZDs, may exert beneficial effects on the development of atherosclerosis through their normolipidaemic and insulin-sensitizing activities. In addition, recent studies (not discussed in this chapter) indicate that PPAR activators also exert direct effects on the vascular wall, resulting in the inhibition of vascular inflammation and thrombogenesis. Such actions are undoubtedly beneficial in the treatment of atherosclerosis. Further studies using new more potent and/or more specific/combined PPAR molecules than the drugs presently available, will certainly provide additional information on the role of PPAR in lipoprotein metabolism, diabetes complications and atherogenesis and should offer new therapeutic perspectives.

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4
Lipid management in acute coronary syndromes

Gregory G Schwartz

Rationale for lipid-lowering interventions in acute coronary syndromes

Scope of the clinical problem

The management of acute coronary syndrome (ACS), defined as unstable angina or acute myocardial infarction (AMI), presents a major challenge to clinicians because of the frequency and clinical importance of these events. In the USA alone there are an estimated 1.5 million hospital admissions each year for ACS —divided approximately equally between unstable angina and AMI[1]—and the problem is of similar magnitude in other Western countries. Despite recent advances in medical and interventional therapy, ACS carries a high risk of early recurrent ischemic events. For example, after presentation with a non-ST segment elevation ACS, the six-month risk of death or recurrent non-fatal AMI is approximately 10% and the risk of death, AMI, or recurrent unstable ischemia is approximately 20%.[2–5] Thus, our challenge, both as investigators and as practitioners, is to identify and employ new treatments that will help to reduce the incidence and improve the outcomes of ACS.

Until recently, lipid-lowering drug therapy was viewed as a long-term strategy to reduce cardiovascular risk, rather than as an intervention to be employed in the short-term management of ACS. This conventional viewpoint was based on experimental and angiographic evidence that lipid-lowering promotes gradual removal of lipid from the core of atherosclerotic plaques (leading to slow, modest regression of arterial stenoses) and on outcome data from three landmark clinical trials (the Scandinavian Simvastatin Survival Study [4S];[6] Cholesterol and Recurrent Events study [CARE];[7] and LIPID[8]). These trials established that lipid-lowering therapy with HMG-CoA reductase inhibitors (‘statins’) reduces the risk of death and non-fatal ischemic cardiovascular events in patients with stable coronary heart disease (CHD). However, event reduction in these trials was a late phenomenon, requiring one-to-two years before an effect of treatment on events could be discerned.

There are two possible explanations for the delayed benefit of statin treatment in these landmark trials: either a prolonged period of drug exposure was required to elicit beneficial effects of statin treatment, or a prolonged period was required for a sufficient number of modifiable events to occur in a stable patient population. With regard to the latter possibility, it is important to recognize that each of the landmark trials excluded patients who had experienced an ACS within three-to-six months prior to randomization—i.e., patients at highest risk for short-term ischemic events were excluded from these trials. In fact, the number of cardiovascular events expected to occur over a period of months following ACS is similar to the number of events expected to occur over a period of years in a stable population. Thus, the question of whether lipid-lowering therapy can reduce cardiovascular events in the early period after ACS remained unanswered by

the landmark trials. The answer to this question hinges upon whether early recurrent ischemic events after ACS are modifiable by lipid-lowering interventions.

Scientific rationale for lipid-lowering after ACS

There are at least three potential mechanisms by which lipid-lowering, and statin therapy in particular, may favorably modify vascular physiology in a manner that reduces early recurrent ischemic events after ACS. These mechanisms include correction of vascular endothelial dys-function, attenuation of vascular inflammation and normalization of a prothrombotic tendency. Experimental data indicate that lipid-lowering therapy may act rapidly through each of these mechanisms to correct abnormal physiology of the vessel wall.

Atherosclerosis is associated with impaired vascular endothelial function. Abnormalities of coronary arterial endothelial function may be more pronounced in patients who present with unstable clinical syndromes, compared with patients with stable angina.[9] Hyperlipidemia contributes to the impairment of vascular endothelial function by reducing the activity of nitric oxide synthase (NOS) and by increasing the catabolism of nitric oxide (NO). Conversely, correction of hyperlipidemia with lipid-lowering therapy can help to restore endothelial function. In addition, statins may exert direct effects to upregulate the expression of endothelial NOS[10] or to increase the population of circulating endothelial progenitor cells involved in vascular endothelial repair.[11] The salutary effects of lipid-lowering on endothelial function may be rapid: an improvement in endothelial function of the brachial artery has been demonstrated within one month of initiation of statin therapy[12] and within hours after apheresis treatment to remove low-density lipoprotein (LDL) cholesterol from plasma.[13] Correction of hyperlipidemia has also been shown to improve the endothelial function of atherosclerotic coronary arteries, but the effects may be slower and/or less pronounced than in nonatherosclerotic vessels.[14–15]

Atherosclerosis is an inflammatory process characterized by infiltration of the arterial wall by macrophages and T-lymphocytes. Oxidized LDL cholesterol is one of several factors that may activate these inflammatory cells, causing them to secrete products that are potentially deleterious to the stability of atherosclerotic lesions. In particular, activated macrophages may secrete matrix metalloproteinases that degrade the fibrous cap of the atherosclerotic plaque and compromise its structural integrity, and tissue factor that is intensely pro-thrombotic. Circulating markers such as C-reactive protein (CRP) may reflect inflammation in the arterial wall and have been shown to affect the prognosis of patients with or at risk for CHD.[16,17]

In two trials of statins in patients with stable CHD, those with elevated levels of circulating inflammatory markers derived greater benefit from treatment than those without elevated markers of inflammation.[17,18] Inflammation of the arterial wall may be particularly important in the pathophysiology of ACS. Coronary atherectomy specimens from patients with unstable angina are more heavily infiltrated with inflammatory cells than specimens from patients with stable angina.[19] Moreover, levels of circulating markers of vascular inflammation such as CRP have been shown to be inversely related to short-term and long-term prognosis after ACS.[20,21] Lipid-lowering therapy with a statin can reduce CRP levels within four weeks,[22] suggesting that initiation of statin treatment shortly after ACS may attenuate vascular inflammation within the period of high risk for recurrent events.

A less well-appreciated, but potentially important, effect of hyperlipidemia is induction of a pro-thrombotic state; conversely, lipid-lowering may reverse this pro-thrombotic tendency. Hyperlipidemia increases platelet activation by altering intracellular pH regulation[23] and may promote thrombosis through decreased production of NO by vascular endothelium or increased secretion of tissue factor by activated

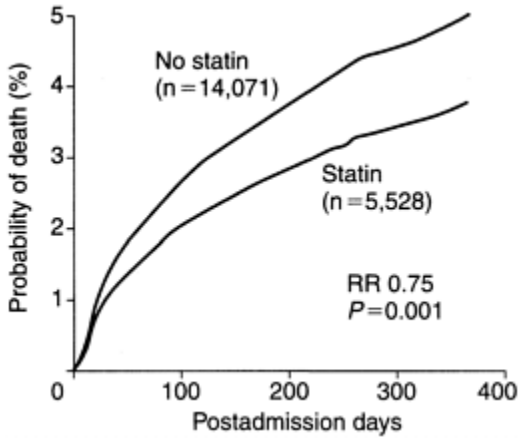

Figure 4.1 Probability of death among patients discharged from Swedish intensive care units after acute myocardial infarction with or without treatment with a statin agent, adjusted for 43 clinical covariates and propensity score for statin use. Statin use was associated with a significant reduction in mortality. Adapted/reproduced with permission from ref. 28.

macrophages.[24] Conversely, treatment of hyperlipidemia with a statin for four weeks has been shown to correct abnormal deposition of platelet thrombus on biological media[25] and the elaboration of tissue factor by activated macrophages.[26]

Clinical evidence supporting lipid-lowering interventions in acute coronary syndromes

Observational studies

Despite mounting experimental evidence indicating several mechanisms by which lipid-lowering therapy might act to stabilize vulnerable coronary plaques, many patients with ACS are discharged from hospital without a lipid-lowering intervention. In one analysis, only 30% of ACS patients overall, and only 50% of ACS patients with diabetes, prior coronary artery disease (CAD), or prior revascularization, were prescribed lipid-lowering therapy at discharge.[27] Are we missing a therapeutic opportunity to improve outcomes for our patients after ACS?

Two large observational analyses have suggested a benefit of early initiation of lipid-lowering therapy after ACS, while another observational analysis indicates no benefit. Taking advantage of the nationwide Register of Information and Knowledge about Swedish Heart Intensive Care Admissions (RIKS-HIA), Stenestrand and Wallentin examined outcomes in a cohort of almost 20,000 patients with first AMI, who were followed prospectively for one year.[28] The main outcome measure was one-year mortality according to statin treatment. Prescription of lipid-lowering agents was uncontrolled and completely at the discretion of treating physicians. A total of 5,528 patients received statins at or before hospital discharge, while 14,071 did not. The investigators attempted to make adjustments for differing baseline characteristics, including a propensity analysis regarding the probability of statin use. In a Cox regression analysis, adjusting for the 42 covariates plus the propensity score, one-year mortality was 3.7% among patients discharged on statin

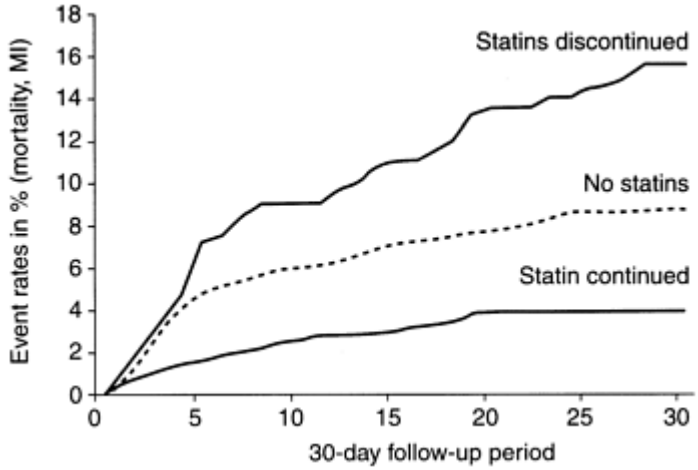

Figure 4.2 Cumulative incidence of death or non-fatal myocardial infarction among patients with ACS enrolled in the Platelet Receptor Inhibition in Ischemic Syndrome Management (PRISM) study. Event rates are shown for 1,249 patients without statin therapy, 379 patients with continued statin therapy, and 86 patients with discontinued statin therapy after hospitalization. Results are adjusted for age, gender, conventional cardiac risk factors, cardiac troponin-T, and assignment to randomized treatment in the parent trial. Discontinuation of statin therapy increased the risk of death or myocardial infarction during the ensuing 30 days. Adapted/reproduced with permission from ref. 31.

treatment, compared to 5.0% among patients not discharged on statin treatment (Figure 4.1). The relative risk was 0.75 (P=0.001).

In another observational study, patients with ACS enrolled in the Global Use of Streptokinase or t-PA for Occluded Coronary Arteries (GUSTO) IIb study and the Platelet Glycoprotein IIb/IIIa in Unstable Angina: Receptor Suppression Using Integrilin Therapy (PURSUIT) study were included in an analysis of death and non-fatal AMI at six months, based on use of a lipid-lowering medication at hospital discharge.[29] As in the RIKS-HIA study, the decision to employ a lipid-lowering medication was left to the discretion of the treating physician. Use of specific classes of lipid-lowering drugs (e.g., statins, fibrates, resins, niacin) was not determined. Of 20,809 patients, only 3,653 (18%) were prescribed a lipid-lowering drug at discharge. After correction for identifiable confounding variables and the propensity to prescribe a lipid-lowering agent, use of a lipid-lowering agent at discharge was associated with a hazard ratio for death of 0.67 (P=0.023). Surprisingly, however, there was no association between use of lipid-lowering medications at discharge and non-fatal AMI at six months (hazard ratio 0.93, P=0.59), suggesting that there may have been an ascertainment bias for AMI among patients prescribed lipid-lowering medications.

In contrast to the RIKS-HIA and GUSTO-IIb/PURSUIT analyses, an analysis of the Sibrafiban vs Aspirin to Yield Maximum Protection from Ischemic Heart Events Post-acute Coronary Syndromes (SYMPHONY) trials found no relation between early statin treatment and outcomes after ACS.[30] After propensity and co-variate adjustment, the 90-day hazard ratio for death, non-fatal AMI, or severe recurrent ischemia was 1.15 (95% Cl 0.99–1.34) among patients who received early statin treatment.

Just as the RIKS-HIA and GUSTO-IIb/PURSUIT analyses suggest that early initiation of lipid-lowering therapy after ACS is beneficial, another observational analysis suggests that discontinuation of lipid-lowering therapy during hospitalization for ACS may be harmful. In a retrospective analysis of 1,616 patients in the Platelet Receptor Inhibition in Ischemic Syndrome Management (PRISM) study, 86 patients had statin treatment withdrawn after hospitalization.[31] Among those patients, the adjusted hazard ratio for

death and non-fatal AMI was 2.93 (P=0.005), compared to 349 patients who continued statin therapy (Figure 4.2).

Each of these non-randomized observational studies suffers from an inherent limitation: the likelihood of unaccounted differences in baseline characteristics of the two treatment groups. Therefore, the findings of these studies, while interesting and provocative, must be viewed as hypothesis-generating, rather than hypothesis-testing. A randomized, placebo-controlled trial was required to provide a more rigorous test of the hypothesis that early initiation of lipid-lowering therapy reduces early, recurrent ischemic events after ACS.

Randomized controlled trials

The MIRACL study

To date, there has been only one published randomized, placebo-controlled trial of lipid-lowering after ACS. The Myocardial Ischemia Reduction with Aggressive Cholesterol Lowering (MIRACL) study[32] tested the hypothesis that early initiation of intensive lipid-lowering therapy after ACS reduces early recurrent ischemic events. In this international multicenter trial, 3,086 patients hospitalized with unstable angina or non-Q-wave AMI were randomized to receive atorvastatin 80 mg daily or placebo, with treatment initiated one-to-four days after hospitalization for ACS and continued for 16 weeks. Exclusion criteria included coronary revascularization that was planned or anticipated at the time of screening, or treatment with other lipid-lowering medications. Importantly, there was no lower limit on either total or LDL cholesterol at entry. In fact, the patients enrolled in the MIRACL study were not hyperlipidemic by usual criteria: at entry, mean total and LDL cholesterol levels were 5.4 and 3.2 mmol/l (207 and 124 mg/dl), respectively. Triglycerides averaged 2.0 mmol/l (184 mg/dl) and high-density lipoprotein (HDL) cholesterol 1.2 mmol/l (46 mg/dl). At the end of the 16-week treatment period, mean LDL cholesterol levels rose by 12% in the placebo group (to 3.5 mmol/l or 135 mg/dl) and fell by 41% in the atorvastatin group (to 1.9 mmol/l or 72 mg/dl). The primary efficacy measure, a composite of death, non-fatal AMI, cardiac arrest or worsening angina with objective evidence of ischemia requiring emergency rehospitalization, was reduced from 17.2% in the placebo group to 14.8% in the atorvastatin group (relative risk 0.84, P=0.048, Figure 4.3). Each component of the composite efficacy measure was at least tendentially reduced in the atorvastatin group, but the greatest effect of treatment was on worsening angina with objective evidence of ischemia requiring emergency rehospitalization (relative risk 0.74, P=0.02). Surprisingly, in light of the short duration of the study, atorvastatin also reduced fatal or non-fatal strokes (0.8% in the atorvastatin group versus 1.6% in the placebo group; relative risk 0.50, P=0.045) (Figure 4.4).[33]

Although a 16% reduction in primary endpoints in the MIRACL trial may be viewed as a modest effect, intensive lipid-lowering after ACS may prevent a substantial number of events in populations at risk, and do so with a high level of efficiency. As discussed above, there are between one and two million hospitalizations for ACS each year in the USA alone,[1] but fewer than half of these patients are discharged from hospital on treatment with a lipid-lowering agent.[27] Based on these statistics and on the point estimates of event reduction in the MIRACL trial, it is possible that 20,000 deaths, non-fatal AMIs, cardiac arrests, strokes, or readmissions to hospital for unstable angina could be prevented annually in the USA if early, intensive lipid-lowering became part of standard care for patients after ACS. The numbers of preventable events worldwide is potentially much greater. In terms of the efficiency of the treatment strategy employed in the MIRACL trial, it would be necessary to treat 35 patients for 16 weeks (corresponding to 11 patient-years of treatment) in order to prevent one primary endpoint event or non-fatal stroke. This compares

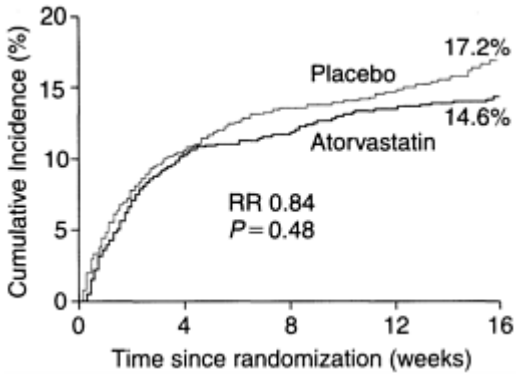

Figure 4.3

Cumulative incidence of death, non-fatal myocardial infarction, cardiac arrest with resuscitation, or worsening angina with new objective evidence of ischemia requiring emergency rehospitalization in patients enrolled in the Myocardial Ischemia Reduction with Aggressive Cholesterol Lowering (MIRACL) trial. Patients were randomly assigned to treatment with atorvastatin 80 mg daily (n=1,538) or placebo (n=1,548) for 16 weeks. Atorvastatin treatment was associated with a significant reduction in the composite endpoint. Adapted/reproduced with permission from ref. 32.

favorably with many other primary and secondary prevention strategies employed in coronary heart disease. In addition, the cost of treating with a statin for four months is relatively modest.

It is noteworthy that the reduction in mortality with intensive lipid-lowering in the randomized, placebo-controlled MIRACL trial was much less than that suggested by the major observational analyses cited above.[28–30] This difference suggests that, despite best attempts to account for identi fiable confounding factors in the observational analyses, unaccounted factors or treatment biases affected the analysis of outcomes. These findings underscore the need for randomized, controlled trials to provide rigorous tests of clinical hypotheses.

In MIRACL, the reduction of recurrent ischemic events by atorvastatin did not appear to depend on the level of LDL cholesterol at the time of the index ACS event. Among patients who had LDL cholesterol levels below the median value of 3.1 mmol/l (121mg/dl) at randomization, a primary endpoint occurred in 15.0% of the atorvastatin group and in 18.6% of the placebo group (relative risk 0.77; 95% confidence interval 0. 59–0.98). Among patients with baseline LDL cholesterol greater than the median value, events occurred in 15.0% of the atorvastatin group and 16.6% of the placebo group (relative risk 0.92; 95% confidence interval 0.71–1.19). This suggests that the reduction of recurrent ischemic events by early intensive lipid-lowering after ACS is independent of circulating LDL-cholesterol concentrations at the time of ACS. These findings are consonant with the long-term findings of the Heart Protection Study,[34] which showed that the benefit of treatment with simvastatin in patients with stable cardiovascular disease was independent of baseline LDL cholesterol levels.

In the MIRACL trial, treatment with atorvastatin 80mg for 16 weeks was generally safe. There was an excess of 1.9% of patients in the atorvastatin group who developed serum transaminase elevation greater than three times the upper limit of normal, but there were no documented cases of myositis. Nonetheless, clinicians must always maintain reasonable caution and vigilance in the use of statins to minimize hepatic dys-function and myositis caused by these agents.

Other randomized controlled trials

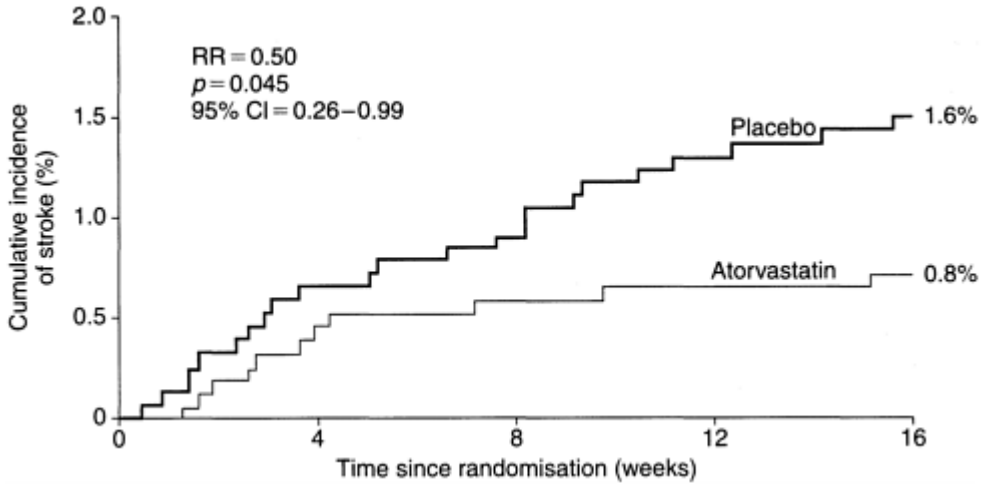

Figure 4.4

Cumulative incidence of fatal or non-fatal stroke among patients randomly assigned to treatment with atorvastatin (80 mg daily) or placebo in the Myocardial Ischemia Reduction with Aggressive Cholesterol Lowering (MIRACL) trial.

Two other randomized, controlled trials presently under way promise to provide additional information regarding the effects of intensive lipid-lowering after ACS. The Aggrastat to Zocor (A-to-Z) study[35] compares 'aggressive care' (simvastatin 40 mg/day for one month, then 80 mg/day) to 'accepted care' (placebo for four months, then simvastatin 20 mg/day). Treatment begins two-to-five days after ACS and continues until a specified number of events have occurred in the trial. A-to-Z will also explore whether there is any benefit of early statin therapy among patients who are also treated with a platelet glycoprotein IIb-IIIa inhibitor (only 1% of the patients in MIRACL) and those who undergo early coronary intervention (an exclusion criterion in MIRACL). The Pravastatin or Atorvastatin Evaluation and Infection Therapy (PROVE-IT) study[36] will attempt to determine the relative benefits of moderate versus intensive lipid-lowering therapy after ACS. The occurrence of ischemic cardiovascular events will be examined in patients randomized to either moderate-intensity treatment with pravastatin 40 mg/day or high-intensity treatment with atorvastatin 80 mg/day, beginning one-to-ten days after ACS and continuing for two years.

Unanswered questions

Despite the recent knowledge gained from experimental and clinical studies, many questions regarding the most appropriate use of statins in the early period following ACS remain unanswered. It is possible that the modest overall benefit of treatment with atorvastatin in the MIRACL trial reflects a substantial benefit of treatment for some patients, but little benefit for others. If so, it would be advantageous to find ways of identifying those patients most likely to benefit from early treatment, while sparing the expense and risks of treatment for those less likely to benefit. In the MIRACL trial, there was no significant interaction between standard demographic or clinical characteristics or baseline levels of standard lipid fractions and the reduction of ischemic events with atorvastatin. Additional studies are under way to determine if circulating inflammatory markers or markers of immune response to oxidized LDL cholesterol provide this information.

The MIRACL trial investigated only a single, high dose of atorvastatin. It is uncertain whether lower doses of this agent or other statins would provide similar benefit. As discussed above, the PROVE-IT and A-to-Z trials may help to answer this question.

Perhaps more important than dose or choice of statin agents is the question of whether non-statin lipid-lowering agents have a role in the early period following ACS. Long-term benefit of fibrate therapy in primary and secondary prevention of coronary heart disease events was demonstrated in the Helsinki Heart and Veteran Affairs High-Density Lipoprotein Cholesterol Intervention trials.[37,38] Acting as activators of the alpha peroxisome proliferator-activated receptor PPARα, fibrates also have the potential to exert anti-inflammatory effects in the arterial wall[39] and attenuate expression of tissue factor by macrophages,[40] effects that might stabilize vulnerable coronary plaques in the short term. At present, however, there is no direct evidence from clinical trials to support the use of fibrates or other classes of non-statin lipid-lowering agents in the period immediately following ACS.

The optimal management of ACS is a rapidly changing frontier. Recent studies support the use of routine early coronary revascularization, platelet IIb-IIIa receptor antagonists, and/or clopidogrel.[2–5] None of these strategies were employed in large numbers of patients in the MIRACL trial. Therefore, further investigation is required to determine if the benefit of intensive lipid-lowering is incremental to the benefit of these other strategies in the overall management of patients with ACS.

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5
Effects of statins on high-density lipoprotein and the implications for coronary heart disease prevention

Philip J Barter

Introduction

Inhibitors of HMG-CoA reductase (statins) have revolutionized the management of coronary heart disease (CHD). Members of this class of drugs include atorvastatin, cerivastatin (no longer available), fluvastatin, lovastatin, pitavastatin, pravastatin, rosuvastatin and simvastatin. The profound lowering of low-density lipoprotein (LDL) cholesterol achieved by statin drugs translates into substantial reductions in future coronary events in all subject groups that have been studied,[1–5] including subjects with and without manifest CHD, regardless of age and gender and regardless of baseline levels of plasma total cholesterol, LDL cholesterol and high-density lipoprotein (HDL) cholesterol. Most of the cardioprotection provided by statins in people without clinical CHD or in subjects with (stable) CHD can be explained in terms of the LDL-cholesterol-lowering achieved by the drugs.[6] There is evidence, however, that statins have potentially anti-atherogenic effects in addition to their ability to reduce LDL cholesterol.[7] One of these additional effects relates to their ability to increase the concentration of HDL cholesterol.

This chapter summarizes the current state of knowledge about the effects of statins on the concentration, composition and subpopulation distribution of HDL. It describes what is known regarding the mechanisms by which statin drugs alter HDL concentration and metabolism and addresses the clinical implications of these effects.

Effects of statins on concentration of HDL cholesterol

Magnitude of effect

All statins elevate the concentration of HDL cholesterol to some extent, with reported increases ranging from about 5% to as much as 30% in

Table 5.1 Lipid levels in the statin trials. Baseline levels (mmol/l) and percentage change on treatment*

Trial (drug)	Total cholesterol (mmol/l)	LDL cholesterol (mmol/l)	HDL cholesterol (mmol/l)	Triglyceride (mmol/l)
WOSCOPS	7.0	5.0	1.14	1.9
(pravastatin)	(−20%)	(−26%)	(+5.0%)	(−12%)
AFCAPS/	5.7	3.9	0.94	1.8

Trial (drug)	Total cholesterol (mmol/l)	LDL cholesterol (mmol/l)	HDL cholesterol (mmol/l)	Triglyceride (mmol/l)
TexCAPS (lovastatin)	(−18%)	(−25%)	(+6%)	(−15%)
4S (simvastatin)	6.8 (−25%)	4.9 (−35%)	1.19 (+8%)	1.5 (−10%)
CARE (pravastatin)	5.4 (−20%)	3.6 (−28%)	1.01 (+5%)	1.8 (−14%)
LIPID (pravastatin)	5.6 (−18%)	3.9 (−25%)	0.93 (+5%)	1.6 (−11%)

*Percentage change on treatment relative to placebo.

different studies. In general, the increase is in the range 5–10% (Table 5.1),[1–5] although it can be greater in patients whose baseline level of HDL cholesterol is low.[8] Statins also increase the concentration of apolipoprotein (apo)A-I,[9] the main protein component of HDL, although the magnitude of the apoA-I increase tends to be less than that of HDL cholesterol.[10]

Dose-response

The recommended starting dose of each of the statins promotes a 20–40% reduction in LDL, with each subsequent doubling of the dose resulting in an additional 6% (approximate) reduction in LDL cholesterol over the whole tolerated dose range.[11] This contrasts with the increase in HDL cholesterol achieved by statin therapy. The HDL cholesterol response is already close to maximal when taking the lowest recommended starting dose of each of the statins (Figure 5.1).[11–13] There is little additional HDL-cholesterol-raising with increasing doses; in some cases, the concentration of HDL cholesterol may actually fall as the statin dose is pushed to higher levels.[14–17] The explanation for this is not known.

Effects of different statins on HDL cholesterol

There have been several reports comparing the HDL-raising effects of different statins, with some evidence that not all statins are equally effective. For example, the HDL increase achieved by simvastatin may be superior to that achieved by atorvastatin,[15,16] especially at higher doses of the two drugs; at the lower 10 mg and 20 mg doses, the increases in HDL promoted by simvastatin and atorvastatin are comparable.[18] However, with increasing doses of the drugs the HDL cholesterol increase achieved by simvastatin tends to be sustained, while higher doses of atorvastatin may lead to a fall in the HDL cholesterol concentration,[15,16] although this difference has not been found in all studies. For example, in one large study involving more than 300 subjects with familial hypercholesterolaemia, two years of treatment with either simvastatin 40 mg or artovastatin 80 mg resulted in a 13% increase in HDL cholesterol in each group.[19]

On balance, it is likely that there are differences between the statins in terms of their HDL-cholesterol-raising capacity, although the explanation is not known. Nor is it known whether the differences have clinical significance.

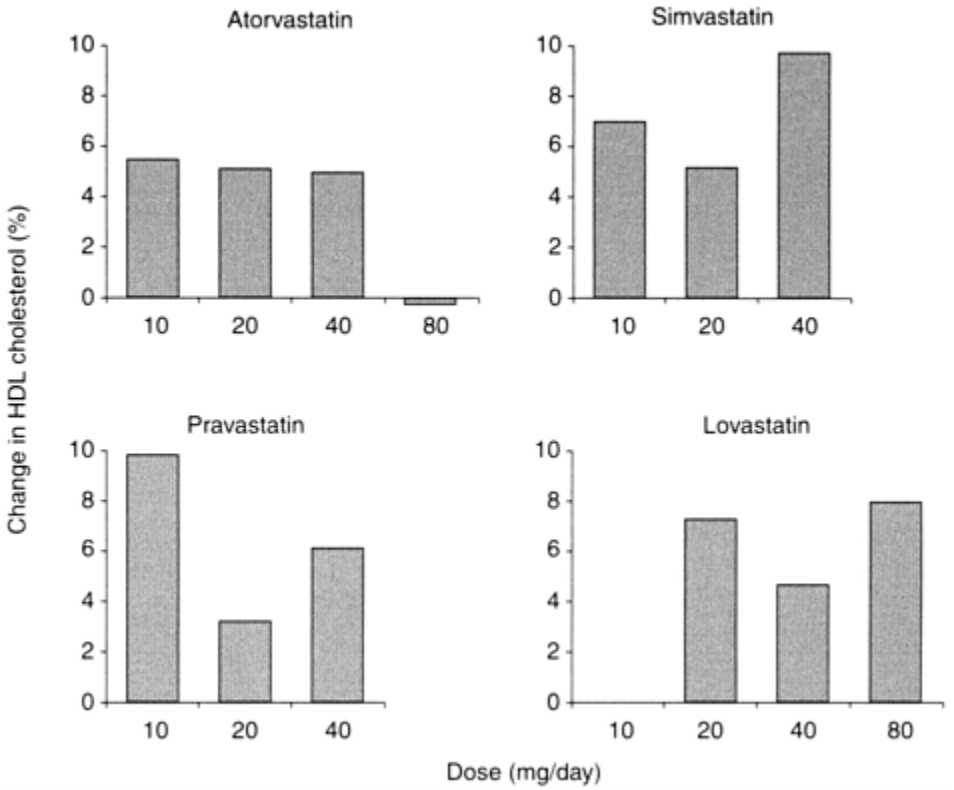

Figure 5.1

Typical dose response curves for statin-induced elevation of HDL cholesterol as reported in the CURVES study.[11]

HDL-raising effects of statins compared with other drugs

Fibrates

There have been many studies comparing the lipid-modifying effects of fibrates and statins.[20–24] Results have depended largely on the baseline lipid profiles of the groups under study. Overall, the results have been predictable from the known effects of the two classes of agents. Statins and fibrates both have the potential to lower plasma total cholesterol, LDL cholesterol and plasma triglyceride and to raise the level of HDL cholesterol. Statins, however, are superior to fibrates in lowering the level of plasma total cholesterol and LDL cholesterol, while fibrates tend to be superior to statins in terms of their ability to lower triglyceride and raise HDL cholesterol.

Niacin

Niacin is the most effective of the currently available drugs for raising HDL cholesterol, with reports of increases up to 30%.[25] Use of this agent has tended to be limited by problems of tolerance, although this has been less of an issue with more recent formulations. Niacin also has the ability to reduce plasma triglyceride by 40–50% and to lower LDL cholesterol by 15–20%.[25]

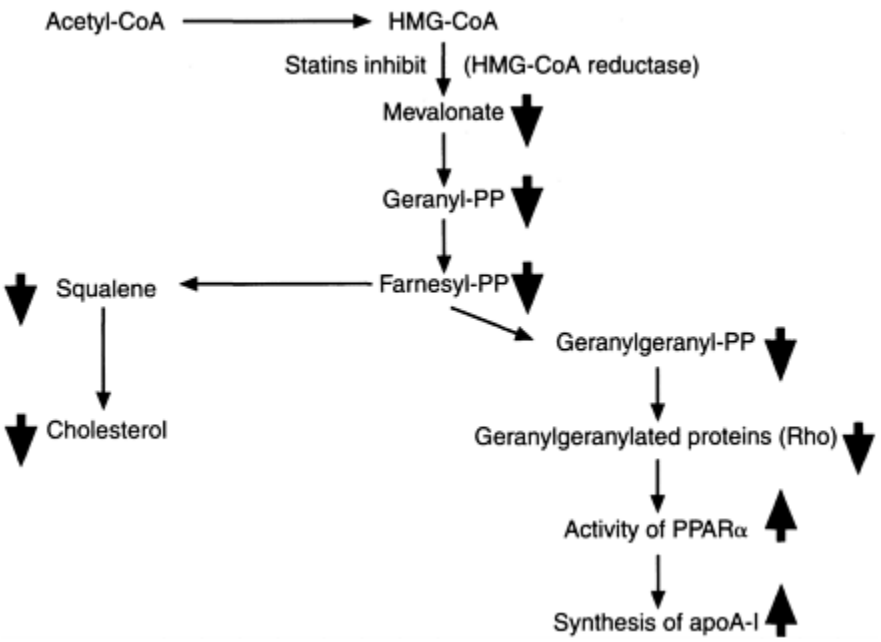

Figure 5.2

Effects of statins on the synthesis of apoA-I. By inhibiting HMG-CoA reductase, statins inhibit all downstream reactions. Thus, not only do they inhibit the synthesis of cholesterol but also the prenylation of Rho. Inhibition of Rho prenylation leads to an increase in activity of PPARα and a consequent increase in the synthesis of apoA-I.[31] The arrows indicate the effects of statins on the various steps.

Effects of statins on the subpopulation distribution of HDL

Statins increase the concentration of HDL cholesterol to a greater extent than apoA-I.[10] Hence, treatment with statins tends to increase the HDL particle size,[26] leading to a preferential increase in the HDL_2 subfraction.[27] Statins also preferentially increase the concentration of the sub-population of HDL that contains apoA-I without apoA-II (A-I-HDL) rather than of those containing both apoA-I and apoA-II (A-I/A-II-HDL).[28] It has been suggested that the A-I-HDL subpopulation has superior cardioprotective properties,[29] although other studies have provided evidence that the protection provided by the two subpopulations may be equivalent.[30]

Mechanism of statin effects on HDL

Increased synthesis of apoA-I

Like fibrates, statins increase the synthesis of apoA-I by increasing the activity of peroxisome proliferator-activated receptor-alpha (PPARα).[31] Unlike fibrates, however, PPARα-activation by statins is achieved via an indirect mechanism involving inhibition of the Rho family of small GTP-ase proteins.[31] Activity of Rho is dependent on a process of prenylation mediated by geranylgeranyl pyrophospate (GGPP), a downstream metabolite of HMG-CoA reductase. Since statins inhibit GGPP produccellular membranes. The

resulting decrease in biological activity of Rho[32] tion, they also inhibit the prenylation of Rho and reduce its attachment to is associated with an increase in the activity of PPARα[31] (Figure 5.2) and a consequent increase in the synthesis of apoA-I.[31] The precise mechanism by which an inhibition of the prenylation of Rho translates into activation of PPARα is not known, although there is evidence that it is related to PPARα phosphorylation.[31]

Secondary to a reduction in triglyceride-rich lipoproteins

The concentration of HDL cholesterol correlates inversely with that of triglyceride-rich lipoproteins (TGRLPs). Thus, the ability of statins to reduce the concentration of TGRLPs should translate into a secondary increase in HDL cholesterol. The reciprocal relationship between the concentrations of HDL TGRLPs is dependent, in part, on the activity of cholesterol ester transfer protein (CETP), the plasma protein that transfers cholesteryl esters from HDL to TGRLP.

Inhibition of CETP

Not only do statins reduce the transfer of cholesteryl esters out of HDL by reducing the concentrations of the recipient TGRLPs but they also inhibit activity of the CETP that mediates the transfer.[33,34] Such an inhibition of CETP may further contribute to the HDL-raising properties of statins.

Thus, statins have the capacity to raise HDL cholesterol by several mechanisms, and which of these mechanisms predominates is not known. Nor is it known how the change in concentration relates to HDL function, whether in relation to the potential for cholesterol efflux or other HDL functions such as their anti-inflammatory properties. Indeed, it still remains to be determined how statin-induced increases in HDL cholesterol translate into clinical benefits.

Mechanism underlying possible differences in the HDL-raising properties of statins

The concentration of HDL cholesterol reflects a balance between the processes of synthesis and catabolism of the HDL particles. It is conceivable that the fall-off in concentration of HDL cholesterol at higher doses of atorvastatin is the consequence of an enhanced hepatic uptake of HDL cholesterol rather than of a decrease in the synthesis of apoA-I. Given that such hepatic uptake is the final step in the pathway of reverse cholesterol transport (RCT) and that its stimulation is potentially anti-atherogenic, it is conceivable that the decrease in HDL cholesterol at higher doses of atorvastatin could reflect an enhancement of an anti-atherogenic process. If, however, the fall in HDL cholesterol reflects a decreased synthesis of apoA-I, it may indicate a reduced anti-atherogenic potential. The true situation is currently not known and awaits further investigation.

Another possible explanation for the observed loss of the HDL-raising effect at higher doses of atorvastatin is the fact that atorvastatin metabolites have potent antioxidant properties.[35] It is known that antioxidants such as probucol reduce HDL cholesterol levels,[36,37] and a mixture of vitamins C and E, beta carotene and selenium has been reported to reduce the HDL-raising effects achieved by the combination of simvastatin and niacin.[38] It has also been reported that the ability of statins to raise the level of HDL cholesterol is markedly influenced by the paraoxonase genotype of the subject.[39] However, further research is required before concluding that the antioxidant properties of atorvastatin metabolites contribute to the loss of HDL-cholesterol-raising when the drug is given at high doses.

Clinical relevance of HDL-raising by statins

There is no doubt that the LDL-cholesterol-lowering properties of statins translate into substantial reductions in atherosclerotic vascular disease.[6] The question arises, does the HDL cholesterol elevation achieved by statins also contribute to the cardioprotective properties of these agents? And if so, what is the magnitude of the benefit relative to that attributable to the reduction in LDL cholesterol?

Relationship between HDL and atherosclerosis in human population studies

A powerful inverse relationship between the level of HDL cholesterol and the risk of developing CHD has been a consistent finding in prospective population studies. Key studies include the Framingham Heart Study,[40–41] the Prospective Cardiovascular Munster (PROCAM) Study,[42] the placebo group of the Helsinki Heart[43] and the Multiple Risk Factor Intervention Trial (MRFIT).[44] The HDL cholesterol data from the Framingham Heart Study, the Lipid Research Clinics Prevalence Mortality Follow-up Study and the MRFIT study have been analysed by Gordon *et al,*[45] who reported that for every 0.025 mmol/l increase in HDL cholesterol, the CHD risk is reduced by 2–5%.

Statin trials

There are two published primary prevention studies: the West of Scotland Coronary Prevention Study (WOSCOPS) using pravastatin in hypercholesterolaemic men[2] and the Air Force/Texas Coronary Atherosclerosis Prevention Study (AFCAPS/TexCAPS) using lovastatin in men and women with average cholesterol levels;[5] and three secondary prevention trials: the Scandinavian Simvastatin Survival Study (4S) using simvastatin in hypercholesterolaemic men and women with existing CHD,[1] the Cholesterol and Recurrent Events (CARE) study using pravastatin in men and women with previous myocardial infarction (MI) in whom the total cholesterol was relatively low[3] and the Australian and New Zealand Long-term Intervention with Pravastatin in Ischaemic Disease (LIPID) study using pravastatin in men and women with previous MI or unstable angina and with average cholesterol levels.[4]

West of Scotland Coronary Prevention Study

WOSCOPS was a double-blind, placebo-controlled trial that included 6,595 hypercholesterolaemic men without clinical CHD at entry into the trial.[2] The active treatment was pravastatin 40 mg daily and the mean follow-up was 4.9 years. The mean plasma total cholesterol was 7.07 mmol/l, the mean LDL cholesterol was 5.0 mmol/l and the mean HDL cholesterol was 1.14 mmol/l. Pravastatin reduced the concentration of LDL cholesterol by 26% and increased HDL cholesterol by 5%. The primary endpoint, a composite of non-fatal MI or death from CHD, was reduced from 7.9% in the placebo group to 5.5% in the pravastatin group ($P<0.001$). The CHD death rate was also reduced from 1.9% to 1.3% ($P=0.042$). A reduction in all-cause-mortality from 4.1% to 3.2% almost reached statistical significance ($P=0.051$).

The magnitude of the reduction in events in the pravastatin group in WOSCOPS was independent of the baseline levels of LDL cholesterol and HDL cholesterol. The event reduction was partly explained by the decrease in LDL cholesterol but was not significantly related to the increase in HDL cholesterol.[46] Furthermore, while treatment with pravastatin largely eliminated the influence of baseline LDL cholesterol as a predictor of coronary events, baseline HDL cholesterol remained as predictive of events in the pravastatin group as in the placebo group.[46] This indicated that while subjects with a low level of HDL

cholesterol derived benefit from treatment with pravastatin, the treatment does not remove the risk associated with low HDL.

Air Force/Texas Coronary Atherosclerosis Prevention Study

AFCAPS/TexCAPS was a double-blind, placebo-controlled trial of 6,605 people (5,608 men aged 45–73 and 997 postmenopausal women aged 55–73).[5] These people had average baseline levels of LDL cholesterol (mean 3.89 mmol/l), low baseline HDL cholesterol (mean 0.94 mmol/l) and were free of clinical CHD at entry into the study. The active treatment was lovastatin, 20–40 mg daily and the mean follow-up was 5.2 years.

Treatment with lovastatin reduced the concentration of LDL cholesterol by 25% and increased HDL cholesterol by 6%. The primary endpoint, a composite of sudden death, fatal or non-fatal MI and unstable angina, was reduced from 9.3% in the placebo group to 6.2% in the lovastatin group ($P<0.001$). There were relatively few deaths in the study, with no difference between the two groups.

The benefits of treatment with lovastatin were unrelated to the baseline level of LDL cholesterol.[47] There was a trend towards a lower event reduction in those with high baseline levels of HDL cholesterol, although, as in the other statin trials, this was not statistically significant. Neither the on-treatment level of HDL cholesterol, nor the increase in HDL cholesterol were predictive of benefit, although the level of apoA-I at one year did predict benefit. A conclusion by the authors of this study that treatment with lovastatin abolished the risk associated with low baseline HDL cholesterol[47] is at variance with all of the other statin trials and may reflect no more than the play of chance.

Scandinavian Simvastatin Survival Study

4S was a double-blind, placebo-controlled trial that included 4,444 hypercholesterolaemic patients with pre-existing CHD (3,617 men and 827 women) aged 35–70.[1] Serum total cholesterol at baseline was 5.5–8 mmol/l and the triglyceride level was <2.5 mmol/l. The level of HDL cholesterol was not an entry criterion. The active treatment was simvastatin (20 mg, up-titrated to 40 mg daily in 37% of the subjects) and the median follow-up was 5.4 years.

Simvastatin treatment reduced the concentration of LDL cholesterol by an average of 38% from a baseline level of 4.87 mmol/l. HDL cholesterol was increased by 8% from a baseline of 1.18 mmol/l. The primary end-point was all-cause mortality, and this decreased from 11.5% in the placebo group to 8.2% in the simvastatin group ($P<0.001$). Coronary death rate was reduced from 8.5% to 5.0% by simvastatin ($P<0.001$). There was also a significant reduction in major coronary event rate from 22.6% in the placebo group to 15.9% in the simvastatin group ($P<0.001$).

The relationship between changes in LDL cholesterol and HDL cholesterol was analysed by Pedersen *et al*[48] who reported that most of the event reduction achieved by therapy with simvastatin was determined by the magnitude of the reduction in LDL cholesterol. The preferred model estimated a 45% reduction in coronary events for the observed 35% reduction in LDL cholesterol; this was close to the observed 40% reduction in events. The relationship between increase in HDL and the reduction in events was statistically significant in some (but not all) of the models that were tested. Overall, it appeared that for each 1% increase in the concentration of HDL cholesterol, there was a 0.6–0.9% reduction in coronary events.[48] It should be noted, however, that treatment with simvastatin did not remove the risk associated with a low concentration of HDL cholesterol.[49]

More recently, Ballantyne *et al.* reported the results in two subgroups of 4S.[50] One group was designated ‘isolated high LDL cholesterol’ and included subjects with the highest quartile of HDL cholesterol and the lowest quartile of triglyceride (n=545). The other group was designated ‘lipid triad’ and included subjects with

the lowest quartile of HDL cholesterol and the highest quartile of triglyceride (n=458). In the group with isolated high LDL cholesterol, the event rate in the placebo group was 20.8%; this was reduced to 18.0% in the simvastatin group. In subjects with the lipid triad (low HDL cholesterol and moderately elevated triglyceride) the event rate was 35.9% in the placebo group, reduced to 19.0% in the simvastatin group. This clearly indicates that subjects who have high LDL cholesterol, high plasma triglyceride and low HDL cholesterol derive substantial benefit from treatment with simvastatin. It does not, however, indicate whether the benefit in this subgroup is derived from a reduction in LDL cholesterol, a reduction in triglyceride or an increase in HDL cholesterol.

Long-term Intervention with Pravastatin in Ischaemic Disease

LIPID was a double-blind, placebo-controlled trial that included 9,014 patients with previous acute coronary syndromes (7,498 men and 1,516 women) aged 31–75.[4] The entry criteria included serum total cholesterol of 4.0–7.0 mmol/l and a triglyceride level of <5.0 mmol/l. The median baseline level of LDL cholesterol was 3.9 mmol/l and the median baseline HDL cholesterol was 0.9 mmol/l. HDL cholesterol was not an entry criterion. The active treatment was pravastatin 40mg daily and the mean follow-up was 6.1 years. Pravastatin treatment reduced the concentration of LDL cholesterol by an average of 25% and increased the HDL cholesterol by 5%. The primary endpoint, CHD mortality, was reduced from 8.3% in the placebo group to 6.4% in the pravastatin group (P<0.001). All-cause mortality was reduced from 14.1% to 11.0% by pravastatin (P<0.001).

The relationship between CHD events and the baseline and on-treatment lipid levels has been analysed by Simes *et al*.[51] The results in the placebo group were similar to those in previous population studies, with an adjusted relative risk per mmol/l of 1.28 for LDL cholesterol and 0.52 for HDL cholesterol. The baseline LDL cholesterol ceased to be a predictor of events in the group treated with pravastatin, while the baseline HDL cholesterol remained predictive even when pravastatin was taken, again indicating that treatment with a statin does not correct the risk associated with a low level of HDL cholesterol.

The on-treatment lipid levels (at 12 months) in the LIPID trial were predictive of events in both the placebo and pravastatin groups.[51] In the pravastatin group the adjusted relative risk per mmol/l was 1.20 for LDL cholesterol and 0.69 for HDL cholesterol. An analysis was made of the proportion of the treatment effect (PTE) that could be explained by the effects of pravastatin on lipid levels. It was concluded that the PTE explained by LDL cholesterol reduction was up to 82%, while that explained by HDL cholesterol increase was 12% or less.[51]

Cholesterol and Recurrent Events

CARE was a double-blind, placebo-controlled trial that included 4,159 normocholesterolaemic patients with previous MI (3,583 men and 576 women) aged 21–75 years.[3] The entry criteria included a serum total cholesterol <6.2 mmol/l, an LDL cholesterol of 3.0–4.5 mmol/l and a triglyceride level of <4.0 mmol/l. The HDL cholesterol concentration (mean of 1.0 mmol/l) was not an entry criterion. The active treatment was pravastatin 40 mg daily and the median follow-up was 5.0 years. Pravastatin treatment reduced the concentration of LDL cholesterol by an average of 32% and increased HDL cholesterol by 5%. The primary endpoint was coronary death or non-fatal MI, which was significantly reduced from 13.2% in the placebo group to 10.2% in the pravastatin group (P=0.003). Mortality differences were not significant.

The relationship between HDL cholesterol and CHD events in the CARE study has been analysed as part of a pooling project that combined the results of the three major studies conducted with pravastatin.[52]

Prospective Pravastatin Pooling Project

The Prospective Pravastatin Pooling Project (PPP) was established to analyse the pooled results from WOSCOPS, CARE and LIPID.[52] In the case of CARE and LIPID, the trial designs were very similar and a pooling of the results has provided a much larger database for analysis than was possible in either trial alone. In an analysis that included 13,173 participants in the combined CARE and LIPID studies, it was found that a low level of HDL cholesterol was predictive of a greater than average event reduction in subjects who also had a low LDL cholesterol (<3.2 mmol/l).[53] However, low HDL cholesterol was not predictive of greater benefit in subjects with higher levels of LDL cholesterol. In subjects with LDL cholesterol <3.2 mmol/l, a 0.26 mmol/l increase in HDL cholesterol (approximately 25%) predicted an event reduction of 29%. In contrast, in subjects with LDL cholesterol levels >3.2 mmol/l, the same 0.26 mmol/l increase in HDL cholesterol (approximately 25%) accounted for only a 10% decrease in events.[53]

The pooling project confirmed the finding from the individual trials that the baseline concentration of HDL cholesterol remained predictive of events during treatment with pravastatin, thus confirming that treatment with a statin does not eliminate the risk associated with a low level of HDL cholesterol.

Impact of statin-induced HDL-raising on CHD

Overall, the relationship between changes in HDL cholesterol and CHD events in the statin trials is unclear, possibly because it is obscured by the major reduction in LDL cholesterol in these trials. If a statin-induced elevation of HDL cholesterol does reduce events, the magnitude of the benefit is small relative to the protection resulting from the reduction in LDL cholesterol. It is also apparent that treatment with statins does not eliminate the risk associated with low LDL, as evidenced by the observations that a low baseline level of HDL cholesterol remains predictive of coronary events in patients treated with statins (Table 5.2). The relatively minor effects of statins on the relationship between HDL and CHD contrast with the results obtained in the fibrate trials in which the benefits of raising HDL cholesterol are substantial.

Relationship between HDL cholesterol and coronary events in fibrate trials

Helsinki Heart Study

The Helsinki Heart Study was a double-blind, placebo-controlled trial that

Table 5.2 Relationship between CHD events and HDL cholesterol in the statin trials

Trial (drug)	Percentage reduction in CHD events	Relationship of CHD events to increase in HDL cholesterol	Baseline HDL cholesterol predicts events	
			On placebo	On statin
WOSCOPS (pravastatin)	31	Not significant	Yes	Yes
AFCAPS/ TexCAPS (lovastatin)	**25**	Not significant	Yes	No
4S (simvastatin)	**34**	**P<0.05**	**Yes**	**Yes**
CARE (pravastatin)	**24**	Not significant	Yes	Yes

Trial (drug)	Percentage reduction in CHD events	Relationship of CHD events to increase in HDL cholesterol	Baseline HDL cholesterol predicts events	
			On placebo	On statin
LIPID (pravastatin)	**24**	Not significant	Yes	Yes

included 4,081 men aged 40–55 years who were free of clinically manifest CHD at entry to the study.[43] The active treatment was gemfibrozil 1,200 mg daily and the mean follow-up was five years. For inclusion, subjects had to be free of clinically manifest CHD or other major illness at the time of randomization and to meet the lipid acceptance criterion of non-HDL cholesterol >5.2 mmol/l. The mean baseline lipid levels were: serum total cholesterol 7.0 mmol/l, LDL cholesterol 4.9 mmol/l, HDL cholesterol 1.22 mmol/l and serum triglyceride 2.0 mmol/l. Treatment with gemfibrozil reduced the concentration of LDL cholesterol by 11% and increased HDL cholesterol by 11%. The primary endpoint (CHD events) was significantly reduced in the gemfibrozil group compared with the placebo group; 2.7 and 4.1 respectively.

In a subsequent robust analysis of the relationship between lipid levels and event reduction, it was concluded that a 1% increase in HDL cholesterol was associated with a 2–3% decrease in CHD events, independent of changes in levels of LDL cholesterol.[54] The main benefit of gemfibrozil in the Helsinki Heart Study was apparent in subjects who had one or more features of the metabolic syndrome, such as a body mass index (BMI) >26, plasma triglyceride >2.3 mmol/l or HDL cholesterol <1.0 mmol/l.[55,56] In subjects without any features of the metabolic syndrome, the benefits were much less pronounced.

The Veterans Affairs High-Density Lipoprotein Cholesterol Intervention Trial

The Veterans Affairs High-Density Lipoprotein Cholesterol Intervention Trial (VA-HIT) was a double-blind, placebo-controlled trial that included 2,531 men aged <74 years with known clinical CHD.[57] To be eligible for inclusion, subjects had to have a low level of both HDL cholesterol (<1.0 mmol/l) and LDL cholesterol (<3.6 mmol/l) and a plasma triglyceride level <3.4 mmol/l. The mean baseline concentrations of HDL cholesterol and LDL cholesterol were 0.83 mmol/l and 2.88 mmol/l, respectively. The active treatment was gemfibrozil 1,200 mg daily and the mean follow-up was 5.1 years. One year after randomization the concentration of LDL cholesterol was unchanged, HDL cholesterol was increased by 6% and the plasma triglycerides were decreased by 31%. These changes were sustained for the duration of the study. The primary endpoint (non-fatal MI or coronary death) was significantly reduced in the gemfibrozil group compared with the placebo group—17.3% and 21.7%, respectively.

The on-treatment HDL cholesterol level was predictive of CHD events in both the active and placebo groups. Multivariate regression analysis showed that, of all of the variables measured, the increase in HDL cholesterol was the only one that predicted benefit.[58] However, the HDL cholesterol increase accounted for only about one quarter of the observed reduction in events. The question arises: what explains the other three-quarters of the benefit? Is it a beneficial effect of fibrates on HDL function beyond the observed increase in HDL cholesterol concentration, or does it reflect direct anti-inflammatory effects of fibrates within the artery wall?

As in the Helsinki Heart Study, the main benefit of gemfibrozil in the VA-HIT study was observed in subjects who had features of the metabolic syndrome; in subjects without features of the metabolic syndrome, the benefits were much less pronounced (S Robins, personal communication).

The Bezafibrate Infarction Prevention study

The Bezafibrate Infarction Prevention (BIP) study was a double-blind, placebo-controlled trial that included 3,090 subjects with clinically manifest CHD (2,825 men, 265 women) aged <74 years.[59] The concentration of plasma total cholesterol ranged from 4.7 to 6.5 mmol/l, the plasma triglyceride concentration was <3.4 mmol/l and the concentration of HDL cholesterol was <1.16 mmol/l. The active treatment was bezafibrate 400 mg per day and the mean follow-up was 6.2 years. Despite a 5% reduction in LDL cholesterol and a 14% increase in HDL cholesterol, there was no significant effect on the primary outcome (the combined incidence of non-fatal MI or death from CHD) at 5 years, or at 6.2 years.[59] Post-hoc analysis, however, suggested a significant benefit in the subset of patients in whom the entry triglyceride level was >2.25 mmol/l. The event rate in this subgroup was significantly lower in the placebo group than in the bezafibrate group 12.0% vs 19.7%, respectively (P=0.02).

Impact of fibrate-induced HDL-raising on CHD

The benefit of treatment with fibrates appears to be greatest in people with features of the metabolic syndrome. In these individuals, the reduction in coronary events attributable to an increase in HDL cholesterol is considerably greater than predicted from the population studies and greater than observed in the statin trials. Whether this reflects an effect on HDL function that is greater than the increase in concentration of HDL cholesterol or whether fibrates have an additional beneficial effect in people with the metabolic syndrome is not known.

Conclusion

On the basis of the evidence currently available, it is possible to draw several conclusions about the effects of statins on HDL.

- All statins increase the concentration of HDL cholesterol by 5–10%, with greater increases possible in subject whose baseline HDL cholesterol is low.
- The HDL-cholesterol-raising potential may vary with different statins, although the clinical significance of this is uncertain.
- The mechanism of the statin-induced increase in HDL cholesterol relates to an activation of PPARα and possibly also an inhibition of CETP.
- Statins reduce coronary risk in all subjects, including those with low levels of HDL cholesterol. They do not, however, remove the risk associated with a low HDL cholesterol.
- It remains to be determined whether a statin-induced increase in HDL cholesterol translates into a reduction in CHD risk.
- The HDL cholesterol increase induced by statins appears to have less of an effect in reducing coronary risk than does a comparable increase in HDL cholesterol induced by treatment with fibrates, at least in people with features of the metabolic syndrome.

In terms of the coronary event reduction achieved with statins, the evidence suggests that most of the benefits of statin therapy are secondary to the reduction in concentration of LDL cholesterol. Any additional risk reduction achieved by the modest statin-induced increase in HDL cholesterol should be viewed as a bonus rather than a major benefit.

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6
Long-term safety of lipid-lowering drugs

Michael Schachter

Introduction

The efficacy and clinical usefulness of lipid-lowering therapies are so clearly established that they no longer need emphasis, even if we are not certain about the mechanisms of their benefits or about how exactly to define the population which will receive these drugs. However, medicine is sadly lacking in drugs that are effective and yet wholly without serious adverse effects. Lipid-lowering drugs cannot be included in such a category. As they have become more clinically important there has been increasing interest in aspects of their safety and tolerability. This is particularly true of the HMG-CoA reductase inhibitors (the statins), which have been used in very large clinical trials in the past decade, most recently in the Heart Protection Study.[1] Understandably, the wider clinical usage of these drugs has paralleled the accumulation of trial data, details of which are discussed in detail in other chapters. An unwelcome impetus to this interest unexpectedly occurred in the summer of 2001 with the abrupt withdrawal of cerivastatin because of many cases of serious, indeed fatal, skeletal muscle damage.[2] While this attracted huge publicity, it is not, as subsequent discussion will show, typical of this class of drugs, still less of lipid-lowering drugs in general. The statins will be the largest single group of drugs discussed in this chapter, but there will of course also be discussion of the other major drug classes: fibrates, bile-acid binding resins, nicotinic acid and the omega-3 fatty acids. In some instances, particularly as regards statins and the fibrates, combination therapy needs separate consideration and discussion.

Statins

The success of the statins, and the clinical trial evidence that has supported it, is a remarkable achievement of modern therapeutics. But it was realised soon after these drugs were introduced that they were capable of causing major adverse effects:

- elevation of liver enzymes
- peripheral neuropathy
- skeletal muscle damage, ranging from myalgia to massive tissue destruction
- hypersensitivity reactions.

Liver toxicity

Asymptomatic elevations of liver transaminases, up to two-to-three times the upper limit of normal, is seen in up to 2% of statin-treated patients.[3] However, most estimates suggest an incidence of about half this figure.[4] Standard protocols for the use of statins recommend that liver enzyme levels are checked as a matter of routine about a month after initiating therapy or whenever doses are increased. In fact, serious liver damage is extraordinarily rare (though a very small number of cases of liver failure have been described), which may reflect the fact that drug treatment is usually discontinued in patients with enzyme abnormalities, in whom levels then revert to normal. It seems highly unlikely that adherence to routine liver function checks is absolutely complete, so one might have expected more frequent liver toxicity to occur. The fact that this is apparently not the case again suggests that this is not a major clinical issue, and some have suggested abandoning continued screening for abnormal liver enzymes,[5] although not the recommendation that the statins should be avoided in patients with active liver disease.

Peripheral neuropathy

Although there has in the past been doubt about whether a link existed between the statins and peripheral neuropathy, it is now generally accepted that this problem does occur, though rarely, during long-term therapy.[6,7] However, two important points add to the significance of this issue. Firstly, when neuropathy occurs, it seems to persist for months after withdrawal of the drug; and secondly, this problem is always associated with long-term statin therapy. We are now at the beginning of a period where patients are committed to statin therapy for many years or even decades. This certainly represents a warning that we do not understand all implications of the prolonged use of these drugs.

Hypersensitivity reactions

A small number of cases of apparent statin-related hypersensitivity have been described, the most serious of which is apparent (reversible) statin-induced pulmonary fibrosis.[8] Again, it is possible that more such problems will emerge with the more extensive and longer-term prescribing of these drugs.

Skeletal muscle toxicity

This problem has attracted increased professional and public attention since the withdrawal of cerivastatin in 2001. Even before the cerivastatin disaster it was appreciated that this was by far the most important adverse effect of the statin drugs. The existence of myopathy, and in extreme cases rhabdomyolysis, was recognized in the mid-1980s, when major clinical studies with the statins were just beginning. Drug-induced rhabdomyolysis is very unusual as a direct adverse effect of other drugs but it is potentially fatal due to acute renal failure and associated hyperkalaemia due to the massive breakdown of skeletal myocytes. It is very important, and rather worrying, to point out that the mechanism of this toxicity is still not well understood (this will be discussed later).

From a clinical point of view the extensive trials involving simvastatin, pravastatin and lovastatin gave little cause for concern, with instances of myopathy very rare and no cases of rhabdomyolysis or of drug-related deaths from any cause. Myopathy, usually defined as a rise in plasma creatine kinase levels to ten-fold or more above the upper limit of normal, occurred in about 0.1–0.5% of patients in the major trials, including the very large and recently published Heart Protection Study.[1] It should be noted that this was in

circumstances where likely drug interactions could be minimized, a crucial point which will be further discussed below.

A review published in the autumn of 2001, written before the withdrawal of cerivastatin, listed 74 case reports of statin-associated rhabdomyolysis, and it was estimated that the frequency of this adverse effect in treated patients, irrespective of the likelihood of drug interactions, was at most 0.2%, with an estimate of 0.04% probably more realistic.[9] Among the published case studies, lovastatin was the most frequently cited of the statins, reflecting its widespread use in the USA. However, a survey published in early 2002 and based on an analysis of adverse event reports to the Food and Drug Administration (FDA), shows a different pattern.[10] Interestingly this survey covered a 29-month period up to March 2000 but even then cerivastatin was the second most commonly mentioned drug after simvastatin (each representing over 30% of total reports), despite the fact that cerivastatin was much less frequently prescribed in the USA than simvastatin. Writing some months after the withdrawal of cerivastatin, an author from the FDA indicated that the reporting rate for fatal rhabdomyolysis per million prescriptions was over 3 for cerivastatin but less than 0.2 for all other statins prescribed in the USA.[11] This is likely to represent under-reporting, as always with adverse drug reactions, but the proportions are very striking. At the time of the withdrawal of cerivastatin there had been at least 31 cases of fatal rhabdomyolysis, but it has since been suggested that the true figure may be two-to-three times greater.

Before considering this special and, one hopes, unique case further,

Table 6.1 Metabolism of the statins

	Cytochrome P450				
Statin	3A4	2A6	2C8	2C9	2D6
Atorvastatin	✗				
Cerivastatin	✗	✗	✗		
Fluvastatin				✗	
Lovastatin	✗				✗
Pravastatin					
Simvastatin	✗			✗	

Involvement of cytochrome P450 sub-types in the metabolism of individual statins. ✗ = significant metabolism.

one must consider the question of *statins and their interactions with other drugs.* All of the statins, with the exception of pravastatin, are metabolized by one or more isoforms of cytochrome P450 (Table 6.1). Pravastatin is the least lipophilic of the statins, with poor penetration into most tissues including skeletal muscle, and has the lowest propensity for causing myopathy.[12] All available data indicate that in the presence of drugs which inhibit cytochrome P450, and hence statin metabolism (Table 6.2), the likelihood of statin toxicity is greatly increased.[13,14] In the few cases where detailed information is available, it would appear that there is a strong positive association between plasma levels of the drug and muscle toxicity. It has been estimated that drugs with the potential to interact increase the likelihood of rhabdomyolysis five-fold. About a third of the patients who suffered from fatal rhabdomyolysis on cerivastatin therapy had also been taking gemfibrozil (despite several warnings that this would increase toxicity),[11] and this fibrate-like drug also increases the myotoxicity of simvastatin and lovastatin by impeding clearance.

A critical issue such as this obviously needs a solution, which raises the question, what is the mechanism of statin-induced myopathy? Perhaps surprisingly, given that this problem was recognized in the 1980s, we really have no convincing answer.[15] The mevalonate pathway inhibited by the statins is, of course, the

source of low-molecular-weight lipids (isoprenoids), which have a central role in the regulation of cell function.[16] Theories regarding the myotoxic effect have included mitochondrial toxicity, interaction with ion channels and altered calcium metabolism but the truth is that none of these has been intensively pursued, probably because the clinical problem was not regarded as especially serious. This issue is made all the more relevant, however, by the cerivastatin debacle. We simply do not know why this agent proved so toxic compared with the other statins, and the ideas advanced so far are unconvincing; for instance, its supposedly greater lipophilicity than other

Table 6.2 Inhibitors of cytochrome P450 3A4

Ketoconazole, itraconazole, fluconazole
Erythromycin, clarithromycin
TCAs, nefazodone, venlafaxine
Fluvoxamine, fluoxetine, sertraline
Cyclosporine, tacrolimus
Omeprazole, lansoprazole
Calcium-channel blockers (esp. diltiazem)
Midazolam
Corticosteroids
Grapefruit juice
Tamoxifen

statins. It seems plausible that a metabolite of cerivastatin is involved, since the drug has an unusual metabolism (Table 6.1), and since its potency was much greater than one would have predicted from the native compound.

Fibrates

Although fibrates have a rather longer history than the statins, they have so far been less intensively studied from the point of view of toxicity. This does not imply that problems are necessarily less frequent. A recent cohort study based on UK general practices suggested that the incidence of *myopathy* was about six times greater in patients on fibrates than in those treated with statins.[17] The first report of fibrate-associated myopathy dates from 1968 and is related to clofibrate, a drug now little used in clinical practice. Since then all of the currently marketed fibrates—bezafibrate, fenofibrate, ciprofibrate and gemfibrozil—have been implicated in causing myopathy. There is surprisingly little information available regarding the incidence of this problem, certainly in comparison to what is known about statins, and it is difficult to assess the incidence and to determine whether it really is more frequent than in statin-treated patients. Certainly it is nowhere near as common as cerivastatin-related muscle toxicity, even if it exceeds the frequency seen with the other statins. Some factors appear to enhance toxicity:

- High doses, especially above recommended levels. As with the statins, toxicity is more closely related to plasma levels of the drug than is efficacy
- Renal impairment, which may of course be associated with the previous point. Fibrates themselves can cause deterioration in renal function (see below)
- Hypothyroidism, untreated or inadequately treated.

No large clusters of cases of fibrate-induced myopathy have been reported. This may represent reporting bias but is more likely to reflect the fact that this is not a major clinical problem.

There may be strong clinical indications for the use of combined statin/fibrate therapy and, with the exception of regimens including gemfibrozil, there is nothing to indicate large excesses of serious adverse events in patients on combination therapy.[18,19] However, particularly close monitoring of such patients is certainly advisable.

Impaired renal function resulting from fibrate therapy has been described in numerous case histories,[20] although not always in the degree of detail that might be desirable. Although the decline in function is usually modest, it can be sufficient to cause major problems in patients with renal transplants. The mechanism is thought to be related to inhibition of renal cyclooxygenase activity.[21] Interestingly, gemfibrozil seems to lack this adverse effect, precisely because it does not interact with this enzyme.[22]

Bone marrow dyscrasias have been reported with fibrate therapy but seem to be extremely rare.

Nicotinic acid

Anyone taking note of the effects of nicotinic acid on the lipid profile (Table 6.3), in comparison with other available drugs, would conclude that this was an almost ideal agent. It is also an inexpensive drug. Since its usage is obviously much more limited than these considerations would suggest—although certainly greater in the USA than in Europe—problems relating to adverse effects and safety of this drug obviously exist; in fact these are numerous and in some cases potentially serious. For the patients the most obvious problem is cutaneous flushing,[23] which may affect over 90% of patients taking the standard formulation of nicotinic acid. About half of these also experience pruritus and rashes. Pre-administration of aspirin can minimize the flushing, as can extended-release formulations of nicotinic acid, especially when taken at night. Gastrointestinal symptoms (nausea, vomiting, dyspepsia, diarrhoea) also affect a significant minority (about 10%) of patients; this too is less troublesome with modified-release preparations.

However, more serious and dangerous adverse effects are also relatively common:[24]

- Liver toxicity
- Impaired glucose tolerance
- Myopathy

Table 6.3 Effects of lipid-lowering drugs

Therapy	TC	LDL cholesterol	HDL cholesterol	TG
Bile-acid sequestrants	↓ 20%	↓ 15–30%	↑ 3–5%	Neutral or ↑
Nicotinic acid	↓ 25%	↓ 25%	↑ 15–30%	↓ 20–50%
Fibrates gemfibrozil	↓ 15%	↓ 5–15%	↑ 20%	↓ 20–50%
Statins*	↓ 15–30%	↓ 24–50%	↑ 6–12%	↓ 10–29%

TC, total cholesterol; TG, triglycerides.
*Statins all given at a dose of 40 mg/day.

- Hyperuricaemia
- Aggravation of peptic ulcer disease.

Hepatotoxicity is potentially the most serious toxic effect of nicotinic acid administration. It is dose-dependent and particularly important at high doses (about 3 g daily) of the immediate-release, unmodified

formulation. In some patients this may take the form of minor asymptomatic rises in liver transaminases but cases of fulminant hepatic failure have also been cause less flushing as well as less severe hepatic reactions, although described. Early modified-release forms of this drug showed greatly increased hepatoxicity.[25,26] A newer formulation (Niaspan®) is said to levels of transaminases still rise in most patients.[27] Impaired glucose tolerance and hyperglycaemia are aggravated in patients with diabetes by standard immediate-release nicotinic acid. For this reason this drug is contraindicated in these patients: even a recent study using very low doses of nicotinic acid excluded patients with diabetes.[28] However, there is now evidence that the newer formulations of nicotinic acid may be safe in these patients.[29] It is not clear whether frank diabetes can be precipitated in susceptible patients by the introduction of nicotinic acid but it is at least a possibility. This of course would be an unfortunate paradox, since it is precisely the type of dyslipidaemia seen in insulin-resistant (but not necessarily diabetic) patients, with low HDL cholesterol and high triglycerides, which might respond best to nicotinic acid.

Myopathy has also been associated with nicotinic acid, usually when given in combination with a statin. A fixed-dose combined formulation of lovastatin and nicotinic acid is, however, currently under development and so far appears to be safe.[30] Myopathy with nicotinic acid monotherapy has been described but appears to be very rare.[31] Hyperuricaemia and associated acute gout are a complication of nicotinic acid therapy but there are no reliable estimates of the extent of this problem. The same is true of peptic ulcer disease, which is said to be induced by nicotinic acid, but there have been no recent reports of this effect.

Anion-binding resins

Cholestyramine and colestipol were the first drugs shown to have significant effects on serum cholesterol levels and for some time were the only drugs in this category. Since their systemic absorption is minimal, most adverse effects have been gastrointestinal (including abdominal distention and diarrhoea) and unpleasant rather than dangerous. If the patient is willing to tolerate these problems—and many are not—the main adverse effects relate to possible malabsorption of fat-soluble vitamins (A, D and K), with the particular risk of a haemorrhagic tendency. There are no recent reports of any major episodes of bleeding attributable to this, one reason for which may be that it is now usual to provide patients with supplements of the affected vitamins to prevent deficiencies. The two resins may also reduce the absorption of some other drugs, but this is rarely of significance. Thyroid hormones are, however, an important exception.

Ezetimibe

This drug was in late development at the time of writing and will soon be marketed in many parts of the world. It is a potent synthetic inhibitor of cholesterol absorption, and monotherapy with ezetimibe can reduce levels of circulating LDL cholesterol by 15–20%.[32] However, its main use will be in combination with statins, where it can significantly reduce the statin dose required, and a fixed-dose combined formulation of ezetimibe and simvastatin is likely to be the main form in which the drug is marketed. Although like the anion-binding resins it has very modest systemic absorption, it does not share the gastrointestinal adverse effects of these drugs and at present appears to be safe and well tolerated, although a few small transient rises in liver transaminases have been reported.

Conclusion

We now have a far wider array of lipid-lowering drugs than would have been envisaged 20 years ago. This does not mean that we can treat all our patients as effectively as we would like, and some will always require non-pharmacological interventions, notably those with homozygous familial hypercholesterolaemia. It is also true that the safety record of these drugs has been generally good. On the whole one can rank them as follows in terms of benefit-to-risk ratios:

Statins>fibrates>nicotinic acid

Much more evidence is available for the statins in terms of clinical outcomes than for any of the others—almost more than for any other class of drugs used in any chronic disease. At the same time, nicotinic acid could be as efficacious. However, we do not have the evidence to support this, while at the same time we do know of its multiple adverse effects. The position of fibrates is intermediate, while the resins are safe but poorly tolerated and disliked by patients.

As in many areas of therapeutics the value of combination therapies is increasingly evident in the treatment of dyslipidaemias. Fixed-dose combinations are likely to be introduced soon, involving statins and nicotinic acid and statins and ezetimibe. There will need to be comprehensive evidence that these do not increase the risk of toxicity. In any case, it is clear that statins will remain pivotal in this therapeutic area for the foreseeable future, with the imminent introduction of one new agent (rosuvastatin) and the possible introduction of a second. Again, following the experience with cerivastatin we need to be aware of unexpected problems. Though some lessons will certainly have been learnt, especially regarding potential interactions and the use of excessive doses, it would also be useful and interesting to understand more about the toxicity of these drugs, especially in the context of myopathy.

Like all effective drugs, and many useless ones, the existing lipid-lowering drugs are not entirely innocuous. They are nonetheless acceptably safe, and even surprisingly so. Anything that supplements, much less replaces, them will need to be not only comparable in efficacy but at least equal in terms of safety. Meanwhile, we should still be cautious. As the title of this chapter indicates, we are particularly concerned with long-term safety because these drugs will be given for years and even decades. Only now are we beginning to acquire really long-term data, as in the continuation to the Long-Term Prevention with Pravastatin in Ischaemic Disease (LIPID) Study, where nearly 8,000 patients were followed for at least eight years. Reassuringly, this confirmed the safety of statin, in this case pravastatin, therapy, with no significant adverse effects observed.[33] We should remember in this context how some of the more lurid fears of a decade ago, concerning the safety of lipid-lowering drugs, have been comprehensively refuted.[34]

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7
Familial hypercholesterolaemia: current strategies and future promise

Andrew Neil and Stephen E Humphries

Introduction

In reviewing current and future strategies for the management of familial hypercholesterolaemia (FH), this chapter critically assesses the application of conventional clinical and DNA-based diagnostic tests, and the extent to which FH fulfils the accepted criteria required by a screening programme. The use of functional foods, advances in drug therapy, drug safety and the determinants of compliance are discussed in other chapters.

Background

FH is an autosomal dominant monogenic disorder with an estimated frequency of 1 in 500 of the population. It manifests as a disorder of lipoprotein metabolism, resulting in an accumulation of low-density lipoprotein of tendon xanthoma, xanthelasma and atheroma.[2–4] Most cases are (LDL) cholesterol in the plasma from birth[1] and subsequent development caused by one of the many different mutations of the LDL-receptor.[1] The gene for the LDL receptor is encoded on chromosome 19p and consists of 18 exons, with corresponding intron, splicing and promoter regions. To date, over 700 different mutations have been reported worldwide,[5] with between 50 and 60 so far identified in patients in the UK.[6] In 3–5% of patients the hypercholesterolaemia is caused by a single mutation in the gene for apolipoprotein B (apoB), which is the ligand for the LDL receptor.[7] The apoB3500 defect is, however, phenotypically almost indistinguishable from FH. Recent evidence suggests that at least one, and possibly two, other gene loci may also cause the disorder.[8,9]

The natural history of the disorder has been well defined by longitudinal studies undertaken before effective lipid-lowering drug therapy was available. In the heterozygous condition the cumulative risk of a coronary event by the age of 60 years without effective treatment is at least 50% in men and about 30% in women,[10,11] with the onset of coronary disease being about 5–10 years later in women and a marked increase occurring post-menopausally. The relative risk of a fatal coronary event is increased nearly 100–fold in young adults aged 20–39 years, although patients who survive through middle age appear to be no longer at substantially increased relative risk.[3] Large extracranial carotid vessels are also affected by atherosclerosis in FH,[12,13] and a small increase in the prevalence of peripheral vascular disease has been noted in some studies.[14,15] The homozygous condition has a much worse prognosis and may be fatal in childhood or adolescence, but is rare, with a frequency of about one in 1,000,000 of the population.

Since FH is an autosomal dominant disorder, on average 50% of the children and first-degree relatives of a patient will also be affected. Clinical management must therefore include both treatment of the index case

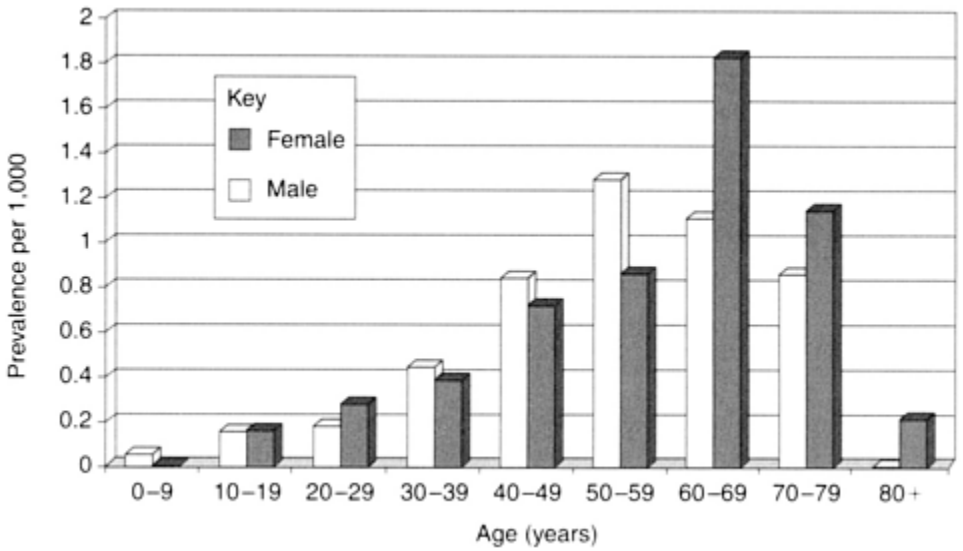

Figure 7.1

Age and sex-specific prevalence of clinically diagnosed familial hypercholesterolaemia in Oxfordshire, UK in 1999. Data from ref. 17.

and the detection and treatment of affected relatives. Although the introduction and widespread use of lipid-lowering drug therapy with HMG-CoA reductase inhibitors (statins) over the last decade has substantially improved the prognosis,[4] it has not been matched by the implementation of systematic screening programmes to detect affected individuals, except in The Netherlands.[16] A UK registry study of FH patients in Oxfordshire recently reported that only about 25% of the cases predicted on the basis of the estimated gene-carrier frequency had been diagnosed,[17] as shown in Figure 7.1; this may be an over-estimate since 52% of the patients had a diagnosis of possible rather than xanthomatous (i.e. definite) FH. Interestingly, although the overall prevalence of diagnosed FH was only 1 in 2,000 (i.e. one quarter of that predicted), the prevalence increased with age in both men and women, peaking at 1.3 per 1,000 in men and 1.8 per 1,000 in women before falling, as would be expected from the early coronary deaths. These data are the first confirmation in the UK of the prevalence of clinically diagnosed FH as being between 1 per 500 and 1 per 750. Figure 7.1 also shows that most cases were only diagnosed in early middle age, probably after the onset of symptomatic coronary disease in the patient or a sibling, emphasizing the need for more effective methods of case ascertainment, particularly to identify children and young adults.

Preventive strategies

In heterozygous FH there is a long enough latent period before the onset of coronary disease for affected individuals to be offered effective treatment. From this it can be seen that a major objective must be to identify affected individuals at an early age, ideally in childhood. However, in the absence of systematic screening programmes, most cases remain undiagnosed and untreated.

Diagnostic test

A venous or capillary blood specimen for measurement of total cholesterol provides a simple, safe, acceptable and adequately precise and accurate[18] biochemical screening test—capillary testing being particularly useful for younger children. An elevated initial test result on screening requires confirmation with a repeat sample in the fasting state for measurement of total cholesterol, high-density lipoprotein (HDL) cholesterol and triglyceride. LDL cholesterol can then be calculated using the Friedewald formula[19] although, in future, direct measurement of LDL cholesterol is likely to become widely available as a screening test.

Clinical diagnostic criteria

FH can be diagnosed clinically from childhood onwards by measurement of plasma lipids and lipoproteins, confirmation of a dominant pattern of transmission of premature coronary artery disease and/or hypercholesterolaemia within the kindred, and the presence of tendon xanthomata, which are pathognomonic, but are usually only present from the fourth decade or later.

Table 7.1 Diagnostic cut-off points for total cholesterol (mmol/l) used in the US MedPed Programme[23]

Age group (yrs)	Degree of relation to closest FH relative			General population
	First	Second	Third	
<20	5.7	5.9	6.2	7.0
20–29	6.2	6.5	6.7	7.5
30–39	7.0	7.2	7.5	8.8
40+	7.5	7.8	8.0	9.3

Diagnostic misclassification

There are, however, no entirely satisfactory diagnostic criteria. There is an overlap between the frequency distribution for LDL cholesterol in the general population and that for patients with FH,[20,21] which leads to false positive and false negative diagnostic rates of 8–18%. The diagnostic cut-off points can be refined by taking account of age, and should be specific to particular populations. Using this approach, the maximum likelihood diagnosis has been calculated based on LDL cholesterol levels in DNA-verified Finnish FH cases.[22] The US MedPed Programme[23] uses criteria that take account of the prior probability of having FH, which will vary for first-, second- and third-degree relatives and the general population. Different cut-off points are then provided for each of these categories for four age groups (Table 7.1). A simpler approach was adopted by the Simon Broome Register Criteria in the UK, which stipulated different total LDL levels, for adults and children (Table 7.2).[3] These criteria also take account of evidence of dominant transmission and the age of onset of coronary disease in the kindred. In 1994 the criteria were expanded to include a DNA-based diagnosis. Using this approach, cases are categorized as ‘definite’ or ‘possible’.

In regions where there is a high prevalence of hypercholesterolaemia and early coronary heart disease (CHD), either because of dietary and lifestyle factors or because of genetic factors, the specificity of the Simon Broome criteria for premature CHD (criterion d) and a family history of hypercholesterolaemia (criterion e) will be poor, leading to ‘over-diagnosis’ of possible FH. By contrast, in regions with a low prevalence of hypercholesterolaemia the criteria will be much more specific, a variance which highlights the

difficulty of developing and applying uniform diagnostic standards. Patients with possible FH are more numerous in most UK clinics than those with xanthomatous (i.e. definite) FH. Since the criteria for possible FH are less specific than for definite FH, possible FH will inevitably include some patients with polygenic hypercholesterolaemia. It

Table 7.2 Simon Broome Familial Hyperlipidaemia Register diagnostic criteria for familial hypercholesterolaemia[3]

A definite diagnosis requires:
(a) Total cholesterol concentration >7.5 mmol/l in adults or a total cholesterol concentration >6.7 mmol/l in children under 16 years *or* an LDL cholesterol concentration >4.9 mmol/l in adults or >4.0 mmol/l in children
PLUS
(b) Tendon xanthoma in the patient or in a first-degree relative
or
(c) DNA-based evidence of an LDL-receptor mutation or of familial defective apoB-100
A possible diagnosis of familial hypercholesterolaemia requires (a) above plus one of the following:
(d) Family history of myocardial infarction from age 50 years in a second-degree relative or before age 60 years in a first-degree relative
(e) Family history of raised total cholesterol concentration >7.5 mmol/l in a first-or second-degree relative

is, however, important to try to distinguish between acquired polygenic hypercholesterolaemia and FH. Patients with FH have sustained elevation of LDL cholesterol from birth and are at a higher risk of coronary disease for any given LDL concentration. Consequently, they warrant more aggressive cholesterol-lowering therapy than suggested by published risk charts based on their age, sex, LDL levels, and other coronary risk factors.

The use of DNA-based mutation testing

A definite diagnosis of FH may be made using DNA-based mutation screening methods. Receptor mutations, however, cannot be identified in all patients with xanthomatous (i.e. definite) FH, with detection rates in case series ranging from 30–80%.[24–26] It is not clear whether differences in detection rates relate to the genetic heterogeneity of the particular case series, the accuracy of the clinical examination for xanthomata or differences in DNA methodology. Using the Simon Broome clinical criteria, one group reported identifying LDL-receptor mutations in 32% of patients with definite and 14% of patients with possible FH,[24] although the frequency was higher than 50% in paediatric cases.[25] Since these varying detection rates were all obtained using the same technique (Single Strand Conformation Polymorphism [SSCP], analysis), the importance of the diagnostic criteria is evident, with fewer confounding causes of extreme hyperlipidaemia being present in childhood.

A DNA-based diagnosis is therefore highly specific but currently has limited sensitivity. However, once the causative mutation in the patient has been found, molecular testing in relatives is possible, allowing unequivocal diagnosis and eliminating false-negative diagnoses. A study comparing diagnostic testing using DNA with cholesterol testing in an extended Irish family showed that 15–20% of family members would have been incorrectly diagnosed based on cholesterol testing alone,[27] while in a Finnish study 10–20% of relatives would have been misdiagnosed.[28] At the present time in the UK, mutation screening for FH is restricted to two-to-three research laboratories, but a pilot project to assess feasibility and uptake has been

running in the DNA diagnostic laboratory at University College Hospital, London, since 1998,[25] and a similar service has been developed in Northern Ireland.[26]

Currently the cost for a complete screen of the LDL receptor gene and a test for the apoB mutation is about £500, with the cost of a single mutation test in a relative of a patient with a known mutation being about £180. Although this is substantially higher than the cost of a full plasma lipid profile at nearly £12,[29] a DNA diagnosis provides an unequivocal result and need never be repeated. Over the next few years its cost is likely to fall significantly as new, automated, higher throughput methods are developed.

Future advances in mutation testing

Over the next two or three years a number of changes in our ability to identify mutations in patients with FH can be expected, and technical advances will increase both the speed and sensitivity of mutation testing. SSCP is believed to be 85–90% sensitive, and the use of either high-pressure liquid chromatography (HPLC) for heteroduplex analysis[30,31] or chip technology[32] will allow rapid screening of all base pairs within the LDL receptor gene. This may be particularly useful as current techniques focus only on the immediate promoter region plus coding exons and only a few bases in the introns, and recently it has been reported that many additional novel sequence changes can be detected in the LDL introns in FH patients.[33] This raises a different problem as the functionality of such intron changes will need to be tested (i.e. their effect on the correct splicing of the LDL-receptor mRNA), and this is beyond the scope of any routine DNA diagnostic laboratory at the present time. It does, however, raise the interesting possibility that a much higher proportion of patients will have a detectable mutation.

The second advance will be in the identification of other genes (apart from those coding for the LDL receptor and ApoB) which cause FH. Two recent reports have demonstrated that one gene is located on chromosome 1[8,9] and that another gene may be located on chromosome 10.[8] Now that the sequence of the entire human genome is available, it is likely that these genes, and possibly others, will be identified soon. It is unclear to what extent mutations in these genes contribute to FH in the UK but they may represent the major part of a genetic cause of FH in patients where no mutations in the LDL receptor or apoB can currently be detected. The application of current or newly developed mutation screening techniques to these genes might therefore increase the mutation detection rate to 90% or higher. Both of these advances will have beneficial implications for the sensitivity and specificity of mutation detection and, therefore, for the cost-benefit analysis of the genetic component of FH screening.

Criteria for screening

FH meets most of the World Health Organization[34] and the UK National Screening Committee criteria for screening.[35] The condition is, as discussed above, an important health problem. The epidemiology and the natural history are well understood and there is a long enough latent period to allow effective preventive therapy. There is a simple, safe, precise and validated screening test, the distribution of the test values is known and suitable cut-off levels have been defined and agreed. Cholesterol testing is widely undertaken and both venepuncture and capillary blood testing are procedures acceptable to the population. Genetic testing requires either a blood or mouthwash sample. Mouthwash samples can provide adequate amounts of DNA and are stable for several days, which enables them to be sent through the post. Whether or not patients respond differently to a clinical and a DNA diagnosis is being investigated in a current UK trial.

There should be an agreed policy on further diagnostic investigations of individuals with a positive test result before screening programmes are introduced. The Joint British Recommendations on Prevention of

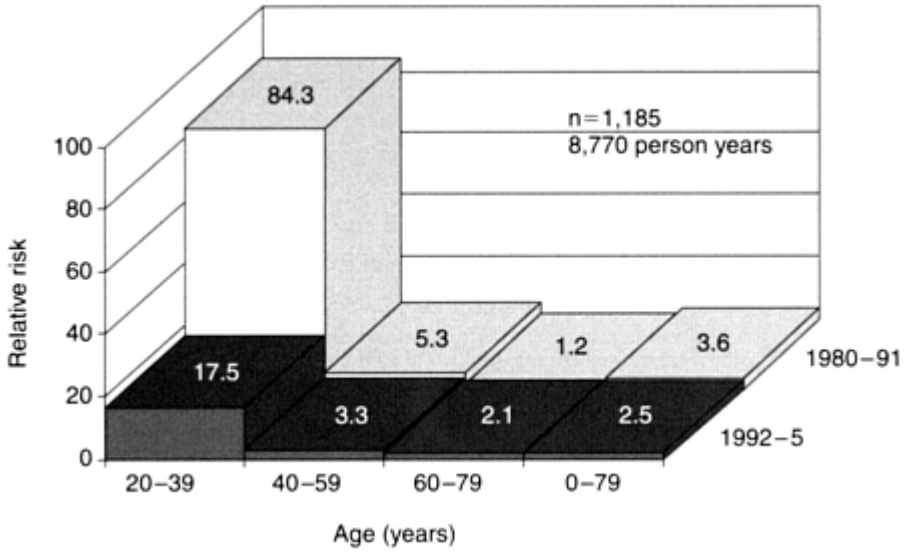

Figure 7.2

Combined analysis for men and women of coronary heart disease mortality for person years accumulated before and after January 1992. Data from ref. 3.

Coronary Disease in Clinical Practice[36] recommend that, in general, the diagnosis and management of FH is best co-ordinated by a specialist. The recommendations stipulate diagnostic cut-off points and specify further investigations. Regardless of age, a positive screening test must be confirmed by measurement of a fasting lipid profile and secondary causes of hypercholesterolaemia must be excluded. Treatment options are, in order of efficacy, HMG-CoA reductase inhibitors (statins), bile-acid sequestrants (resins) and diet. Separate guidelines for children have been published jointly by the British Hyperlipidaemia Association and the British Paediatric Association,[37] which recommend the adoption of family tracing (cascade screening). The guidelines suggest that children should be tested by the age of ten years, and that the age for testing should take account of parental wishes, the age of onset of coronary disease in the index case and other affected family members, and the treatment options available.

Treatment

Screening presupposes that there is an effective treatment or intervention for patients identified through early detection. There are few data about the effect of lipid-lowering drug therapy on the risks of coronary disease in heterozygous patients[38–40] and it would no longer be ethical to conduct placebo-controlled clinical endpoint trials to obtain more information. Clinical management is therefore based largely on the evidence from a number of observational studies and an extrapolation from the results of clinical trials of lipid-lowering drug therapy conducted in patients with polygenic hypercholesterolaemia. Since the introduction of statins in the early 1990s, drug treatment of hypercholesterolaemia has changed dramatically. These drugs have been shown to reduce coronary event rates by about a third in clinical trials of both primary and secondary prevention of coronary heart disease.[41–46] As illustrated in Figure 7.2, the relative risk of coronary mortality in a large cohort of heterozygous FH patients has also been shown to have

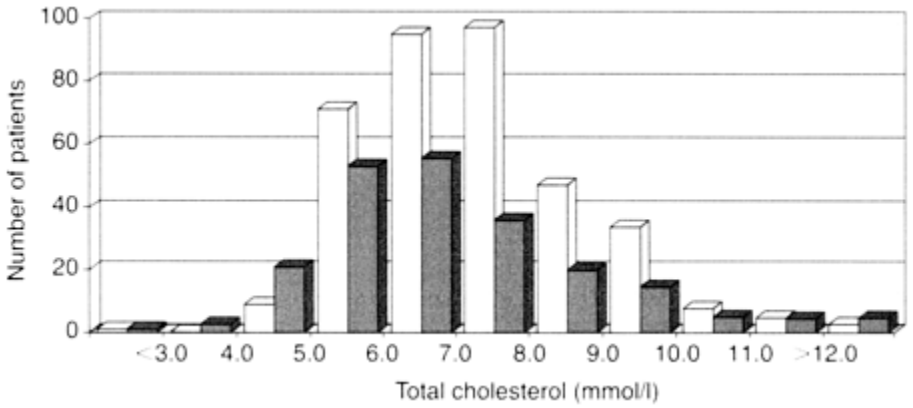

Figure 7.3

Treated total cholesterol concentrations (mmol/l) in 217 heterozygous FH patients with CHD (solid bars) and 370 without CHD (open bars).

declined for those aged 20–59 from an eight-fold higher risk before 1992 to 3.7–fold higher risk thereafter, compared with the general population of England and Wales.[3] This improved prognosis corresponds with the widespread use of statins, better access to coronary artery bypass grafting (CABG) and percutaneous transluminal angioplasty (PCTA), and more extensive use of cardioprotective medication. Drug therapy in FH has been shown to arrest progression and promote regression of coronary atherosclerosis.[38–40] Rigorous treatment with statins has also been shown to result in regression in carotid intimal thickness, leading to the suggestion that LDL cholesterol should be reduced by at least 45% in routine clinical practice.[47]

Statins are the firstline of treatment for FH; side-effects are uncommon, and serious adverse reactions are rare.[46] Patients are also advised to follow a diet equivalent to a National Cholesterol Education Programme Adult Treatment Panel III (NCEP ATP III) Therapeutic Lifestyle Changes (TLC) diet,[48] although the additive cholesterol-lowering effect is only about 5%.[49] A maximum reduction in LDL cholesterol of 55% or more can be achieved with atorvastatin 80 mg[50] and a smaller reduction of about 47% with simvastatin 80 mg,[51] while rosuvastatin 80 mg reduces LDL cholesterol by up to 65% but is, as yet, unlicensed.[52] Other statins at currently licensed dosages achieve smaller reductions in LDL cholesterol levels. Statins result in a modest elevation of HDL cholesterol levels of 6–10%, and a reduction in triglyceride levels of 10–15%, although larger reductions of 30% or more may be achieved in patients with hypertriglyceridaemia.[53,54] Although resins can achieve a reduction in LDL cholesterol of about 20–30% with good drug adherence,[55] reductions of about 10% are more usual, probably because these drugs are inconvenient to take and somewhat unpalatable.

In practice, cholesterol-lowering dietary advice and monotherapy with high-dose statins often fail to reduce LDL cholesterol concentrations adequately. Unpublished data from the Simon Broome Register Group showed that of 587 treated heterozygous patients attending six large UK lipid clinics between 1997 and 1999, total cholesterol concentrations exceeded 7.0 and 8.0 mmol/l in 47% and 25% of patients, respectively (Figure 7.3). Combination therapy is therefore needed to achieve target LDL-cholesterol levels. However, combination therapy with statins and nicotinic acid, fibric acid derivatives or bile-acid sequestrants is limited by the increased potential for side-effects, intolerance and drug interactions. Co-administration of a statin to inhibit endogenous cholesterol synthesis and a plant stanol- or sterol-enriched spread to reduce exogenous cholesterol absorption can lower LDL cholesterol by an additional 10–15%,[56,57]

equivalent to two dose titrations with a statin, and is well tolerated. Ezetimibe, the first of a new class of selective cholesterol absorption inhibitors, at a 10 mg dose in combination with simvastatin 10 mg results in a 17% additive reduction in LDL cholesterol compared with statin monotherapy.[58] Co-administration of ezetimibe or a plant sterol-enriched spread with a statin may therefore fill an unmet need in the management of FH. LDL apheresis is usually reserved for patients not responding to conventional drug treatment or with homozygous FH.[39]

Gene therapy

Although the majority of patients with heterozygous FH respond to statin therapy with adequate lipid-lowering, in general, patients with homozygous FH do not. This is because they tend to have a complete (or almost complete) deficiency of functioning LDL receptors, which therefore cannot be 'upregulated' by statin therapy. It has been proposed that homozygous patients would be good candidates for gene therapy. To date, the results of gene therapy have been reported in a total of six patients.[59,60] All were treated by the same group in the USA, who used an *ex-vivo* procedure, in which the liver is partly removed and hepatocytes transfected with a vector carrying the LDL receptor gene. Cells were then re-injected into the patient where they are then involved in the recovery growth of the liver, such that a proportion of the new liver cells then express the LDL receptor. Although this was well tolerated, the treatment achieved only modest lipid-lowering (in some of the patients) which declined somewhat with time.[61] One of the current problems is that expression of a normal human LDL receptor in the liver cells of a homozygote that has no LDL receptors will cause an immune response that will result in the designation of these cells as 'foreign' and, thus, their rapid destruction without immune suppression. At the present time, therefore, it appears that gene therapy is not a viable option for homozygous FH, and to be successful will require major advances in vector technology.

Screening programmes

Before implementing a screening programme, there should, if possible, be evidence from high-quality randomized controlled trials that the pro gramme is effective in reducing mortality or morbidity. No such evidence is available for FH; only very long-term trials could provide this, and their feasibility and ethical acceptability is questionable.

There should also be evidence that the complete screening programme, including the screening test, diagnostic procedures and treatment, is clinically, socially and ethically acceptable to health professionals and the public. The professional acceptability of such a programme can be inferred from routine clinical practice, where family tracing is already undertaken, albeit in a fragmented and unsystematic fashion, and from a systematic study in Manchester where a nurse-led case finding was undertaken over a ten-year period.[62] In The Netherlands, cascade screening has been effectively implemented on the basis of DNA testing.[17] There are, however, some unresolved issues regarding the relative merits of different methods of approaching the relatives of index patients.

Research ethics committees in the UK hold conflicting views on whether relatives can be approached directly by a researcher or only through the index case. In clinical practice in Australia, the USA, and The Netherlands, but not in Scandinavia, family members are approached directly by the physician. However, these differences are not based on evidence of the acceptability to the public of either procedure. Without further research, it is impossible to determine whether a direct approach from a physician or nurse would provide a higher yield or which approach relatives of the index case prefer. So far, this question appears to

have been addressed by only one study, conducted among the general public in Norway. This found that of those respondents who were 'interested in knowing whether they had inherited a high cholesterol', 74% wanted to be approached directly by the physician.[63] A further uncertainty is that the names of relatives to be contacted for testing are likely to be stored on computer file, and the acceptability of storing names provided by a third-party remains uncertain. At present there is insufficient evidence to draw firm conclusions about these and other aspects of a complete screening programme.

The benefits from the screening programme should outweigh the physical and psychological harm caused by the test, diagnostic procedures and treatment. Although adverse psychological effects have been reported in FH, most studies conclude that these effects are transitory and relatively minor.[64,65] Identification of a psychologically vulnerable group might allow educational counselling—to ameliorate these deleterious effects—to be targeted at such patients, but the utility of the strategy has not been evaluated. Educating the public and the insurance sector[66,67] may be necessary to avoid unnecessary stigmatization and discrimination of those testing positive, but, again, the evidence for the existence of stigmatization and discrimination is weak. Evidence-based information explaining the consequences of testing, investigation and treatment should be made available to potential participants to assist them in making an informed choice. This is accepted as good clinical practice, and it may help individuals understand the potential risks and benefits. However, the precise contents of counselling and education sessions need evaluating. Patient self-help groups can also provide information leaflets and other advice about the potential benefits and disadvantages of testing.

The opportunity cost of the screening programme needs to be economically balanced in relation to expenditure on medical care as a whole. A recent Health Technology Assessment Review undertook economic modelling to determine the benefits and costs of different screening strategies to identify and treat patients with FH.[29] It examined:

- Universal screening
- Opportunistic screening in primary care
- Screening of premature myocardial infarction admissions
- Tracing family members of affected patients.

It concluded that tracing family members to identify the affected relatives of known FH patients would be a cost-effective strategy (£3,097 per life year gained) and that only 2.6 individuals need to be screened to identify one case, at a cost of £133 per case detected. If the genetic mutation was known within the family then the cost per life year gained was only slightly increased by genetic confirmation of the diagnosis (£4,914). For each strategy it was more cost-effective to screen younger people and women. Targeted strategies were more expensive per person screened, but the cost per case detected was lower. Population screening of 16-year-olds was as cost-effective as family tracing (£2,777) assuming that such a programme was clinically, socially and ethically acceptable to health professionals and the public, and there was at least a 55% uptake among the 16-year-olds invited for screening.

Adequate staffing and facilities for testing, diagnosis, treatment and programme management need to be provided for screening. Family testing (cascade screening) of relatives of newly diagnosed index patients can be undertaken with modest resource implications by a nurse based in a hospital clinic. The feasibility of this approach, which is based on the research model developed by the US MedPed programme,[24] has already been demonstrated in the UK.[62] Additional resources are required in the short-term if cascade screening is extended to cover the relatives of previously diagnosed patients with FH (the prevalent backlog). The cost of the drug component of the programme is likely to fall as statins begin to come off

patent and generic prescribing becomes available. Plans would also have to be developed for managing and monitoring a cascade-screening programme and an agreed set of quality assurance standards would be required. Unlike some other screening programmes, such as cervical or breast cancer, there would not be the public pressure for widening the eligibility criteria or reducing the screening interval, particularly if the diagnosis was based on definitive demonstration of an LDL-receptor mutation.

Finally, before screening programmes are implemented there need to be agreed, evidence-based policies covering which individuals should be offered treatment and the appropriate treatment to be offered. There is a broad consensus that boys should be treated with statins from their late teens and women from their mid-to-late twenties or early thirties onwards, with this approach being modified if the family history is particularly adverse.[68] Bile-acid sequestrants may be prescribed for younger patients since they are not absorbed systemically. There is an increasing tendency for paediatricians to begin treatment with a statin, although there are no long-term safety or outcome data available in children, and a clinical trial is needed to assess this approach.[68] Affected individuals and families should have access to a specialist dietician,[36] and older children and adult patients are advised to follow the equivalent of an NCEP ATP III TLC diet,[48] and should receive appropriate lifestyle advice. It is crucial that children should be discouraged from ever starting to smoke. Unpublished evidence from Simon Broome Register showed that nearly two-thirds of patients with coronary artery disease had a history of smoking, while two-thirds of those without clinical disease were life-long non-smokers.

Conclusion

The prognosis for FH has improved since the introduction and widespread use of HMG-CoA reductase inhibitors, and newer drugs will allow LDL cholesterol to be reduced more effectively. However, the majority of individuals with FH remain undiagnosed and untreated and, thus, at high risk of early CHD. The challenge for the next five years is to identify these patients by general or targeted screening strategies. Technical advances over the next few years will increase the speed and sensitivity of DNA-based LDL-receptor mutation testing and substantially reduce its cost. Other genes causing FH, apart from LDL receptor and apoB, will be identified. The routine application of DNA-based testing in clinical practice will provide a definitive diagnosis and overcome the diagnostic misclassification, which reduces the cost-effectiveness of family tracing. FH fulfils the principal criteria required of a screening programme, and cascade screening of relatives of people with FH has been demonstrated to be the most effective way of detecting cases across the whole population and to be feasible using either biochemical or DNA-based testing.

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8
Functional foods in lipid-lowering and coronary prevention

Audrey Brynes and Gilbert R Thompson

Introduction

For the past couple of decades nutritionists have concentrated on developing guidelines to reduce or prevent the incidence of coronary heart disease (CHD), such as low-fat, low-salt, and high-fibre diets in addition to ways to reduce excess body weight. More recently, functional foods have gained popularity as a means of retarding the onset and progression of cardiovascular disease. The emphasis has ceased to be solely on measures to reduce plasma lipids—today's research also investigates ways in which diet may affect risk factors such as lipoprotein oxidation, platelet aggregation, thrombotic mechanisms, plaque formation and cardiac arrhythmias. Other areas of enquiry have included epidemiological studies of various diets from around the world, such as the Mediterranean diet, as well as of specific vitamins, minerals and essential fatty acids.

This chapter outlines some of the functional foods that are available, and reviews the evidence on their effectiveness in the prevention of CHD. The term functional food is used to describe foods that, by virtue of physiologically active components, provide health benefits beyond basic nutrition. The Functional Food Science in Europe (FUFOSE) group propose a working definition of functional foods as 'foods that can be satisfactorily demonstrated to affect beneficially one or more target functions in the body, beyond adequate nutritional effects, in a way relevant to an improved state of health and well being and/or reduced risk of disease'.[1] Two categories of claims are also proposed: foods that enhance function (type A) and those which reduce risk of disease (type B). This chapter focuses on type B claims, in particular those relating to reduction of cardiovascular risk.

It is difficult to decide where the limits of functional foods lie. For the purpose of this chapter we have restricted our discussion to plant stanols and sterols, fish and omega-3 (ω-3) fatty acids, garlic, soya and phytoestrogens, soluble fibre such as oat gum, psyllium and guar gum, and folic acid. We have excluded vitamins (apart from folic acid) and anything that requires a prescription or which needs to be given in pharmacological amounts to achieve an effect. Some companies market traditional products with natural levels of functional components, such as Quaker Oats (soluble fibre) or John West Mackerel Fillets (ω-3 fatty acids), while others manufacture products with added levels of active ingredients such as Benecol (plant stanol ester) or Flora Pro-activ (plant sterol ester). In the UK, foods becoming available include ω-3 eggs (the diet of hens is enriched with powdered sea algae to produce eggs high in docosahexaenoic acid), high-fibre juices such as Ribena Juice & Fibre and breads with added folic acid. The market for functional foods is now well established and looks set to expand. This applies especially to products with lipid-regulating properties such as Benecol, which was first introduced into Britain in 1999.

Cholesterol-lowering potential of plant sterols and stanols

Plant sterols are the botanical analogues of cholesterol and differ from it structurally only in their side chain. Saturated sterols, which lack a Δ-5 double bond in the B ring, are called stanols. Thus, saturation of cholesterol gives rise to cholestanol and saturation of sitosterol, the most commonly occurring plant sterol, gives rise to sitostanol. Plant sterols are found in vegetable oils, nuts and seeds and their dietary intake varies from 150–350 mg daily, whereas the dietary intake of plant stanols is usually only about 50 mg daily. The absorption of these compounds differs also, plant sterols being absorbed one tenth as well as cholesterol and plant stanols only one tenth as well as plant sterols.[2]

The use of foods containing plant sterols or stanols, known collectively as phytosterols, as a means of reducing plasma cholesterol dates back almost 50 years, to when Pollak[3] administered crude sitosterol to humans. Concomitant studies in rabbits showed that sitosterol, when given in excess, blocked the absorption of cholesterol and thereby prevented atherosclerosis. One of the first descriptions of the therapeutic use of phytosterols was by Lees and Lees,[4] who used a commercial preparation derived from soybean oil, which consisted of a mixture of sitosterol and campesterol. Doses of 15g daily lowered plasma low-density lipoprotein (LDL) cholesterol by 14% but markedly increased plant sterol levels in plasma, especially that of campesterol, which is better absorbed than sitosterol. This raised concerns over safety in the light of the knowledge that the recessively inherited disorder sitosterolaemia, characterized by excessive absorption and high plasma levels of plant sterols, causes premature atherosclerosis.

The use of plant stanols to lower plasma cholesterol, first demonstrated by Heinemann *et al*[5] seemed free from this drawback in that administra tion of capsules of sitostanol 1.5g daily lowered LDL cholesterol by 15% without any rise in plasma sitostanol. However, a similar but larger study by Denke,[6] who administered sitostanol 3 g daily, showed no lowering of LDL cholesterol. It was subsequently pointed out that the capsules used contained only one twentieth of the amount of sunflower oil needed to solubilize the sitostanol and that this would have impaired its dissolution in the contents of the small intestine.

The negative outcome of Denke's study drew attention to the importance of the physicochemical state of plant stanols in determining their efficacy. The solubility of free plant stanols in margarine is <1%, but esterification with long chain fatty acids increases their lipid solubility and enables them to be incorporated into a variety of foods. This process was patented by a Finnish company, Raisio, in 1989 and subsequently led to the marketing of Benecol margarine.

Mechanism of action of phytosterols

During fat absorption the small intestinal contents consist of an oil phase and an aqueous phase, the latter comprising aggregates of monoglycerides, ionized fatty acids and bile salts—termed mixed micelles. Non-polar sterols such as cholesterol and fat-soluble vitamins must be incorporated into mixed micelles as a prerequisite to their absorption. Recent studies suggest that the uptake of cholesterol from micelles into the intestinal mucosal cell is mediated by a saturable transporter. It has further been shown that cholesterol absorption is regulated not only by controlling influx but also efflux; the latter process is determined by adenosine triphosphate (ATP)-binding cassette transporter proteins (ABCs), upregulation of which increases cholesterol efflux. Defects of this pathway result in increased absorption of cholesterol and plant sterols, as occurs in sitosterolaemia.

Phytosterols become incorporated into mixed micelles to the same extent as cholesterol, and competitively decrease micellar uptake of the latter.[7] *In vitro* there was no difference between sitosterol and sitostanol in this respect, a fact borne out *in vivo* by a study in which addition of 1.5 g of sterol or stanol esters to a high-cholesterol diet reduced cholesterol absorption to a similar extent (by about one third) in

each case.[8] It remains to be shown whether micellar competition is the sole mechanism or whether phytosterols exert an additional inhibitory effect on the mucosal phase of cholesterol absorption.

Inhibition of cholesterol absorption by plant sterols or stanols results in compensatory increases in cholesterol synthesis of similar magnitude[8] and an accompanying upregulation of LDL receptor expression.[9] The only kinetic studies performed suggest that the ensuing reduction in LDL cholesterol reflects decreased formation rather than increased catabolism of LDL. Complete blockade of absorption resulted in a 37% decrease in LDL cholesterol despite doubling cholesterol synthesis.[10] This underlines the importance of the absorptive pathway, via which both dietary and endogenously synthesized biliary cholesterol return to the liver, as a determinant of plasma cholesterol.

Efficacy of phytosterols in lowering LDL cholesterol

A meta-analysis of 14 randomized trials in which plant sterols and stanols or their esters were used to lower serum cholesterol was published in 2000 by Law.[11] He found a dose-response relationship up to about 2 g per day of sterol or stanol, but no further reduction in LDL cholesterol above this dose. At this level of intake, reductions in LDL cholesterol were in the region of 9–14% and did not differ significantly between plant sterols and stanols. Law suggested that long-term decreases in LDL cholesterol of this magnitude at the population level would be expected to reduce the risk of CHD by 25%.

Subsequent to Law's meta-analysis, a further 14 randomized trials have been published, details of which are shown in Table 8.1. These included two trials in children and two in hypercholesterolaemic adults receiving statin therapy. Six trials involved stanols, four used sterols and four compared both. In most instances the phytosterols were dissolved in margarine but the most recent trials used other food vehicles, including yoghurt, bread and cereals, with apparently similar efficacy to margarine.

Examination of the data in Table 8.1 and those from earlier studies[11] suggests that the minimum effective dose of phytosterols is approximately 1 g per day, usually given in divided doses. However, one study has shown that a single daily dose of stanol ester was as effective as three divided doses.[12] Most of the trials were of one-to-two months' duration, apart from the North Karelia study, which showed that the LDL-lowering effect of stanol ester 2.6 g daily was more marked at one year than at six months.[13] It remains to be shown whether the same applies to sterol esters.

The lipid-lowering effects of phytosterols and the background diet are additive, as too are their effects when given together with statins. The age, gender and lipid status of the consumer has no obvious influence on outcome but there are suggestions that subjects with an apolipoprotein (apo)E4 allele, who are known to hyperabsorb cholesterol, may show an enhanced response. Such individuals have low rates of cholesterol synthesis and tend to respond poorly to statins;[14] consumption of phytosterols, which upregulate cholesterol synthesis, may improve their response to these drugs.

Comparative studies of plant sterol and stanol esters

It is appropriate to conclude this section with the outcome of studies in

Table 8.1 Randomized trials of phytosterols, 1999–2002

Date	1st Author	Reference	Design	Subjects (n/y)	Methods	Results
1999	Nguyen	*Mayo Clin Proc;* 74; 1198–206	R, DB, PC, I I	318/*53*	US marge ±stan est 2 or 3 g, 8 wk	LDL-C −4, –10, −5% on US

Date	1st Author	Reference	Design	Subjects (n/y)	Methods	Results
					Euro marge ±stan est 3 g, 8 wk	2, 3 g and Euro 3 g v P
1999	Andersson	*Eur Heart J Suppl;* 1 (Suppl S): S80–90	R, DB, PC, I I	61/*55*	Usual diet ±stan est 1.9 g, 8 wk Low fat diet ±stan est 1.9 g, 8 wk	LDL-C −12% on UD+stan est; LDL-C −8% and −15% on low fat± stan est, Δ –7%
1999	Ayesh	*Food Chem Toxicol;* 37: 1127–38	R, DB, PC, I I	24/*36*	Marge±sterol est 5 g, 3–4 wk	LDL-C −22% v P
1999	Williams	*J Am Coll Nutr;* 18: 572–81	R, XO	19/*2–5*	Marge+stan est 3 g, 4 wk or Fibre 5–10 g, 4 wk	LDL-C −16% on stan est v b'line
2000	Tammi	*J Pediat;* 136: 503–10	R, PC, DB, XO	72/*6* (low fat diet)	Marge±stan est 1.5 g, 3 mths each	LDL-C −8% v P
2000	Blair	*Am J Cardiol,* 86:46–52	R, PC, DB	167/*56* (on statins)	Statin and marge±stan est 3 g, 8 wk	LDL-C −10% v P marge
2000	Hallikainen	*J Nutr;* 130: 767–76	R, SB	22/*51*	Stan est 0, 0.8, 1.6, 2.3, 3.2 g, 4 wk	LDL-C v 0 were −2, −6, –10, −10% 2.3 & 3.2 g NS better than 1.6 g
2000	Jones	*J Lipid Res;* 41:697–705	R, DB, PC, XO	15 *37–67*	Marge±sterol est v stan est 1. 8 g, 3 wk each	LDL-C −13% sterol est, −6% stan est v P
2000	Hallikainen	*Eur J Clin Nutr;* 54: 715–25	R, DB, PC, XO	34/*30–65*	Low fat diet +marge±sterol est v stan est 2 g, 4 wk each	LDL-C −10% sterol est −13% stan est v P
2001	Neil	*Atheroscler;* 156:329–37	R, DB, PC, XO	32 on diet, 30 FH on statin/ *53*	Marge±sterol est 2.5 g, 8 wk	LDL-C −15% 4 wk, −10% 8 wk v P
2001	Davidson	*J Am Coll Nutr;* 20: 307–19	R, DB, PC, I I	77/*18–65*	Marge±sterol est 3, 6 or 9 g, 8 wk	LDL-C −4%, −2%, −8% v P. Diffs NS
2001	Tikkanen	*Am J Cardiol;* 88: 1157–162	R, DB, PC, I I	71/*57*	Various foods ±sterol 0.9, 1. 9, 4.2 g, 5 wk each	LDL-C −5%, −5%, -81 v P
2001	Nestel	*Eur J Clin Nutr;* 55: 1084–90	R, SB, PC, XO	22/*60*	Various foods ±sterol est v	LDL-C −14%, −8% v P

Date	1st Author	Reference	Design	Subjects (n/y)	Methods	Results
					stan, 2.4 g, 4 wk each	
2002	Mensink	*Atheroscler;* 160:205–13	R, DB, PC, I I	60/*36*	Yoghurt±stan est 3 g, 4 wk	LDL-C −14% v P

Abbreviations: R, randomized; DB, double blind; SB, single blind; PC, placebo controlled, XO, crossover; I I, parallel group; n, number; *y,* age (mean or range); P, placebo; FH, familial hypercholesterolaemia; wk, weeks; mths, months, stan, stanol; stan est, stanol ester; marge, margarine; g, grams of stanol or sterol; LDL-C, LDL cholesterol; b'line, baseline; NS, not significant.

Table 8.2 Comparative studies of plant sterol and stanol esters at equivalent intakes (Reprinted with permission from: Thompson GR. Significance of cholesterol absorption: inhibitor role of plant sterols and stanols. In: Carr T, Descheemaeker K (eds). *Nutrition and Health.* Oxford: Blackwell Science Ltd, 2002: 27–33.)

Source	Subjects			Diet		Dose g/day		Duration
	Type	n	Age (yrs)	Fat (g)	Chol (mg)	Sterol	Stanol	
Weststrate[14]	NC/HC	95	45	42	230	3.1	2.7	3 weeks
Jones[15]	HC	15	37–61	35	ns	1.8	1.8	3 weeks
Hallikainen[16]	HC	34	49	30	175	2.0	2.0	4 weeks

NC, normocholesterolaemic; HC, hypercholesterolaemic; ns, not stated.

which sterol and stanol esters have been compared on a head-to-head basis. Three such studies have been reported to date,[15–17] the salient features of which are shown in Table 8.2. In each study similar amounts of sterol and stanol esters were fed in random sequence to the same individuals for periods of three-to-four weeks, except in the study of Weststrate *et al*[15] where the dose of stanol was less than that of sterol.

The LDL-lowering efficacy of sterol and stanol esters in the three studies is shown in Figure 8.1, only minor differences being evident between them apart from the small study by Jones *et al*,[16] which favoured sterol

Table 8.3 Changes in plasma plant sterol levels in subjects consuming sterol and stanol esters (Reprinted with permission from: Thompson GR. Significance of cholesterol absorption: inhibitory role of plant sterols and stanols. In: Carr T, Descheemaeker K (eds). *Nutrition and Health.* Oxford: Blackwell Science Ltd, 2002: 27–33.)

Diet	Source	Change in plasma ratio v control	
		Campesterol/TC	Sitosterol/TC
Sterol ester	Weststrate[14]	89%	52%
	Jones[15]	93%	39%
	Hallikainen[16]	47%	34%
Stanol ester	Weststrate[14]	−11%	−33%
	Jones[15]	−12%	−10%
	Hallikainen[16]	−28%	−30%

TC, total cholesterol.

esters. The weighted means gave values of 12.4% for sterol esters and 11.9% for stanol esters, the difference being insignificant. In contrast, as shown in Table 8.3, plant sterols and stanols differed in their

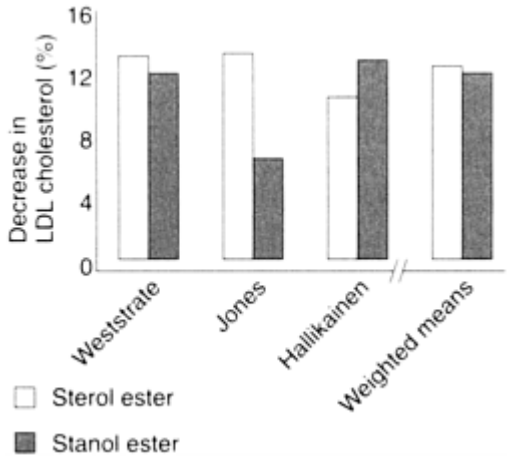

Figure 8.1

Comparative efficacy of plant sterol and plant stanol esters (Reprinted with permission from: Thompson GR. Significance of cholesterol absorption: inhibitory role of plant sterols and stanols. In: Carr T, Descheemaeker K (eds). *Nutrition and Health.* Oxford: Blackwell Science Ltd, 2002: 27–33.)

effects on plasma plant sterol levels; plasma campesterol: cholesterol and sitosterol: cholesterol ratios increased on sterol esters but decreased on stanol esters.[15–17] Whether these differential effects have any clinical significance probably depends on the genetic background of the individual, as described recently.[18] Plant sterols and stanols are both contraindicated in sitosterolaemia but although heterozygous carriers of this disorder hyperabsorb plant sterols, they reportedly excrete them sufficiently rapidly to avoid accumulating them in plasma. On the other hand, statin therapy does cause plasma plant sterol levels to rise in hypercholesterolaemic subjects, an effect which is mitigated by concomitant administration of plant stanols but compounded by plant sterols.[19] Hence, despite their equal efficacy in lowering LDL cholesterol in short-term studies, plant sterols may prove to be less beneficial than plant stanols in the long term.

Plant sterol and stanol esters are well tolerated whether given in margarine or, in the case of stanol esters in the UK, in yoghurt, milk and cereal bars. The only safety concern is that consumption of either sterol or stanol esters in a dose of 2 g daily over periods of one-to-twelve months results in a 20–30% decrease in plasma carotenoid levels. However, carotenoid: LDL cholesterol ratios remained within the normal range, and decreases in vitamins A, D, E or K were not observed.[20] Furthermore, a recent study has shown that the reduction in beta carotene levels induced by phytosterols can be prevented by an additional daily serving of fruit or vegetables with a high carotenoid content.[21] Thus, a regular intake of phytosterols provides a useful adjunct to the dietary and pharmacological management of dyslipidaemia, as endorsed by the latest guidelines from the USA.[22]

ω3 fatty acids

ω3 fatty acids occur in the diet as long-chain, polyunsaturated triglycerides derived from plant and marine sources. The three main compounds regarded as functional foods are α-linolenic acid (ALA or 18:3ω3), eicosapentaenoic acid (EPA or 20:5ω3) and docosahexaenoic acid (DHA or 22:6ω3). ALA is derived mainly from certain vegetable oils, such as soya, rapeseed, and walnuts, while EPA and DHA are derived

from oily fish. None of these compounds can be synthesized *de novo* so they are considered to be essential fatty acids. However, a limited amount of interconversion takes place in normal subjects, roughly 25% of EPA in plasma being available for synthesis of DHA, although <1% of ALA is converted to EPA.[23]

Metabolic effects

The potential health benefits of ω3 fatty acids were first recognized more than 30 years ago in Greenland Eskimos, whose diet consisted largely of seal, whale and fish, all of which are rich in EPA and DHA. The incidence of CHD seemed to be much lower in these Eskimos than in those living in Denmark on a Danish diet, and this was attributed to beneficial changes in their serum lipids, notably a lower level of triglyceride.[24] A corresponding decrease in very-low-density lipoprotein (VLDL) as well as the marked anti-coagulant action of ω3 fatty acids was noted by Sinclair,[25] who lived solely on seal, fish and water for three months. Similar decreases in serum triglyceride and platelet aggregability, accompanied by increases in EPA and DHA and a decrease in the linoleic acid (18:2ω6) content of plasma lipids, were observed under less extreme conditions in subjects consuming 150–200 g of fish daily, mainly mackerel, herring and salmon.[26] Other potentially beneficial metabolic effects in these individuals include decreased production of pro-inflammatory cytokines and a reduction in blood pressure.[27] The mechanism of these effects and the role of ω3 fatty acids in the prevention of CHD have been reviewed recently.[28]

Studies of ω3 fatty acid intake and risk of CHD

In the light of the apparent protection from CHD seen in Eskimos, several prospective studies have examined the relationship between ω3 fatty acid intake or frequency of fish consumption and the incidence of CHD in other populations. The first to demonstrate such an effect was the Zutphen Study, which showed a 50% decrease in CHD mortality in middle aged Dutchmen consuming 30g fish per day compared with those who ate none.[29] This effect was independent of major risk factors such as total cholesterol, blood pressure and smoking, but triglycerides were not measured. However, an extension of this study, the Zutphen Elderly Study, failed to find any relationship between ALA intake and CHD.[30]

The large US Health Professionals Follow-up Study found no difference in the incidence of CHD in men in the top versus the bottom quintiles of ω3 fatty acid or fish intake,[31] but in the US Physicians Health Study, consumption of fish at least once a week significantly reduced the risk of sudden cardiac death but not other CHD events.[32] The Nurses Health Study showed that frequent fish consumption in women reduced the risk of cardiac death more than the risk of non-fatal myocardial infarction (MI),[33] whereas the Western Electric Study had earlier shown an inverse correlation between fish consumption and non-sudden death from MI.[34]

Despite the somewhat contradictory evidence from observational surveys, data from two case-control studies support the protective role of certain ω3 fatty acids. Plasma levels of both EPA and DHA were inversely correlated with risk of MI in a Norwegian study,[35] and an analysis of the Physicians Health Study[36] showed a protective effect of plasma DHA levels on the risk of sudden death; a similar trend was evident for EPA but did not reach statistical significance. Neither study showed any evidence of a protective effect of ALA.

Randomized controlled trials

The suggestive but inconclusive results from epidemiological studies served to emphasize the need for randomized controlled clinical trials to test the effect of ω3 fatty acids on CHD outcome. The first such trial, the Diet and Reinfarction Trial (DART), involved over 2,000 male survivors of MI in the UK, who were randomly allocated to receive or not receive dietary advice to increase their intake of ω3 fatty acids by eating fish twice weekly or taking 1.5 g of fish oil daily.[37] Those receiving this advice showed a significant decrease in total mortality, due mainly to a decrease in fatal MI; it was suggested that this might have been due to a protective effect against ischaemia-induced ventricular fibrillation.

The Lyon Heart Study[38] was another secondary-prevention trial during which the treated group increased their intake of ω3 fatty acids, mainly by substituting rapeseed oil margarine, rich in ALA, for butter and cream. Total mortality decreased by 70% versus the control group, without any differences in serum lipids or platelet aggregation to explain this effect. A protective effect against the risk of cardiovascular death without much change in serum lipids was also seen in GISSI-Prevenzione, a secondary-prevention trial in which treated subjects received supplemental EPA plus DHA in a dose of 1 g daily, with or without additional vitamin E.[39]

A meta-analysis of the results of these three trials and of eight others which have assessed the effects of dietary and non-dietary ω3 fatty acid supplements in patients with CHD has recently been published.[40] Overall, the risk of fatal MI was reduced by 30% ($P<0.001$) and total mortality by 20% ($P<0.001$) in those receiving ω3 fatty acids, irrespective of whether this was from dietary or non-dietary sources (Figure 8.2). The wider confidence limits of the dietary trials reflect that there were only two compared with nine non-dietary trials. In five of the trials there was a 30% reduction in sudden death ($P<0.01$). Triglycerides decreased by an average of 20% during ω3 fatty acid supplementation but little change was observed in LDL and HDL cholesterol.

Mechanism of the cardioprotective action of ω3 fatty acids

Data from several sources, most notably from the randomized trials, suggest that ω3 fatty acids protect against fatal CHD, especially sudden death, rather than non-fatal events. The results of a second GISSI-Prevenzione trial, published just recently, show that the protective effect of ω3 fatty acids against sudden death is evident within four months.[41] The effect is observed at relatively low intakes of ω3 fatty acids, and appears to be much greater with EPA and DHA than with ALA. These features suggest that the mechanism involved is not anti-atherogenic but is either anti-thrombotic or anti-arrhythmogenic.[40]

Experimental evidence strongly supports the latter explanation, as discussed by Leaf and Kang.[42] EPA and DHA have both been shown to prevent lethal arrhythmias in dogs with induced myocardial ischaemia by stabilizing the cell membrane of myocytes. This is achieved by their incorporation into membrane phospholipids and consequent promotion of calcium ion (Ca^{2+}) efflux.

Data from the GISSI-Prevenzione studies[39,41] suggest that prevention of fatal arrhythmias post-MI, if that is indeed the protective mechanism involved, is achieved by consumption of 1g daily of ω3 fatty acids, equivalent to 100 g of oily fish. Further studies are needed to ascertain the minimum consumption of ω3 fatty acids required for cardioprotection but epidemiological data suggest it may be less than 1 g daily; this is arguably the level of intake above which ω3 fatty acids should cease to be regarded as functional foods and become pharmacological compounds. In the latter context, doses of 1–4 g daily of EPA and DHA are prescribed to treat severe hypertriglyceridaemia.

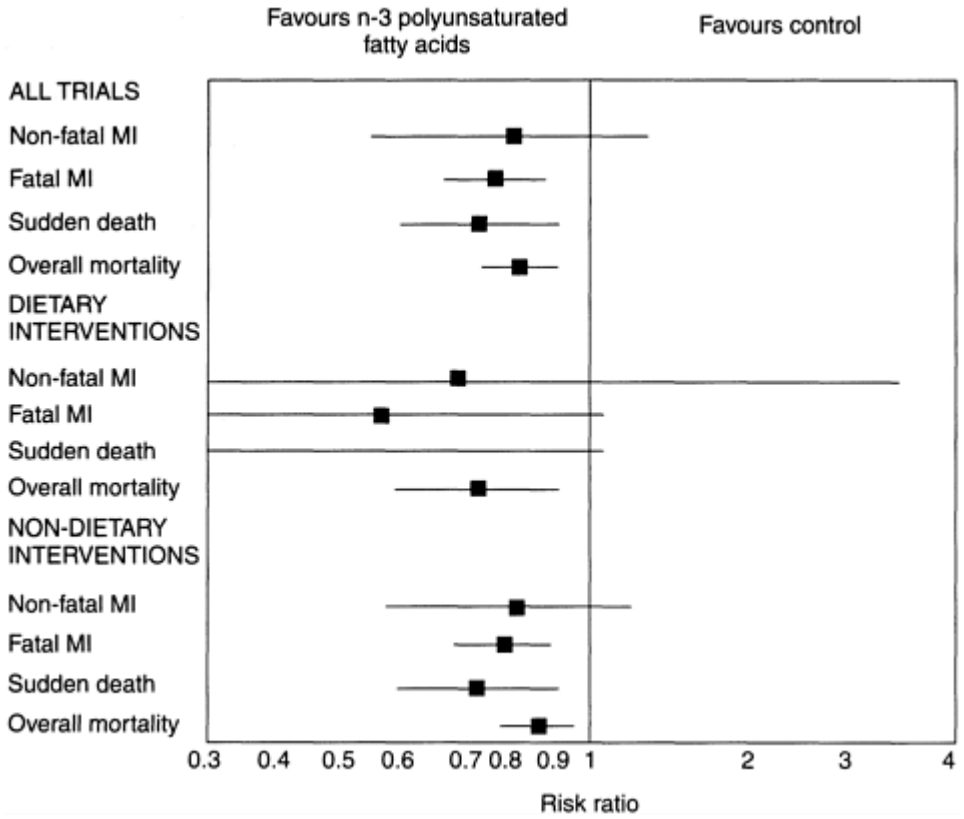

Figure 8.2

Pooled risk ratios and 95% confidence intervals for the different endpoints in randomised controlled trials of dietary and non-dietary supplementation with n–3 polyunsaturated fatty acids versus control or placebo (modified from ref. 40, with permission from Excerpta Medica Inc., © 2002). MI, myocardial infarction.

Population guidelines

Current guidance in Britain and the USA is that it is healthy to consume 1.5–1.8 g of ω3 fatty acids per week, either as fish or fish oil supplements.[43,44] Whether there is any benefit to begained from increasing the dietary intake of ALA remains to be established, especially since it does not get converted to EPA to any significant extent, but a recent study suggests that ALA is no more effective than linoleic acid in the primary prevention of CHD.[45] This outcome and the conflicting results of the Lyon Heart Study[38] and the Zutphen Elderly Study[30] underline the need for further data before the role of ALA as a functional food can be properly evaluated.

Garlic

Garlic *(Allium sativum)* shows some promise for improving certain cardiovascular risk factors. Alliin, a sulphur-containing compound, is broken down by the enzyme allinase and converted to allicin when the garlic bulb is crushed. Commercial compounds are standardized according to the alliin content but the actual

active ingredients and the mechanism of action remain unknown. Two meta-analyses of randomised controlled trials have been published recently. Stevinson *et al*[46] included 13 studies published before 1998 and reported a modest but significant reduction in total cholesterol compared to placebo. The weighted mean difference was −0.41 mmol/l (95% Cl, −0.66 to −0.15 mmol/l) or 4–6% reduction of total cholesterol, on a range of intakes of between 600 and 900 mg of garlic preparations standardized to 1.3% alliin (equivalent to 2.7 g of fresh garlic or 1 clove/day), over periods of 8–24 weeks (ten of 13 studies used the same powder). In contrast to these positive results, a study using garlic oil found no effect on serum lipids.[47]

Ackermann *et al*[48] looked at 45 randomized trials published between 1966 and 2000 and found small reductions in total cholesterol at one month and at three months but not at six months. Changes in LDL and cholesterol paralleled those in total cholesterol, and no changes were noted in HDL cholesterol. The review also suggests benefits of garlic on antiplatelet factors and variable effects on blood pressure. Proven adverse events include bad breath and body odour. Concerns about the true effects of garlic remain, due to the inadequate definition of active ingredients and the unpredictable activity of commercial products.[48]

Garlic has been proposed also as a treatment for asthma, *Candida* infections, colds, diabetes and cancer but its claimed hypolipidaemic activity has been the main focus of research. The implication for clinical practice is that although it may not be the most effective dietary means of reducing lipid levels, if patients enjoys garlic then they should be encouraged to continue using it.

Soya protein and phytoestrogens

Soya proteins have been consumed in China and Asian countries for hundreds of years but they are fairly new in European diets. Although the availability of soya and soya-based foods such as tofu, soya milk and soya yoghurts is increasing, consumer and physician awareness of its use in the therapy of cardiovascular disease is limited.[49] Its use in cooking also appears to be limited, and manufacturers need to provide more acceptable soya-based foods for the consumer.[50] Substituting soya for animal protein can result in lower saturated fat and cholesterol intakes, thereby resulting indirectly in a more beneficial cardiac risk profile.[51]

The efficacy of soya and soya derivatives in lowering total and LDL cholesterol was recently endorsed by the US Food and Drug Administration (FDA) which, in 1999, finalized a rule that authorized the use on food labels and in food packages under FDA jurisdiction of the following health claim concerning the association between soya protein and reduced risk of CHD: *'25 g of soya protein a day, as part of a diet low in saturated fat and cholesterol, may reduce the risk of heart disease'*[52] In practical terms this means that all products containing 6.25g of soy protein per serving (i.e. four intakes per day) may make the health claim on the label.

A meta-analysis of 38 clinical trials found that LDL concentrations can be lowered by about 13%, plasma triglycerides by 10% and HDL increased by about 2% (these reductions are even greater for individuals with high pretreatment LDL concentrations).[53] it is unclear if the benefits come mainly from the phytoestrogens, also known as isoflavones, found in soya (e.g. diadzein and genistein) or from the soy protein itself.[54,55] The available evidence regarding the role of isoflavones on lipids suggests a stronger effect when the isoflavones are given together with soya protein. Consuming isoflavones without soya protein may not lower cholesterol but might provide other cardiovascular benefits. One study has found a dose-dependent improvement in vascular function with the infusion of genistein,[56] with an increase in nitric oxide-dependent brachial vasodilatation; this suggests that the effects of isoflavones are not limited to lipids. In addition, soya has been used to prevent cancer (breast, endometrial, prostrate), to treat osteoporosis, constipation and diarrhoea and to relieve menopausal symptoms.[57]

Fibre

The term dietary fibre refers to non-starch polysaccharides and lignin present in plant products. These are a diverse group and in general are not always digested in the upper gastrointestinal tract. There are many ways of defining dietary fibre from a metabolic view but it is probably best classified as either water-soluble (i.e. hemicelluloses, pectin, gums, mucilages, carrageenan or agar), with the ability to form viscous gels, or insoluble (i.e. celluloses or resistant starch) in water. Insoluble fibres have less of an effect on cholesterol levels.[58] Water-soluble fibres found in foods such as beans and pulse vegetables have been reported to reduce total cholesterol mainly by lowering LDL concentrations. The key water-soluble fibres are listed below.

Dietary fibre was the first of the functional ingredients to be a commercial success, and the sudden rise in drinks containing high levels of fibre in the late 1980s is considered to be the start of the functional foods market in Japan and the rest of the world.[59] The product credited with being the first Japanese functional food is a dietary-fibre-containing soft drink called Fibre-Mini (Otsuka Pharmaceuticals), launched in 1988, which uses water-soluble polydextrose as its functional ingredient and is marketed for regulation of gut function.

Oat gum

Based on a meta-analysis of ten studies, Ripsin *et al*[60] concluded that the daily consumption of approximately 3 g soluble fibre from oat products lowers serum total cholesterol by about 0.15 mmol/l. This effect was positively related to baseline cholesterol concentration. The US FDA approved a health claim in 1997 for 0.75 g of β-glucan per serving, on the assumption that four servings a day would reduce cardiovascular disease risk.[61] Jenkins *et al*[62] have recently shown that although this effect is relatively small in terms of patient treatment, the reduction in cardiovascular disease risk is likely to be significant on a population basis. There are many ways of including oats or oat products in the diet. This health message appears to be relatively well understood and accepted by both the consumer and the physician.

Psyllium

Of the viscous soluble fibres, psyllium appears to be one of the most effective in lowering serum cholesterol and LDL-cholesterol concentrations, with few adverse effects. In 1998 the FDA acknowledged the likelihood that soluble fibres from sources other than oats could affect blood lipids and thus reduce the risk of heart disease. Psyllium fibre comes from the dried psyllium seed husk (PSH), which is cultivated primarily in India and is known as blond or Indian psyllium. Its health claim was approved at a dose of 1.78 g per serving on the assumption that four servings a day would reduce cardiovascular disease risk.[63] Currently in the UK it is found in breakfast cereal products such as Kellogg's Bran Buds and in a variety of dietary supplement products promoted for increased fibre intake and as aids for weight loss. Some foods containing PSH can be difficult to swallow and because of this the health claim on the label must also have a statement advising on the need to consume the food with adequate amounts of liquid.

Guar gum

It is fair to say that guar gum had a bad press in the late 1980s, mainly due to its marketing as a powder product which needed to be properly hydrated before being consumed, in order for it be effective. It also had some gastrointestinal side-effects. However, recent work from Imperial College School of Medicine in

collaboration with RHM Technology and King's College, London, has produced a palatable bread with a high guar gum content. Initial studies suggest this is effective in reducing postprandial lipids and insulin sensitivity in people at risk of CHD,[64] and further work is under way. This would suggest that these type of products work best when mixed intimately and hydrated within a food matrix.

Other studies suggest that increased soluble fibre intake may decrease blood pressure slightly, assist in weight management, alter blood clotting factors and increase insulin sensitivity. Intake of dietary fibre and complex carbohydrate appears to have a protective role in the prevention of CHD and should be encouraged.[22]

Folic acid

Elevated plasma homocysteine has been shown in many studies to be an independent marker of increased risk of cardiovascular disease.[65] Homocysteine is a sulphur-containing amino acid produced during the metabolism of dietary methionine, a process which is dependent on the presence of four B vitamins (vitamin $B_{12)}$ vitamin B_6, folate and riboflavin). A preventive role for folic acid has been postulated on the basis that increasing folate intake can reduce elevated plasma levels of homocysteine. However, to date no intervention trials have been completed which show that lowering plasma homocysteine levels will decrease risk of CVD.

Reduced homocysteine is a highly reactive molecule, and hyperhomocysteinaemia is associated with lipid oxidation and vascular damage, manifested by endothelial dysfunction and smooth-muscle proliferation. Men with plasma homocysteine levels in the upper 5% of the reference range (5–15 μmol/l) have a three-fold increase in risk of MI, and there is a similar increase in risk of stroke in those with values above the 75th percentile. There is also increasing evidence that elevated homocysteine levels are an independent risk factor for dementia, including both vascular dementia and Alzheimer's disease. Homocysteine levels are increased by numerous factors, including mutations of the enzymes methylenetetrahydrofolate reductase and cystathionine-β-synthase, dietary folate deficiency, high intakes of coffee or alcohol, renal failure and iatrogenic causes.[66] Folic acid supplements have been shown to reduce homocysteine levels,[67] irrespective of the underlying cause, but are not a substitute for lifestyle and dietary change.

To try and prevent a raised homocysteine level, people should be encouraged to have five portions of fruit and vegetables each day to ensure that adequate amounts of vitamins and minerals are consumed. The combination of vitamins that most effectively lowers homocysteine levels has yet to be determined. If supplementation with B vitamins is going to form part of a public health strategy to prevent vascular disease, it is important that the lowest effective dose is used to avoid toxicity. In the USA, grain products are fortified with folic acid at a level of 1.4 μg/g product. Although this is intended primarily to prevent neural tube defects, it is hoped to be beneficial also for vascular disease prevention. Promotion of foods high in the appropriate B vitamins should therefore be encouraged within a healthy diet. However, doubts over dietary supplementation will remain until further research has been completed and specific information on target groups is available.

Conclusion

This chapter has provided an overview of those functional foods with the strongest evidence for the reduction of plasma lipid concentrations and prevention of CHD, notably phytosterols and ω3 fatty acids. In addition there are many other foods marketed for which claims about disease reduction are only tentative. These include prebiotics and probiotics, nuts, flaxseed, and certain types of tea, such as green tea. Further research will determine whether any or all of these can be regarded as true functional foods. Ethical

marketing of products aimed at promoting health or preventing disease must be based on evidence of efficacy and safety.

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9
Compliance with lipid-lowering drugs

Grace M Lindsay and Elizabeth Tolmie

Introduction

Since the demonstration of the positive benefit of lipid-modifying drugs in the prevention and treatment of coronary heart disease (CHD) and other vascular problems,[1–4] guidelines incorporating evidence generated from these trials have been compiled to help practitioners improve the management of CHD.[5,6] The potential benefit, however, of any innovation or treatment is limited by the extent to which practitioners and patients follow advised therapeutic regimens. Evidence exists that guidelines are not being implemented and that not all patients eligible for a hyperlipidaemic drug receive one.[7–9] Consequently, target levels are not being achieved. It is not clear why underprescribing occurs but evidence that it does illustrates the point that responsibility for achieving optimal compliance does not always rest solely with the patient. Thus, the problem of poor compliance needs to be tackled in a way that will both encourage practitioners to comply with guideline recommendations and improve patient compliance.[10] In this chapter, the concept of compliance, the extent of the problem, and a systematic review of factors relating to compliance with lipid-modifying therapy are presented. In addition, behavioural theories developed to help explain health-related intentions and actions will be reviewed and their application to understanding the nature of compliance behaviour discussed.

Compliance with lipid-modifying therapy: the extent of the problem

The literature highlights low compliance to lipid-modifying drugs,[11–14] with reported discontinuation rates of 6.5–60%. Even with the relatively well-tolerated statin drugs,[15,16] the problem persists, with reported discontinuation rates as high as 34.5%.[16] Unfortunately, the bias towards using a quantitative methodology in studies investigating compliance with hyperlipidaemic drugs has meant that the reasons why patients do not comply with their prescribed drug regimen, from the perspective of the patient, have been investigated only superficially or not at all. As poor compliance with hyperlipidaemic drugs has clear implications for both cost and effectiveness of CHD prevention, insights into why could help guide appropriate education, communication and health care delivery practices.

Meaning of compliance

The complex nature of compliance with drug regimens is reflected in the diversity of professional groups (psychology, psychiatry, sociology, pharmacology and medicine) who have attempted to measure, predict, prevent or improve compliance. In recent years, the term ‘compliance’ has fallen out of favour because it

may imply obedience; an expression which is clearly inappropriate in contemporary health care where individuals are expected to and expect to be involved in decisions that may affect their health. Alternative terms such as 'adherence' and 'concordance' have been proposed.[17,18] The former is defined in the Collins English Dictionary as 'to follow closely or exactly' and the latter as 'a state or condition of agreement or harmony,[19] illustrating subtle but clinically important differences. In contrast, 'compliance' is simply 'to act in accordance with'.

Sackett[20] defined compliance as 'the extent to which a patient's behaviour co-incides with the clinical prescription (or therapeutic recommendation) provided by a physician, nurse practitioner, physician's assistant, or other health care practitioner'. Therefore, for the purposes of this chapter, the term 'compliance' is considered to be more appropriate than the others and is used to describe the level to which an individual will act, consciously or unconsciously, on 'expert' advice and in accordance with the definition applied by Sackett.

Optimal compliance

An effective level of compliance is that which will achieve the expected treatment goal, and will be dependent on the therapeutic regimen. In non-pharmacological therapeutic regimens the goals set will vary widely and will include, for example, weight lost or gained, reduction in alcohol units consumed, and other such measures. In pharmacological regimens, the effective level of compliance will depend on pharmacodynamics and pharmacokinetics. It has been suggested that a minimum compliance goal of 80% is justified, on the basis that it is founded on the dose-response function and incidence of symptomatic and adverse side-effects obtained through clinical trial safety and efficacy data supporting new drug registration.[14] But in clinical practice, a goal of 80% may be too narrow because it does not take account of the individual circumstances that influence drug action and response. A broader definition that characterizes different aspects of compliance is therefore required. This should encompass the fact that the drug be taken according to the specific instructions, at the correct dose and the correct time and without any omissions or additions.

Assessing compliance levels

A number of methods have been used to assess compliance behaviour. These include patient self-report, practitioner judgement, pill count, electronic monitoring systems, record review, pharmacological assays of drug levels, and blood lipid levels. None is 100% reliable and most are too impractical or costly to implement in clinical practice on a routine basis. From a purely pragmatic point of view, patient self-report is simple, cost-effective, and under the correct circumstances, reliable if determined in consultation with the patient (and where appropriate the patient's family) after the treatment goal has been agreed. Agreed goals, however, should be explicit and attainable. There is little point in practitioners specifying a goal if the patient does not understand why that goal has been set, cannot for some reason attain it, or has no intention (whether overtly or covertly) of trying to attain it, regardless of how achievable that goal is. However, identifying poor compliance and the extent to which a patient is not compliant may prove difficult, particularly if the patient feels the need to rationalize their behaviour by proposing adverse effects as the reason for poor compliance,[21] when there is in fact another motive. Therefore, if we are to gain access to legitimate compliance information from patients, we need to communicate in a non-judgemental and non-threatening manner. Otherwise, patients may simply deny poor compliance or justify it by offering a reason they believe will be acceptable.

Compliance with hyperlipidaemic drugs

The following section presents the results of investigations conducted within or across a primary care setting in which factors relating to compliance with prescribed hyperlipidaemic drugs, in particular statin therapy was either the main purpose of the study or investigated as part of the study. The review was conducted according to the standards for assessing methodological rigour of published compliance studies.[22]

Criteria for considering studies for the review

Because of the different research designs appropriate for the conduct of compliance investigations, studies of qualitative, quantitative, and mixed design were reviewed.

Inclusion and exclusion criteria

Investigations of compliance with lipid-lowering therapy that included at least one HMG-CoA reductase inhibitor were included. Paediatric studies, intervention studies, clinical trials and studies that did not include at least one HMG-CoA reductase inhibitor were not included in this review.

Data extraction

The review extracted data relating to the main purpose of the investigation; the definition and measurement of compliance used; sample characteristics; study inclusion and exclusion criteria; study design and the main results and conclusions of the investigator(s).

Study results

Design and purpose of original studies

Thirteen original studies published between 1993 and 2001 met the inclusion criteria for this review. The design of the available studies varied according to the purpose of the study. The main purpose of six of the studies was compliance rates.[11,12,15,23,24] Others focused on prescribing patterns,[7] the extent to which lipid level goals were achievable[13] or achieved,[26] the effects of hospital discharge prescription on long-term compliance,[9] and patients' values and preferences in terms of additional lifespan with and without drug therapy.[25] Three studies restricted their investigation solely to statins,[8,9,23] but most[11–13,15,16,24,25] conducted the investigation using more than one class of lipid-regulating drug. Only three studies specifically investigated the factors associated with compliance.[8,16,21]

Sampling

Sample size varied widely across studies (n=35−7, 287) Twelve studies used a non-probability sample of men and women and one used a randomized list.[24] One (open label) study restricted the sample to men.[23]

Study definitions

The terms used to define and measure 'compliance' varied across studies but were clearly specified in all but two investigations.[8,25] Compliance was defined as discontinuation of therapy or persistence (%) with prescribed dose over a specified time period. One study[15] did not clearly distinguish between patient-initiated discontinuation of therapy and physician-initiated discontinuation. Measurement of compliance

was conducted using record review, pill count or self-report. Only three studies used more than one measure of compliance.[12,23,24]

The purpose of this review was to report investigations of compliance with hyperlipidaemic drugs and to summarize the factors associated with intentional and non-intentional compliance. The review identified 29 factors associated with decreased compliance or discontinuation of hyperlipidaemic drugs, 19 factors associated with increased compliance and 29 factors that made no difference. For the purpose of this chapter, these are broadly categorized into eight categories: demographic and social; biological markers and adverse effects; communication; drug regimen and compliance history; and psychological (see Table 9.1).

Demographic and social characteristics

The extent to which individual demographic and social characteristics impact on compliance behaviour is reported inconsistently across studies and, consequently, remains inconclusive. For example, compliance has been associated with increasing age,[12,16] with young age [21] and to have no association with age at all.[11,15] Similarly, female gender and drug cost have been associated with increased compliance in some studies[12,16,21] but decreased compliance in others.[8,16] This suggests that poor compliance occurs regardless of age or gender and that perhaps social, cultural or personal characteristics are more important determinants. Inconsistencies with regard to drug costs and compliance may be explained by the variations in prescription payment and reimbursement across different health care systems. In the UK, for example, drug cost may only inhibit patients who need to pay prescription fees. Even then, it may not negatively affect compliance in patients who pay an annual pre-payment fee (currently £89.00 per year) regardless of the number of drugs they receive. In other countries, however, drug charges may deter a greater number of people.[8]

Physical

Biological parameters

As with demographic and social characteristics, the relationship between compliance and some of the biological parameters measured is unclear, with results dependent on the criteria used to define and measure compliance. Kiortsis[21] used data obtained from routine medical examination to examine the relationship between high (100% pills taken), intermediate (<6% pills missed) and poor (>6% pills missed) compliance to hyperlipidaemic drugs and a number of biological parameters (body mass index [BMI], blood pressure, blood glucose, serum triglyceride levels). An association was found between increased compliance and higher systolic blood pressure. No association was found between compliance and diastolic blood pressure or any other biological parameter. One study by Avorn,[16] discussed more fully in the following section, detected an association between increased compliance and hypertension. However, the criteria for hypertension in Avorn's study were not explicit and the definition of compliance (<80% and ≥80%) differed from that used by Kiortsis.[21]

Adverse effects

The most consistently reported reason for poor compliance with hyperlip

Table 9.1 Factors associated with compliance to hyperlipidaemic drugs as reported in primary care investigations.

	Demographic and social characteristics	**Biological markers and adverse effects**	**Communication**	**Drug regimen and compliance history**	**Psychological**
Decreased compliance/ discontinuation	Increasing age[12,16] Younger age[21] Female gender[15] High cost of drug[8] Poverty[16]	Adverse events/ side-effects[8,11–13,21,24] Concomitant serious illness[11,12] Hospitalization[11] Absence of atherosclerosis at coronary angiography (physician)[24] Adequate response to diet (physician)[24] Poor efficacy of drug (physician)[11,12,24]	Patient or physician unaware therapy lifelong[24] Lack of instruction on drug use[8] Breaking appointments with physician[21]	Increased no. drugs prescribed[15,16,21,24] Increased no. doses LL drugs prescribed[15] >16 products per year prescribed[16] Treatment perceived as inconvenient[21] History of poor adherence[11] Discontinuation of previous LL therapy[11] Prescribed a fibrate[12] Prescribed antidepressants[12] Overtime[16]	Unconvinced of need for ongoing treatment[12] Depression[24] Anxiety about potential side effects[11] Preference for non-pharmacological methods[11] High vitality score (SF-36)[15]
Increased compliance		Decreased disability[15] Hypertension[16] Higher systolic blood pressure[21] Diabetes[16] Coronary artery disease[16] Less body pain[15]	Discharged from hospital on therapy[9] Perception of time physician spent discussing cholesterol and CVD[21] Good relationship with doctor[21] Ten days before clinic visit (toothbrush effect)[23]	Lower dose frequency[15] Incorporated into daily routine[21] Self reported history of good adherence[15] Long-term use of LL therapy[16] Prescribed analgesics[12] Prescribed antidiabetic drugs[7] Prescribed cardiovascular drugs[7,12]	Perceived additional life span[25] Belief that drug will be effective in preventing future CVD event[21]

Table 9.1 continued

	Demographic and social characteristics	Biological markers and adverse effects	Communication	Drug regimen and compliance history	Psychological
No significant difference	Age[11,15] Gender[12,16,21] Cost of drug[21] Drug cost subsidized[12] Alcohol consumption[21] Employment[15] Educational attainment[15] Ethnicity[15] Marital status[15]	Sideeffects frompreviously LL drugs[15] Drugclasseffects[15] Blood glucose levels[21] Cardiovascular disease (CVD)[23] Body mass index[21] Diastolic blood pressure[21] Hospitalization[15] Serum triglyceride levels[21]	Source of prescriptionor type of prescriber[12] Satisfaction with GP advice[15] Patients' knowledge of cholesterol[21] Satisfaction with pharmacy services[15] Patronage of a single pharmacy[15]	Time between diagnosis and treatment[21] Previous use of LL therapy[12] Use of an HMG-CoA reductase inhibitor[15]	Mood or stress[21] Personal beliefs about role of cholesterol in CVD[21] Beliefs about personal risk of a future CVD event[21] Symptoms attributed by patients' to high cholesterol levels[21]

LL, lipid lowering.

idaemic medication is drug-associated adverse effects.[8,12,13,16,21,23,24,26] Most common are those associated with the gastrointestinal (GI) system. Rash and flushing, particularly with niacin, are reported less frequently than GI problems but more so than other adverse effects such as gout, depression or myalgia. A study,[11] primarily conducted to determine if there was a difference between the rate of discontinuations reported in clinical trials (n=5) and that reported in primary care facilities (n=2), identified that adverse effects contributed to 56% of all patient- or physician-initiated discontinuations and that primary care had the highest discontinuation rate. This study, which reviewed retrospective data from 2,369 men and women with a mean age of 56.5, showed statins to have a lower discontinuation rate at one year than niacin, fibrate, or bile-acid sequestrants (15% as opposed to 37–40%). Overall, the highest discontinuations were with niacin (46%) and bile-acid sequestrants (41%). Nevertheless, despite the fact that side-effects attributed to statins appear less problematic[11,27] than those attributed to other agents, the study identified that 35 of 54 (65%) statin discontinuations were attributable to adverse effects.

The higher rate of discontinuation with niacin and bile-acid sequestrants is also reported in a study[24] that compared persistence with hyperlipidaemic drugs across two primary care facilities. This study, discussed in more detail later in this chapter (see Consultation style and compliance), identified a significantly higher rate of discontinuation, in both facilities at one year, with cholestyramine (18% and 64%) or gemfibrozil (30% and 84%) when compared with statins (50% and 87%). Similarly, a study[16] using retrospective prescription data of 7,287 men and women aged over 65 years to compare compliance rates across different class of hyperlipidaemic drugs (n=6), found statin compliance to be higher (64.3%±29.8% of days) as opposed to compliance with bile-acid sequestrants (36.6%± 29%) or any other class of hyperlipidaemic drug.

That statins are generally better tolerated than alternative lipidmodifying drugs[16] may explain, at least to some extent, their lower discontinuation rate in comparison to alternative hyperlipidaemic drugs.[12,13,24,26] Nevertheless, despite their apparently greater tolerability, only around 50–60% of patients persist with statin therapy beyond the first year.[9,12,23]

Drug scheduling/compliance history

Complexity of drug regimen

A number of studies, discussed more fully elsewhere in this chapter,[15,6,21,24] have shown that the more complex the drug regimen in terms of the number of drugs or doses prescribed,[15] the less likely patients are to comply with it. It is not clear if and at what point the number of drugs prescribed negatively influence compliance, although it has been sug gested that this may be at a level of ≥16 drugs per year.[16] It is likely, however, that the extent to which individuals are affected by the complexity of a therapeutic regimen will differ. Furthermore, despite the simplicity of statin regimens in comparison to other agents, compliance seems to decrease over time.[9,12,23] In a small open-label study[23] involving 39 men aged 20–70 years, compliance to dose, days and time of a single dose of fluvastatin 40 mg daily was measured using an electronic measuring device (MEMS), and pill count. Although compliance remained at >80% and >93% according to MEMS and pill count, respectively, a downward trend was noted over the six-month study period.

Regardless of the regimen prescribed for hyperlipidaemic drugs, the overall prescription provided for an individual may impact on compliance in a positive or negative way. A prospective survey[12] of pharmacy dispensing data from men and women (n=610) aged 22–84 years found that patients prescribed either a fibrate or a statin were more likely to comply with hyperlipidaemic drugs if prescribed concomitant cardiovascular drugs or analgesics than those prescribed antidepressants. While the relationship between compliance and antidepressants may result from factors associated with a patient's mental state, such as lassitude, anxiety or lack of motivation, the extent to which mental state impacts on compliance may vary according to either the cause of depression or the criteria used to define it.

Past use of lipid-lowering drugs

Sung[15] suggested that past use of hyperlipidaemic drugs was not associated with compliance and that around 50% of patients discontinued therapy within the first three months. The problem of early discontinuations may go some way towards explaining the association noted between long-term drug use and good compliance.[16] Long-term users have better compliance than new users simply on the basis that because they continue therapy they can be identified as long-term users. In addition, the relationship between past and existing compliance behaviour[11,15,24] may be due to existing health beliefs. Nevertheless, access to previous compliance behaviour may provide practitioners with a guide as to how a patient will respond when supplied with a prescription for hyperlipidaemic drugs or when the class of drug previously prescribed is amended. However, it should be noted that changing one prescription or regimen for hyperlipidaemic therapy to another might alter existing compliance behaviour.

Daily routine

Patients are significantly more likely to comply with hyperlipidaemic therapy if taking the medication can be incorporated into daily routine.[21] However, this contributing factor may be applicable only to patients with a positive attitude towards their medication. Patients who are willing to take their prescribed medication go to great lengths and use a number of strategies to remind themselves about their medicine, until taking it becomes part of their daily routine (Tolmie, Lindsay *et al* unpublished work). Being able to take medication at a time that fits in with one's lifestyle and ongoing commitments may help this process. However, it is unlikely that patients who have, for whatever reason, reservations about taking a particular drug will make any attempt to integrate drug-taking into daily life. In such circumstances, the patient's rationale for non-compliance will need to be identified.

Consultation style and compliance

Misinterpretation of instructions for use

The importance of the interaction that takes place between practitioners and their patients is illustrated in a study[8] which highlighted how even simple communication failures impact on compliance. The study, which reviewed 207 patient records to identify the reasons for poor compliance with statin therapy, found that although only 54 (26%) drug discontinuations or instances of irregular use were patient-initiated, 17 of these were due to patients misunderstanding physicians' instructions for taking the medication. These results are supported by Wirebaugh[24] who, from a sub-sample (n=100) of study participants, identified that 11 out of 30 patient-initiated, and 6 out of 42 physician-initiated drug discontinuations occurred because the patient or physician did not know that therapy was life-long.

Wirebaugh[24] compared persistence with hyperlipidaemic medication in men and women (n=400) who had previously undertaken an intensive cardiology prevention education programme, with that of a randomized sample (n=400) of individuals who had not. The higher rate of discontinuation at one year after commencement of therapy in the latter group highlights the benefits of the programme. However, as the intervention programme was intensive—consisting of a teaching programme covering many aspects of cardiovascular disease, clinical trial results, and prevention and treatment—the practicalities and potential costs associated with implementation and maintenance are likely to prohibit the use of such a programme in clinical practice.

Patient education

Educational programmes appear to result in better compliance[24,28] although it is not clear if improvements are due to an increase in patients' knowledge, to the emphasis placed by practitioners on specific aspects of the problem, or to patients' positive perceptions that the greater the time invested in them, the more significant the problem. Kiortsis[21] (see next section) found no association between compliance and patients' knowledge about cholesterol. Regardless of knowledge, patients who considered their physician to have spent 'a lot' as opposed to 'enough' or 'insufficient' time discussing cardiovascular disease (CVD) and cholesterol with them were significantly more likely to comply with therapy. These findings seem to suggest that the quality of the consultation and patients' perceptions of the quality of the consultation may impact more on compliance than the quantity of information provided. It may be that during an effective consultation, patients' beliefs will be expressed more easily and that, consequently, misunderstandings and misinterpretations that have the potential to affect compliance in a negative way, will be avoided. This may be particularly relevant in circumstances where patients search for clues about their condition[29] without asking directly for the information, or when patients are prescribed hyperlipidaemic drugs during a consultation that is primarily for another purpose (e.g. a diabetic or thyroid check). If practitioners prescribe hyperlipidaemic drugs while focusing their attention on issues pertaining to the primary purpose of the visit, or are inattentive to patients' concerns, the significance of hyperlipidaemia, and thus the need for continuing treatment, may be minimized (Tolmie, Lindsay, Kerr *et al,* unpublished work).

Psychological factors

Perceived efficacy of treatment

A number of studies investigating compliance with drug regimens have shown that some patients are doubtful about the need for medication.[12,30,31] Simons[12] found that of 365 patients who had discontinued

treatment for elevated cholesterol, 115 (32%) had done so because they were unconvinced about the need for ongoing treatment. These results are supported by Kiortsis[21] who surveyed 193 hyperlipidaemic men and women aged 52.7±12.9 to determine the factors associated with compliance and to determine patients' beliefs about the role of cholesterol in CVD. A questionnaire with a ten-point scale and multiple-choice questions was used to assess compliance and evaluate patients' beliefs about cholesterol and the drugs used to treat hyperlipidaemia. A significant association was found between compliance and patients' beliefs about how effective the drug would be in preventing CVD, but no association was identified between compliance and symptoms attributed by patients to elevated cholesterol or between compliance and patients' beliefs about personal risk. These findings may indicate that patients' perceptions about the efficacy of the drug have a greater impact on compliance than their perception of the threat CVD poses for them.

Perceived health status

Beliefs about the value of hyperlipidaemic drugs may be influenced by personal perceptions of the significance of elevated cholesterol in relation to other health problems. Sung[15] identified an association between compliance and chronic disease and between compliance and bodily pain (a health status domain of the Health Status Questionnaire—Short Form 36 [SF36]) in a survey conducted to identify the reasons for non-compliance. Data—via pharmacy records, health care records and a patient questionnaire—were obtained from 772 men and women aged 34–82 years. The questionnaire was based on a modified version of the SF36, which identifies health status as perceived by the patient and by the validated Morisky scale.[33] The Morisky scale, consisting of four questions requiring a Yes/No response, elicits information on drug omissions due to forgetfulness, carelessness and feelings of well-being. Results indicated that there was an association between chronic disease and decreased compliance and between less bodily pain and increased compliance. However, as chronic disease was measured using a score based on the overall number of hyperlipidaemic and chronic disease drugs prescribed, the association between existing disease status remains inconclusive. In addition, because perceptions of bodily pain may be inconsistent and influenced by use of analgesics, anxiety or other factors, this too remains inconclusive (see Table 9.1).

Concomitant illness

As with other factors discussed throughout this chapter, relationships between compliance and existing health problems are not always clear nor study results consistent. While one might expect individuals with chronic disease, particularly CHD, to be more compliant, this is not always the case. Avorn[16] detected higher compliance in individuals with coronary artery disease, hypertension, and diabetes. In contrast, Schwed[23] (see drug scheduling) and Kiortsis[21] found similar rates of persistence with statin therapy in men with and without CHD. Possible explanations for this inconsistency are that experience of symptoms may positively influence compliance to a greater extent than a diagnosis of CHD, or that drugs prescribed at the time of an acute event or diagnosis will be taken more readily than those prescribed some time later. It is clear then that there is a need to emphasize the significance of hypercholesterolaemia and that this may be particularly pertinent in the absence of symptoms. Nevertheless, the presence of symptoms may not be sufficient a cue for patients to realize the need for therapy.

Although men and women prescribed statin therapy at discharge from hospital after a diagnosis of CHD (n=343) were nearly twice as likely to be persisting with therapy two years after diagnosis than those commenced on statin therapy after discharge,[9] only 47% (n=162) continued to take their medication regularly. As illustrated in this study, some patients do not comply with statin therapy even when diagnosed with CHD. It could be argued that not having the drug prescribed until some time after discharge may have minimized patients' perceptions of the need for therapy. However, Kiortsis[21] found no difference in

compliance between patients treated 'immediately', and those treated within 'weeks' or 'months' of diagnosis.

Patients' perceptions of elevated cholesterol and its potential effect may be influenced by existing health problems and by the priority in which each problem is placed. A survey[34] of 815 hyperlipidaemic men and women aged 65–74 years who had suffered an acute myocardial infarction (MI) found that patients with cerebrovascular disease or hypertension believed cholesterol-lowering to be 'very important' more frequently than those with congestive heart failure or diabetes. Although the reason for this is not clear, it may be that symptoms associated with congestive heart failure and diabetes, and the relief of those symptoms, overshadow other issues. Alternatively, it may be that patients with particular health problems such as congestive heart failure or diabetes associate elevated cholesterol with diet but consider other aspects of their diet to be of greater consequence to their health than cholesterol.

Perceptions of the cause of coronary heart disease

Beliefs about the relationship between health and lifestyle factors such as diet and smoking and the cause of ill health may help explain why, even when evidence of personal risk exists, some patients will choose not to follow their prescribed drug regimen. Interviews[35] with 105 patients admitted to hospital following a first diagnosis of acute MI, or for coronary angiography, found that most patients believed diet and smoking to be the primary risk factors for CHD in general and for themselves as well, with MI patients citing diet significantly more often.

Summary of trial results

Only a small number of primary care studies have investigated compliance with prescribed lipid-lowering agents, and even fewer have limited their investigation to prescribed statin therapy. As illustrated in this chapter, results of studies that have looked at compliance vary according to the purpose of the study, methods used, drugs prescribed and the country in which the study originated. Although many different factors have been associated with compliance, results remain inconclusive. Most studies fail to acknowledge the multi-dimensional and potential interactive relationship between factors. Those that have focused exclusively on either quantitative factors or psychological factors, without considering the other aspects of the problem, are limited and prohibit determination of the extent to which the factors identified act as inhibitors or determinants of compliance.

The results of this review show that poor compliance may be unintentional, occurring because of uncontrollable factors such as age or serious illness, or intentional, when the individual actively chooses not to take the drug. Although discontinuation of treatment might appear illogical to some practitioners,[12] patients will utilize the inherent or learned information available to them to come to a decision about whether or not to take their medication. While there may be little scope for changing many factors such as age, or the presence of chronic disease, the patient's perspective remains to be fully explored. We need to improve our understanding of individuals' attitudes and beliefs regarding proposed therapy and ask what motivates some to comply with their prescribed drug regimen whereas others only comply partially or discontinue their medication altogether. The factors that may impact on health behaviour are explicated in the theoretical models developed to help explain health behaviour. These models may offer some explanation as to patients' rationale for discontinuing medication and may add support to the view that these reasons are rational rather than irrational and that they are influenced by many interacting components.

Theoretical models

Compliance behaviour is complex, with patients' views, beliefs, understanding of risks and benefits of treatments and misconceptions central to planned and sustained health advice in general and to medication-taking specifically.[31,36] In addition, decision-making can be further influenced by the way in which the advice is delivered and subsequently interpreted.[37,38] A number of theories have been proposed which provide conceptual maps that take into account a range of factors such as individual beliefs of causation, views of health professionals, risks and benefits of treatments, and motivation to comply with advice and treatment. These theoretical perspectives relating to health beliefs and actions are presented in brief in the following sections.

Health locus of control

Locus of control beliefs has been described as the extent to which an individual uses internal or external attributions to explain events and actions related to their own health.[39] The Health Locus of Control (HLOC) model[39] has been developed to measure the extent to which individuals believe that their health is influenced either by their own behaviour (internal locus of control) or by external forces (external locus of control). The latter may be luck, chance or other powerful forces beyond a person's direct control.

The concept has been derived from social learning theory, and consists of four basic constructs—behavioural potential, expectancy, reenforcement value and psychological situation—which were developed to account for human behaviour in complex situations.[40] The theory proposes that the potential of a specific behaviour to occur in any given situation is a function of the individual's expectancy that the behaviour will lead to a particular re-enforcement. Expectancy, however, is a component of past experience in a similar situation or, if there is no past experience on which to draw, is generalized. Therefore, future behaviours, in a broad range of activities, are considered to be determined by socialization experiences.

Internal locus of control beliefs have been related in a positive way to health practices and general well-being, with the converse being true for external locus of control beliefs. However, these relationships have not been confirmed universally in studies that have assessed and evaluated measures of locus of control and health behaviour intentions.[39] The HLOC construct has been applied in a wide range of behaviours. Some studies have documented a positive relationship between internal HLOC beliefs and indices of preventive health practices,[41,42] but other studies have, by contrast, failed to establish such a relationship.[43] HLOC remains of debatable use to health care professionals in terms of predicting behaviour changes. Developments to incorporate HLOC assessment in which the three concepts of the health value, self-efficacy and locus of control are combined into a more general theory of health behaviour are being investigated.[44]

Health beliefs

Theories describing health-related behavioural change are based on the premise that if an individual has sufficient information and understands the relationship between an activity and its effect on health, this will in turn influence behaviour. The health belief model[45] (Figure 9.1) is probably the best known theoretical model highlighting the function of belief in health care decision-making. It has been used to predict protective health behaviour such as screening or vaccination uptake and compliance with medical advice.[46]

The model suggests that whether or not a patient changes their behaviour will be influenced by an evaluation of both the feasibility of making changes and the benefits likely to be accrued against the actual costs. According to this theory, an individual must believe that they are capable of carrying out the intended behaviour.

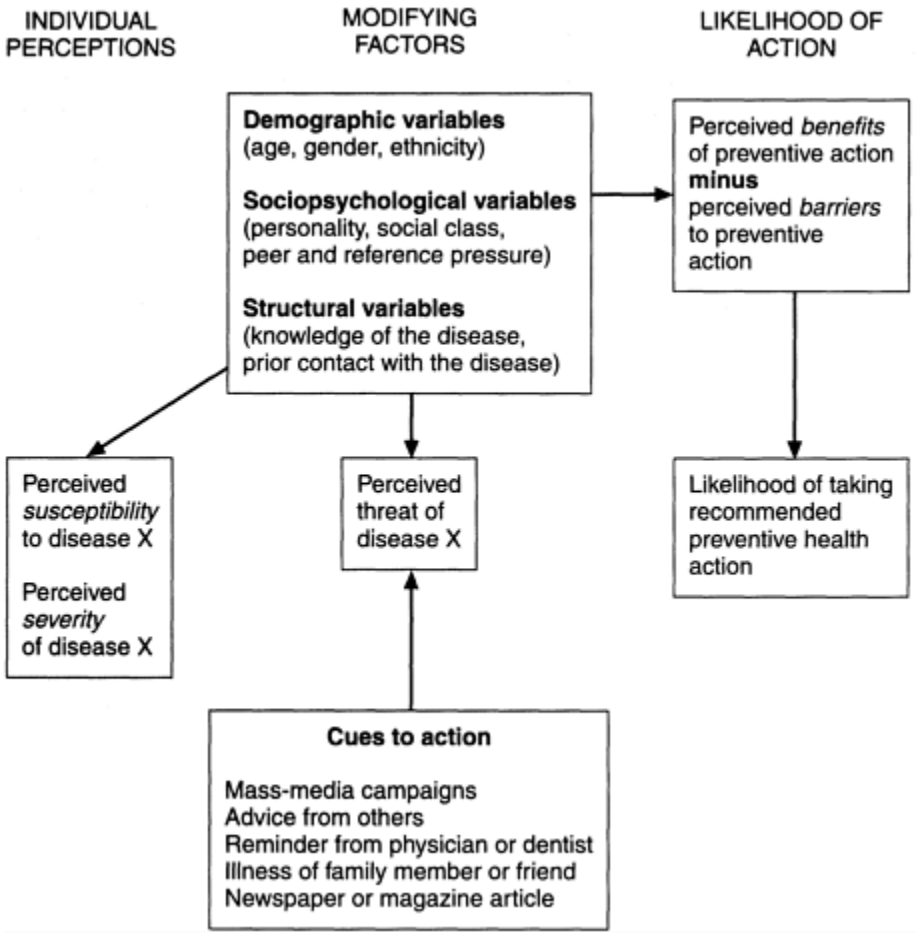

Figure 9.1

The health belief model. Adapted with permission from ref. 45.

For behavioural changes to take place, the model outlines four areas to consider:

- There is an incentive to make change
- The patient is threatened by current behaviour
- Change would have few adverse consequences
- The patient is competent to carry out the changes.

In terms of incentives to make change, individuals must perceive themselves to be susceptible to a particular illness or injury and consider the consequences to be serious. An individual's perception and assessment of risk is therefore important and is likely to be influenced by their previous personal experience, experience of others, ability to change the situation and a general feeling that illness or danger has drastic consequences. It is suggested that people need to have some kind of cue before action is taken. The issue

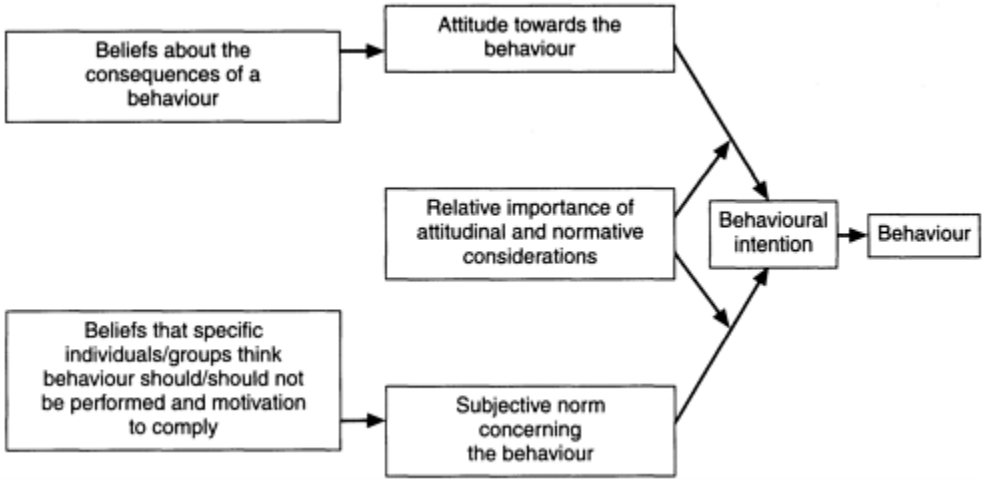

Figure 9.2

Factors determining an individual's behaviour. Adapted from ref. 47.

needs to become relevant to the individual and this may be the result of appreciating the adverse effects of their own lifestyle, or being closely involved in the illness of others.

Theory of reasoned action

The Theory of Reasoned Action, first introduced in 1967 and subse quently re-tested and refined over time,[47] makes the assumption that individuals are rational beings who weigh up the consequences of their behaviour before taking action (Figure 9.2). The intention to behave in a particular way, however, does not necessarily result in the intended behaviour being performed. Whether or not the intention actually occurs is influenced by the individual's attitude towards the behaviour, that is, an evaluation of the positive and negative aspects of its outcome (performance outcome) and their perception of how 'significant others' will view the behaviour (subjective norms).

Behavioural change theory

Motivation to change adverse aspects of lifestyle and to adopt a healthier perspective has been described within the context of the Stages of Change model[48] (Figure 9.3). This approach evaluates an individual's readiness to make changes, and bases interventions on that assessment; i.e. those who were willing to make changes were helped to do so, whereas those who were resistant to change were given general advice and information. Individuals who are receptive to making changes are encouraged to evaluate the positive and negative aspects of their lifestyle and, through endorsement of the positive aspects, are supported in making changes to less healthy behaviours.

Limitations of theoretical models

Although none of the theoretical models of health behaviour are highly predictive, they do offer some understanding as to why individuals adopt certain attitudes or act in a particular way towards preventive health

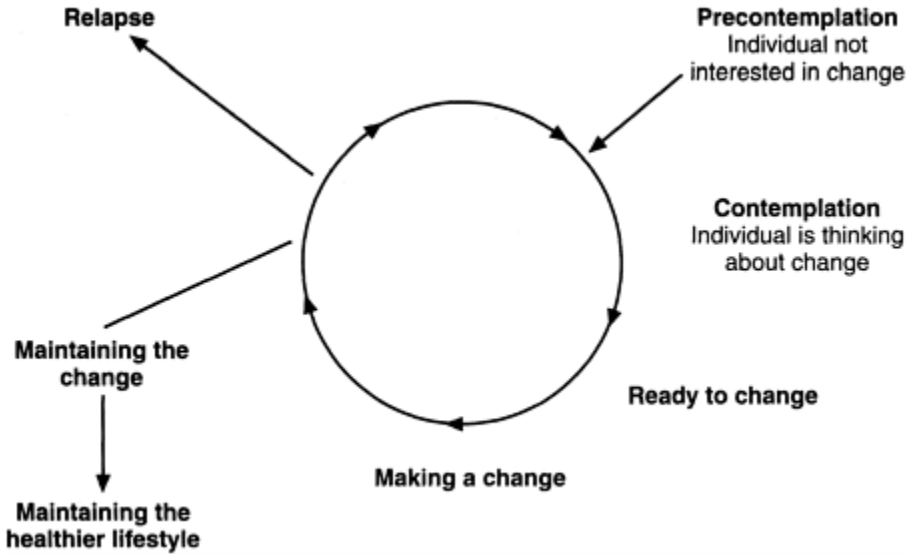

Figure 9.3

The stages of change model. Adopted from ref. 48.

messages or advice. Thus they may help us understand non-compliance as more than merely irrational behaviour by providing some explanation for the many factors that impact on medication-taking. As such, they may help guide patient consultation, information giving and follow-up strategies.

Conclusions

It is clear that for optimal compliance to occur, practitioners must identify, assess and treat patients in line with guideline recommendations, and patients must have the desire, motivation and ability to act on the advice offered. Nevertheless, regardless of how well practitioners may comply with their part of the bargain, the effectiveness of any advised therapeutic regimen will rest with the patient's ability to follow the advised regimen and, ultimately, on the decision they make regarding the extent to which they will comply with the regimen. If the patient/NHS partnership[49] is to evolve in a way that truly involves the patient, treatment goals will need to be agreed in collaboration with the patient. This means that practitioners must be aware of and accept the many factors that impact on patients' perceptions of their health and the information that they are given. Merely supplying information may not be enough to effect and sustain changes in health behaviour. Unless the issues that result in poor compliance are identified and addressed from the patient's perspective, efforts to improve compliance are unlikely to be successful.

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10
Lipid guidelines come of age?

Chris Isles

Introduction

In the beginning there was only opinion, usually strongly held. Then there was evidence, and after evidence came guidelines. And the medical profession were divided: some regarded guidelines as a good thing created by experts in their field who spent long hours sifting through the results of clinical trials in order to help the rest of us make evidence-based treatment decisions, while others considered guidelines a straitjacket or worse, a form of cookbook medicine designed primarily to curtail clinical freedom. Those for and those against guidelines are unlikely ever to be completely reconciled, but even the hardened sceptic would probably concede that guidelines in general, and lipid guidelines in particular, are now coming of age.

The big leap forward in the evolution of lipid guidelines came when we moved from the 'know your number' philosophy or the belief that treatment decisions should be based on the level of serum cholesterol alone, to 'know your risk', namely the realization that those at greatest risk of a vascular event have the most to gain by treatment, irrespective of the level of their serum cholesterol. As a direct result of this evolutionary process, the latest lipid guidelines from both sides of the Atlantic emphasize vascular risk over serum cholesterol in the decision to prescribe a lipid-lowering drug.[1–3]

Epidemiology

The burden of disease

In 1990 there were an estimated 50 million deaths worldwide, of which 14 million (28%) were due to cardiovascular disease. Most of these were a result of coronary heart disease (CHD) (6.2 million) or stroke (4.3 million)—the first and second leading causes of death, respectively. By 2020 the global burden of cardiovascular disease is expected to grow even further. It is anticipated that by this time there will be around 68 million deaths per year worldwide, of which 25 million will be due to cardiovascular disease, 11.1 million to CHD and 7.7 million to stroke.[4]

Risk factors

Atherosclerosis is a lifelong process that begins in adolescence and is more common in people with cardiovascular risk factors who are not genetically protected against it. The lipid risk factors are a high serum level of low-density lipoprotein (LDL) cholesterol[5,6] and a low serum level of high-density

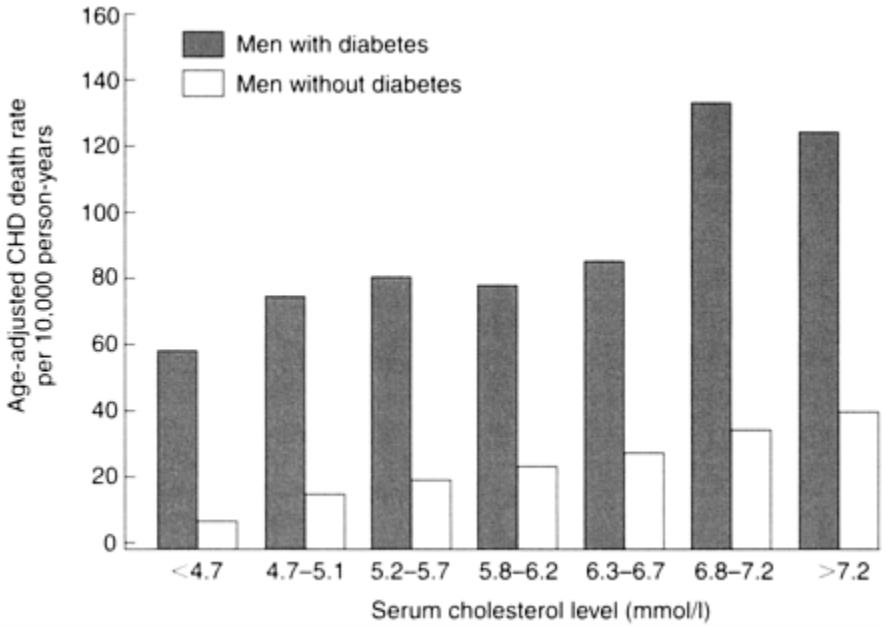

Figure 10.1

Age-adjusted coronary heart disease (CHD) death rates by serum cholesterol level for 347,978 men with and without diabetes at initial screening for the Multiple Risk Factor Intervention Trial.

lipoprotein (HDL) cholesterol,[7,8] the latter often associated with a high serum level of triglycerides. Taken together with other modifiable (smoking, diet, physical inactivity, obesity, diabetes and alcohol) and non-modifiable (age, male gender and heredity) risk factors, these define an individual's chance of developing both coronary and non-coronary vascular disease (Figure 10.1). Even patients with borderline elevation of risk factors such as cholesterol and blood pressure have an increased risk of CHD.[9]

Benefits of treatment

The early statin trials

Five statin trials published between 1994 and 1998 have shown convincingly that treatment with simvastatin,[10] pravastatin[11–13] andlovastatin[14] reduced CHD events, and cardiovascular and all-cause mortality in patients whose baseline risk of fatal or non-fatal myocardial infarction (MI) ranged from below 5% to around 20% over five years. It has since been estimated from these trials that CHD risk is reduced by 15% for each 10% reduction in plasma LDL cholesterol[15] or by 25% for a 1 mmol/l reduction in plasma total cholesterol.[16] Numbers needed to treat to save one fatal or non-fatal MI are shown in Figure 10.2. Follow-up studies have confirmed the benefit for important subgroups within these trials, including women,[17–18] the elderly[17,19,20] and patients with diabetes.[21–23]

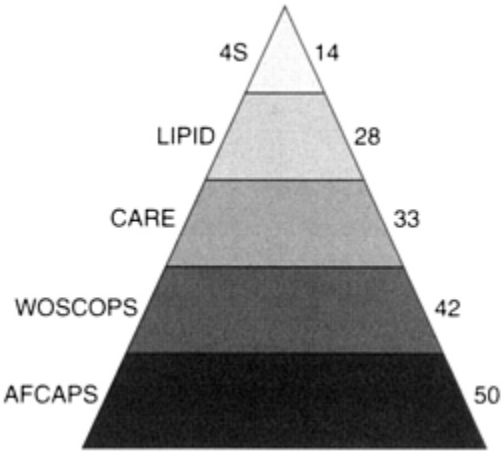

Figure 10.2

Number needed to treat (NNT) to save one fatal or non-fatal myocardial infarction in the early statin trials. NNT is the reciprocal of the absolute risk reduction. The patients who benefited most were those at highest risk initially.

Heart Protection Study

This recently published trial seems likely to rewrite the lipid guidelines once its main findings have been digested.[24] As a result of this study, several grey areas now look considerably less grey. Patients with CHD, other occlusive arterial disease or diabetes who received 40 mg simvastatin daily in addition to their existing therapy showed substantial reductions in both relative and absolute risk, which extended to men and women over 75 years of age and to those with baseline serum cholesterol of 3.5–5 mmol/l. An important finding was that the size of the benefit related more to the pre-treatment risk of an event than to the pre-treatment level of cholesterol. Benefits were shown for every category of participant tested, including women, those with low HDL less than 0.9 mmol/l, high triglyceride greater than 4 mmol/l, renal impairment, smokers and treated hypertensives. Moreover, the benefits were in addition to those recorded for patients already taking other secondary prophylactic drugs. These results clearly indicate that a larger proportion of the population might enjoy the benefits of statins than currently receive them.

Lipid-lowering guidelines

Europe, UK and USA

A condition such as CHD, which is common, often fatal and is to a large extent preventable (or at least delayable), has attracted guideline development groups, who have not failed us in their efforts to convert evidence into recommendations for treatment. The three main guidelines relevant to the treatment of high blood cholesterol include the Second Joint European Task Force Report on the Prevention of Coronary Heart Disease in Clinical Practice,[1] the Joint British Recommendations for Prevention of Coronary Heart Disease in Clinical Practice[2] and the Third Report of the National Cholesterol Education Programme Expert Panel on the Detection, Evaluation and Treatment of High Blood Cholesterol in Adults.[3] There are many other excellent guidelines, for example the Scottish Inter-collegiate Guidelines (SIGN) on Primary[25] and

Secondary Prevention,[26] the UK National Service Framework (NSF) for CHD[27] and the National Assembly for Wales Guidelines on CHD,[28] but in the context of this review it seems sensible to concentrate on the three that best represent current practice in Europe, the United Kingdom and the USA.

Without exception, these guidelines are sending out clear and consistent signals: namely that risk is continuously distributed; that patients with established vascular disease are at high enough risk to justify lipid-lowering drug therapy even if their serum cholesterol is only modestly elevated; that firstline drug therapy should be a statin; and that management of patients with hyperlipidaemia is but one aspect of an approach to cardiovascular disease which must also include strategies to reduce vascular risk imposed by other modifiable risk factors, particularly diet, obesity, smoking, physical inactivity and alcohol. For patients who do not yet have clinical evidence of CHD, each guideline recommends that the decision on drug therapy should be driven by an assessment of CHD risk using a form of global risk assessment based on the Framingham risk equation.

There are of course some differences in the detail (Figures 10.3 and 10.4). While all three guidelines use age, gender, smoking status and systolic blood pressure to calculate CHD risk, the lipid component of the risk score varies: the US guideline requires a fasting lipid profile for LDL, total and HDL cholesterol; the European guideline uses serum total cholesterol only in the risk score, while acknowledging that risk will be underestimated if serum HDL cholesterol is less than 1 mmol/l; the UK guideline needs a total cholesterol: HDL cholesterol ratio in order to calculate risk. The presence or absence of diabetes is handled differently by the three guidelines (see section on special groups, below) and there are differences in the way the three guidelines arrive at a decision on drug therapy. This is driven by CHD risk and a complex sliding scale of LDL cholesterol in the US guideline, but essentially by CHD risk alone in the European and UK guidelines, which state that if CHD risk is high enough to justify drug therapy then this should be given to all patients whose serum total cholesterol exceeds 5 mmol/l.

Thresholds for intervention

The currently recommended threshold for intervention by drug therapy is a ten-year CHD risk exceeding 20% in the US guideline, a ten-year CHD risk that exceeds 20% or (importantly) will exceed 20% if projected to age 60 in the European guideline, and (as a minimum standard of care) a ten-year CHD risk exceeding 30% in the UK guideline. The UK guideline goes on to recommend that individuals with CHD risk greater than 15% over ten years should also receive statins when economic considerations allow. Clearly, the constraints imposed by cost and volume in a health service that has neither the infrastructure nor the resources to deliver moderately expensive drugs to large numbers of people mean that it is not currently possible to offer treatment to all UK patients who might benefit.

Lipid targets

The US guideline also opts for a sliding scale of LDL cholesterol goals, in contrast to the European and UK guidelines which both state that the goal of therapy should be total cholesterol <5 mmol/l (which is equivalent to LDL cholesterol <3 mmol/l) or a 1 mmol/l fall in total cholesterol with treatment, whichever is the greater. The results of the Heart Protection Study suggest that the greatest good can be done for the largest number of patients simply by giving a statin to all those who either have or are at high risk of vascular disease, implying that a lipid target might not matter. Subgroup analysis of this remarkable trial, however, shows that the benefits of treatment were the same in patients whose LDL cholesterol was reduced from around 3 to 2 mmol/l as in those whose LDL decreased from around 4 to 3 mmol/l (Figure 10.5). This

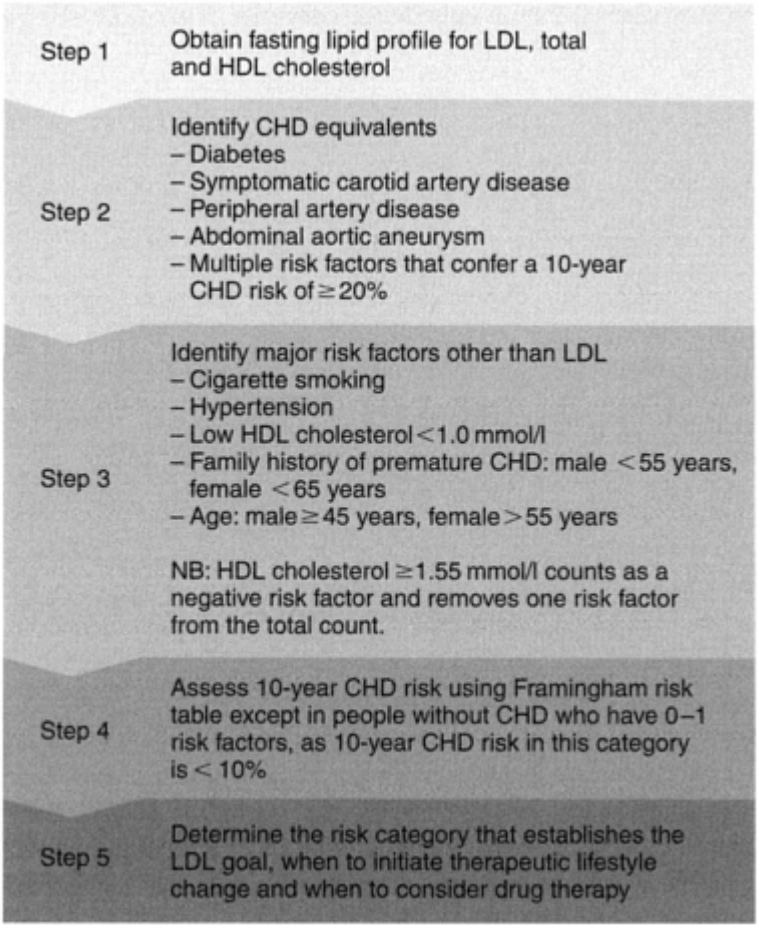

Figure 10.3

Stepped approach to risk assessment and lipid-lowering drug therapy recommended by the National Cholesterol Education Programme (NCEP) Expert Panel on detection, evaluation and treatment of high blood cholesterol in adults (Adult Treatment Panel III). (Reproduced with permission from ref. 3.)

suggests that an even greater good might be achieved by more intensive therapy in individual patients. This possibility is currently being explored in the Treating New Targets (TNT), and Incremental Decrease in Endpoint through Aggressive Llipid-Lowering (IDEAL) trials (referenced by Deanfield).[29]

Special groups

Hyperlipidaemia in the elderly

There remain a number of grey areas in the lipid guidelines, not least of which are those that relate to the treatment of hyperlipidaemia in the elderly. If we accept that cholesterol-lowering drugs should be offered to all those who will benefit, then age per se ought not to be a barrier to therapy. The reality is of course

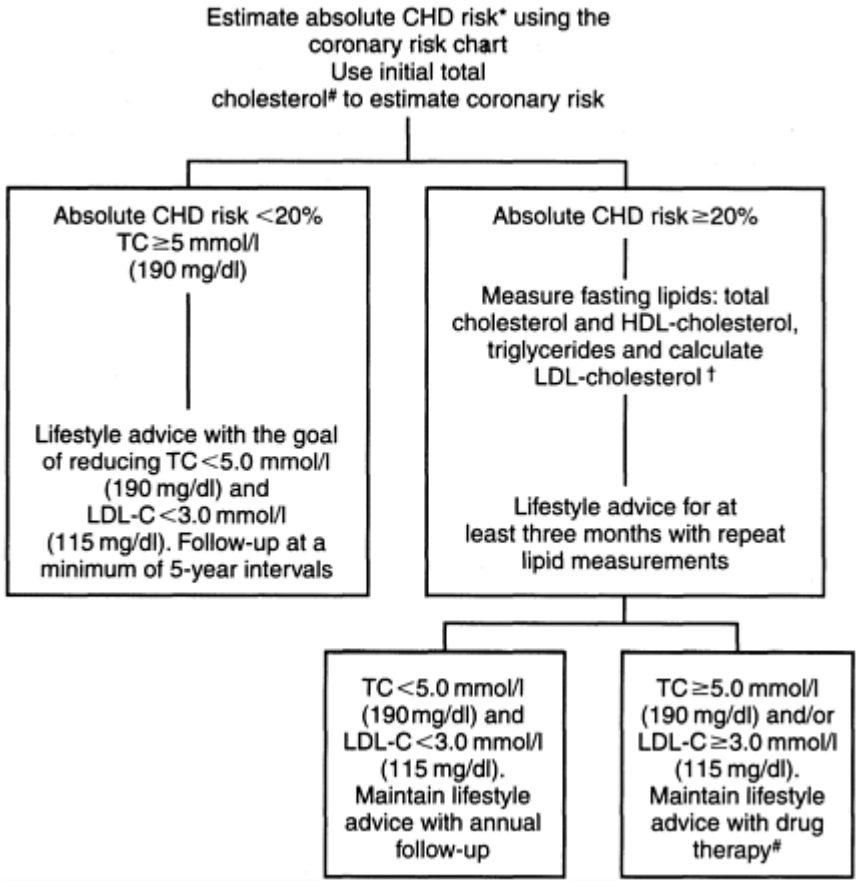

Figure 10.4

The European Societies primary prevention guide to lipid management. *High CHD risk ≥20% over 10 years or will exceed 20% if projected to age 60 years. †HDL cholesterol <1.0 mmol/l (40 mg/dl) and fasting triglycerides >2.0 mmol/l (180 mg/dl) are markers of increased coronary risk. # Consider genetically determined hyperlipidaemias (total cholesterol usually >8.0 mmol/l (above 300 mg/dl) with stigmata of hyperlipidaemia and a family history of premature CHD) and causes of secondary hyperlipidaemia such as obesity, diabetes, alcohol, hypothyroidism, liver and renal disease. If appropriate refer to a specialist.

more complex given the epidemiology of CHD in the elderly, the current lack of trial data in men and women over 75 years, and financial and other considerations imposed by health services that have still to show they can deliver effective preventive health care to the under–75s.

First, the epidemiology. Most of the deaths attributed to CHD in Europe, the UK and USA occur in the elderly. Longitudinal studies show that serum total cholesterol increases in men and women up to 50 years before plateauing and then falling at a rate of about 1% per year.[30] High serum cholesterol and low HDL continue to predict CHD in older persons: relative risk (the disease prevalence in subjects with hyperlipidaemia relative to those without) falls with age but attributable risk (an estimate of how much risk might be reduced if hyperlipidaemia were corrected) increases.[31] Thus, the epidemiology predicts that benefits of cholesterol-lowering therapy may well be greater in the elderly. Is there likely to be an upper age

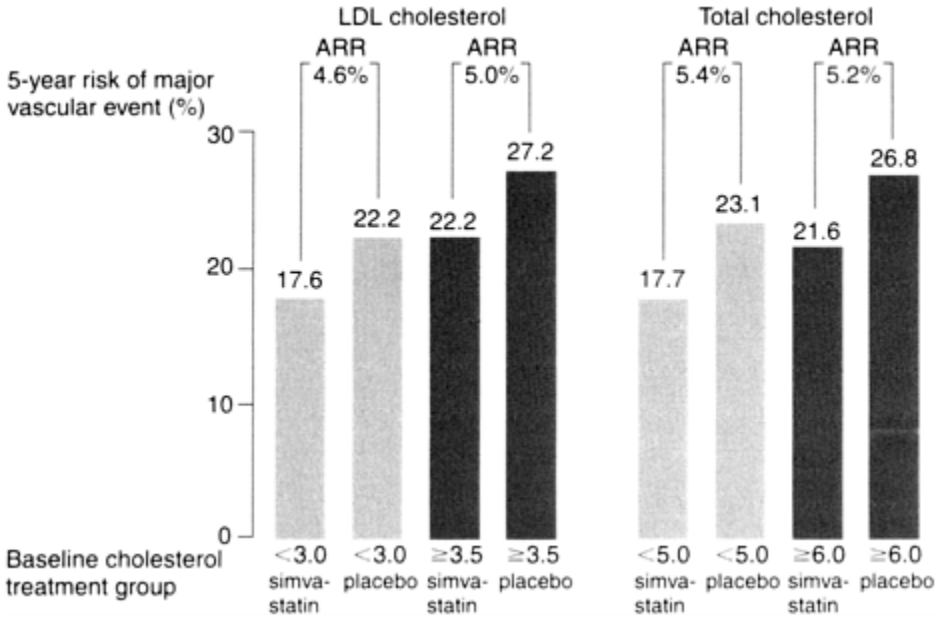

Figure 10.5

Subgroup analysis showing risk of major vascular events in the Heart Protection Study by treatment group and by baseline LDL and serum total cholesterol. (ARR=absolute risk reduction)

limit for this benefit? We do not know for certain but the results of the Leiden study and Honolulu Heart Programme are worth noting: among the very elderly there appears to be an inverse association between cholesterol and mortality with significantly lower death rates among those with high serum cholesterol.[32,33]

Next, the trial results. Of the primary prevention trials, only the Air Force Coronary Atherosclerosis Prevention Study (AFCAPS) recruited anyone over the age of 65.[14] Although the authors reported that the elderly benefited just as much as younger patients, this claim was based on a sub group analysis using median age as the cut-off point. The median ages of men and women in AFCAPS were 57 and 62 years, respectively—hardly very elderly. By contrast, the Scandinavian Simvastatin Survival Study (4S), Cholesterol and Recurrent Events (CARE) trial and Long-Term Prevention with Pravastatin in Ischaemic Disease (LIPID) study did include subjects with a reasonable claim to being old, and all three have published follow-up analyses limited to patients aged over 65 years at trial entry, which show that the benefits of pravastatin and simvastatin on cardiovascular events and mortality in older patients with CHD are equal to or exceed those in younger patients.[17,19,20] The two most recent statin trials, the Heart Protection Study (HPS) and the Prospective Study of Pravastatin in the Elderly at Risk (PROSPER) both included large numbers of elderly subjects. The 1,200 subjects aged 75–80 years in the HPS experienced an impressive reduction in a combined endpoint of fatal and non-fatal CHD and stroke, and of coronary and non-coronary revascularization from 32.3% to 23.1% (Collins R, data presented at European Society of Cardiology, Berlin 2002). PROSPER was a shorter trial of only three years' duration, which also showed benefits for cholesterol-lowering in the 75–80 years age range. There was, however, no reduction in the risk of stroke in this trial.[34]

Those looking to guidelines for inspiration may feel dissatisfied by what they find. The UK guideline recommends treatment of hyperlipidaemia up to 70 years in high-risk primary prevention and up to 75 years in secondary prevention.[2] This advice now appears rather conservative. The UK National Service Framework (NSF) for CHD does not specify an upper age limit for statins in primary or secondary prevention but implies that these drugs should be made available to people at high risk and to those under 75 years with established vascular disease and total cholesterol >5 mmol/l.[27] Predictably, the European and US guidelines impose no age restrictions on the grounds that older persons in the statin trials showed significant risk reductions with drug therapy.[1,3] Most authorities do qualify their recommendations by stating, quite sensibly, that patients with limited life expectancy due to severe debilitating conditions such as malignancy, dementia, disabling stroke or chronic lung disease, should not be regarded as candidates for drug therapy.

Patients at risk of stroke

Recent guidelines also recommend the use of statins for primary prevention of stroke or transient ischaemic attack in patients with CHD.[35,36] These recommendations have been made on the strength of subgroup analyses of the statin trials,[10,37,38] supported by four meta-analyses of the effects of statins on stroke.[39–42] Table10.1 confirms that stroke reduction by statins is possible, but also puts this into context: by comparing stroke reduction by statins with that achieved by antihypertensive therapy,[43–45]

Table 10.1 NNTs for stroke, MI and major vascular events in recent hypertension and statin trials

Table gives the number needed to treat (NTT) for five years to save one event. For trials of duration < or > five years, the five-year event rate was extrapolated from published data, assuming that benefits were cumulative and linear. NNT=the reciprocal of the absolute risk reduction. NNTs for major vascular events are not strictly comparable because the definition of a major vascular event differed in these trials. Major vascular events were: MI, stroke or death from any vascular cause in PROGRESS, HOPE, LIFE; nonfatal MI or CHD death, coronary bypass or angioplasty in CARE; MI, stroke or death from any cardiovascular cause, coronary or non coronary revascularization, including amputation, in HPS; any fatal or non-fatal coronary, cerebrovascular or peripheral vascular event in 4S. There was no analysis by major vascular event in LIPID.

Trial	Active treatment	Control	NNT for stroke	NNT for MI	NNT for major vascular events
PROGRESS	Perindopril/ indapamide	Placebo	21	61	17
LIFE	Losartan	Atenolol	55	No benefit	55
HOPE	Ramipril	Placebo	64	41	26
HPS	Simvastatin	Placebo	68	38	19
4S	Simvastatin	Placebo	81	11	9
CARE	Pravastatin	Placebo	84	32	17
LIPID	Pravastatin	Placebo	143	32	Not available

and also by comparing the effects of lipid-lowering on the risk of stroke and MI. The main conclusions are that antihypertensive therapy is a highly effective way of preventing a second stroke, and that statins are particularly good at preventing a second MI. Statins do not appear to save as many strokes as MIs, but this may be because the risk of stroke in the statin trials was low. It remains to be determined whether statins are

as effective as antihypertensive therapy in the secondary prevention of stroke. This important question will be addressed by the Stroke Prevention by Aggressive Reduction of Cholesterol Levels (SPARCL) study.[46]

Diabetes

Diabetes, particularly type 2 diabetes, is a powerful risk factor for coronary death and non-fatal coronary events in patients with and without established coronary disease.[6,44] Indeed, the possibility that a patient with diabetes but with no clinical evidence of vascular disease may have the same cardiovascular risk as a non-diabetic who has already experienced an MI[44] has led to the view, now widely held, that all patients with type 2 diabetes already have vascular disease and should therefore be given a statin.[3]

What, if any, support does this view gain from the guidelines? The US guideline has given diabetes without clinical evidence of CHD the status of a CHD risk equivalent,[3] the European guideline states that diabetes doubles the risk of CHD in men and more than doubles the risk of CHD in women,[1] while both the European and the UK guidelines recognize the importance of diabetes by giving risk tables for diabetic and non-diabetic and to not qualify for a statin, it is certainly true that most type 2 diabetes patients, separately.[1,2] While it is probably just possible to have diabetes patients will be at sufficiently high risk of a coronary event to justify the use of a lipid-lowering drug, if a CHD risk exceeding 20% at ten years is chosen as the threshold for intervention.

Metabolic syndrome

The latest US guideline[3] (but not the European or UK guidelines yet) recognizes the metabolic syndrome as a secondary target for treatment. The metabolic syndrome is driven by abdominal obesity and physical inactivity, which together promote development of insulin resistance and in turn lead to hypertension, high triglyceride/low HDL cholesterol dyslipidaemia and glucose 'intolerance.[48] The diagnosis of metabolic syndrome is made when three or more of the risk factors listed in Table 10.2 are present.[3] Abdominal obesity is more closely correlated with the metabolic risk factors than is an elevated body mass index (BMI), which is why measurement of waist circumference is recommended to identify the body weight component of the syndrome. Treatment is primarily by

Table 10.2 Features of the metabolic syndrome

Metabolic syndrome is diagnosed when three or more features are present. Some male patients can develop multiple metabolic risk factors when waist circumference is only marginally increased in the range 94–102 cm. Such patients may have a strong genetic contribution to insulin resistance.

Abdominal obesity		
	–men	Waist circumference>102cm
	–women	Waist circumference>88 cm
Elevated triglycerides		≥1.69 mmoll (150 mg%)
Low HDL cholesterol		
	–men	<1.03 mmol/l (40 mg%)
	–women	<1.29 mmol/l (50 mg%)
Raised blood pressure		≥130/≥85 mmHg
Fasting blood glucose		≥6.1 mmol/l (110 mg%)

lifestyle change, namely weight reduction and increased physical activity. Firstline drug therapy for the high triglyceride/low HDL cholesterol dyslipidaemia of metabolic syndrome should be a statin for those whose ten-year CHD risk exceeds 20%, unless serum triglycerides are greater than 5.64 mmol/l (500 mg%), in which case a triglyceride lowering/HDL raising drug such as a fibrate or nicotinic acid is preferred.[3]

Estimation of risk

A new paradigm

The traditional view of cardiovascular disease is that people either have or do not have a condition such as CHD, hypertension, hyperlipidaemia or diabetes, and that it is therefore possible to distinguish primary from secondary prevention strategies. This view is gradually being replaced by a new paradigm, namely that there is a continuum of risk which is determined to a large extent by one's lifetime exposure to environmental risk factors (Figure 10.6). Some high-risk individuals will already have asymptomatic disease, and both patients at high risk and those with asymptomatic disease may carry the same event risk as patients with clinically overt disease. From this it follows that the distinction between primary and secondary prevention is somewhat artificial, and that we should simply be targeting individuals at high risk.

Framingham risk scores

If we accept that most patients at high risk will benefit from lipid-lowering drug therapy, then there remains a need to develop more sophisticated means of identifying such individuals. Patients with existing vascular dis ease and people aged over 75 years do not need risk charts because their CHD risk already exceeds 20% at ten years. The development of risk scoring methods for the remainder of the population, pioneered in New Zealand[49] and used in the European,[1] UK[2] and US[3] guidelines, means that it is no longer necessary to estimate CHD risk simply by counting risk factors. Based on the Framingham risk function these three risk scores represent a major advance in risk assessment (see Figure 10.7 and Figure 10.8 in the Appendix to this chapter, on pp. 184–7). Comparison of the utility of the Joint British Chart with two other risk scoring methods available in the UK suggests that the Joint British Chart may be the most suitable for use by general practitioners and nurses in Britain.[50]

All guidelines on treatment of lipids in primary prevention of coronary disease emphasise that risk will be underestimated in certain groups of patients: those with familial hypercholesterolaemia or a strong family history of premature coronary disease, patients with electrocardiographic left ventricular hypertrophy, patients with type 1 diabetes and type 2 diabetic patients with nephropathy, British Asians and patients who have only recently stopped smoking or started antihypertensive therapy. Moreover, for individuals whose CHD risk is borderline for drug treatment by one of the risk assessment methods described, there is an additional need for better discriminators of CHD risk in order to more precisely target therapies. The measurement of C-reactive protein,[51] carotid intima media thickness,[52] lipoprotein(a),[53] LDL particle size[54] and homocysteine,[55] while not yet incorporated into existing risk scores, may further refine the process of risk assessment in selected cases.

The SCORE project

The recent demonstration that the Framingham risk score may also over-estimate risk in low-risk populations[56,57] suggests it is time for a new approach to risk assessment.[58] The SCORE (Systematic

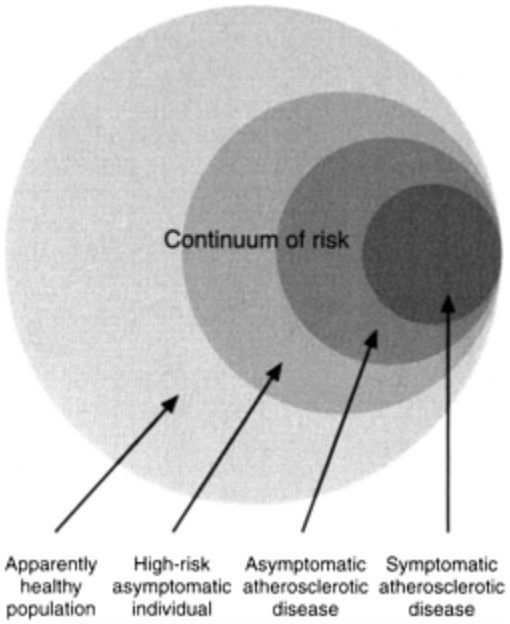

Figure 10.6

The continuum of risk from healthy population to symptomatic atherosclerotic disease.

Coronary Risk Evaluation) project is an analysis of risk and outcome in 200,000 subjects in 12 European cohorts. It will become the recommended form of risk assessment in the Third European Task Force Report, which is due to be published in September 2003. SCORE differs from the Framingham risk function in a number of important respects. Because it is based on a far larger number of endpoints, estimates of risk will be more precise. SCORE will be used to predict total cardiovascular risk rather than just CHD risk, allowing stroke risk to be separated from CHD risk, if required. This may be important in lower-risk areas of Europe, where stroke contributes proportionately more to cardiovascular mortality. The particular strength of the SCORE project is that, given reasonably accurate national mortality statistics and survey data on risk factor distributions, the risk chart can be recalibrated for individual European countries. Furthermore, it is also possible to incorporate the effect of new risk factors such as CRP or homocysteine.[59] When combined with a sophisticated management tool such as the Danish PRECARD system, a fully electronic, flexible, upgradeable and interactive approach to risk factor, management finally looks possible.[60]

Implementation

Treatment gap

Despite well-documented evidence of benefit of statins in patients with CHD, there remains a significant 'treatment gap' between those patients in whom treatment is indicated and those who actually receive it. The two EUROASPIRE studies allow a direct comparison of risk factor status and use of drug therapies during the period 1995–1996 and 1999–2000 in nine countries (Table 10.3).[61] The UK figures are for 1999/

2000 only, as the UK was not represented in EUROASPIRE I.[62] Prescription of secondary prophylactic drugs has increased in Europe, and although the UK NSF targets are currently met for aspirin only, trends for the other classes of drugs are either encouraging (because they show improvement) or discouraging (because they do not yet meet current recommendations),

Table 10.3 Prevalence of risk factors and use of medication in coronary patients in Europe and the UK

Risk factor profile and prophylactic drug status (percentages) were ascertained by interview at least six months after discharge from hospital in men and women under 70 years with diagnoses of myocardial infarction, myocardial ischaemia, CABG or PTCA. Results shown are for 3,659 EUROASPIRE I patients, 3,379 EUROASPIRE II patients and the UK cohort of 362 patients in EUROASPIRE II.

	EUROASPIRE I 1995–1996	**EUROASPIRE II 1999–2000**	**UK Cohort 1999–2000**
BP 140/90 mmHg	55.4	53.9	52.4
Cholesterol≥5 mmol/l	86.2	58.8	53.5
Current smoker	19.4	20.8	17.7
BMI≥30 kg/m^2	25.3	32.8	38.4
Antiplatelet therapies	81.2	83.9	80.9
Betablockers	53.7	66.4	43.8
ACE inhibitors	29.5	42.7	27.4
Lipid-lowering drugs	32.0	62.9	69.0

depending on one's point of view (Table 10.3).

These data do not, unfortunately, tell us how well the UK is doing in relation to the rest of Europe, as we do not know whether the two UK centres in EUROASPIRE II were representative of the UK as a whole.[62] There is much evidence to suggest that they were not. An observational study of statin-prescribing in 288 general practices in England and Wales showed that of patients with a general practitioner diagnosis of ischaemic heart disease, only 13.3% of men and 8.2% of women received a prescription for a statin in 1996.[63] In a more recent UK survey of 24,431 patients with CHD, conducted in primary care in 1997 and 1998, a third of the men and more than half of the women had never had a cholesterol measurement. Of those whose cholesterol had been measured, 75% had total cholesterol >5 mmol/l, but only 16% of patients were receiving statins.[64]

The Americans may prescribe more statins than we do in the UK but even they might have difficulty in meeting the UK NSF target, which states that 80–90% of CHD patients should be taking statins.[27] Lipid-lowering medications were part of the discharge regimen in only 31.7% of 138,001 patients with acute MI discharged from 1,470 US hospitals in 1998 and 1999.[65] In a multivariate analysis, factors independently related to lipid-lowering use included history of hyperlipidaemia, cardiac catheterization during hospitalization, care provided at a teaching hospital, use of a beta-blocker and smoking-cessation counselling. Lipid-lowering medications were given less often to patients who were older (65–74 years), those with a history of hypertension and those undergoing coronary bypass surgery.[65] A difficulty with all of these studies is that the data are historical i.e. two-to-three years out of date, and are, therefore, unlikely to reflect current practice, by the time they are published.

Barriers to implementation

So why have robust evidence and best practice guidelines failed so far to achieve national targets? Resistance to change on the part of the physician or GP is often cited as a reason why valid research-based recommendations and clinical guidance are not routinely adopted in practice.[66] Ten years ago, lack of interest among doctors may have been a barrier to prescribing prophylactic drugs following MI, but this seems less likely now given the high profile accorded to secondary prevention of CHD by the NHS both locally and nationally. The results of EUROASPIRE II suggest that progress is being made in this area, albeit more slowly than most of us would wish.[61] Lack of resources is also usually quoted as a barrier to implementing guidelines. The cost of treating CHD patients with statins in 1999 was estimated to lie between £5,100 and £8,200 per life year gained.[67] The authors argued that this was as cost effective as many other treatments in wide use, for example bypass surgery for men with severe angina and three vessel disease.[67] Statins are certainly more cost effective than recent measures taken to improve safety of rail travel in the UK. If, as a country, we can afford one, then why not the other?

Another important barrier is that a significant minority of the public may not wish or be able to comply. This politically incorrect observation is supported by the results of several recent studies which suggest that patients in the UK may be less willing than their counterparts in the US and Europe to change their lifestyles and take prophylactic medication.[68] Failure to adhere to advice and treatment does not necessarily imply a deliberate wish on the part of the patient to sabotage the efforts of clinicians in reducing coronary risk, but reflects a complex web of choices and influences on patients' behaviour. A wealth of research in the fields of compliance and adherence has identified factors influencing the likelihood that patients will engage in behavioural change: these include knowledge (though it is quite clear that knowledge alone does not change behaviour), health beliefs, readiness to change, perception of benefits, social and cultural factors and the doctor-patient relationship. Successful secondary prevention will need to take all of these into account.

Start statins in hospital

Against this background, an increasing body of evidence supports the view that statins should be started in hospital at the time of the acute coronary syndrome. Four observational studies[69–72] and two randomized trials[73,74] suggest that such a strategy will not only increase compliance[69,70] but will also improve outcome.[69–74] In-hospital initiation of lipid-lowering therapy means that treatment is started when the minds of patients and their carers are concentrated on their cardiovascular risk, which in turn may strengthen the perception that this therapy is essential for the prevention of recurrent events.[75] One of these studies—a quality improvement programme which focused on implementing secondary prophylactic medical therapy with lifestyle advice—increased statin prescription at discharge from 6% to 86% of patients with MI.[69] Treatment rates one year after discharge were 10% and 91%, respectively. These were associated with an increase in patients achieving an LDL cholesterol <2.6 mmol/l (6% versus 58%) and a reduction both in recurrent MI (7.8% versus 3.1%) and total mortality (7.0% versus 3.3%). This achievement was all the more remarkable because the study was conducted as long ago as 1994.

Therapy options

Statins

An increasing body of evidence supports the use of statins as firstline lipid-lowering drug therapy for patients with hyperlipidaemia. The evidence is strongest for patients with isolated elevation of LDL cholesterol but is no longer limited to this subgroup. Thus, we now know that patients with raised LDL, low HDL and high triglycerides benefited in 4S,[76] as did diabetics with low LDL, low HDL and raised triglycerides in CARE and LIPID.[77] The Heart Protection Study has confirmed substantial benefits for statins in patients with low HDL <0.9 mmol/l and in those with raised triglycerides >4 mmol/l.[24] The choice of statin should be based on the outcomes of large prospective randomized trials. The withdrawal of cerivastatin because of death attributed to rhabdomyolysis is a timely reminder that cheaper is not always better, and that although there may be a class effect for efficacy, the same does not apply for safety.[78]

Fibrates

The evidence for fibrates as therapy for hyperlipidaemia rests with three trials: the Helsinki Heart Study,[79] the Veterans Affairs High-Density Lipoprotein Intervention Trial (VA-HIT)[80] and the Bezafibrate Infarction Prevention Study.[81] Although fibrates raise HDL and lower triglycerides to a greater extent than statins, the cardiovascular benefits were less consistently positive in these trials. As such it seems likely that the future role of fibrates will be as firstline therapy for patients with triglycerides >5.6 mmol/l, and as add-on drugs to statin-treated patients whose hyperlipidaemia is inadequately controlled, when additional lipid-lowering is indicated.[3]

Conclusion

The European and US guidelines probably represent the way most UK physicians would treat themselves if they had hyperlipidaemia, whereas current UK guidelines are clearly a compromise between what is desirable, affordable and achievable in the UK. Future guidelines are likely to acknowledge the continuity of risk rather than attempt to draw a line between primary and secondary prevention; and to address total cardiovascular risk rather than CHD risk only, as we come to realize that the benefits of statins extend beyond the coronary circulation to the cerebral and peripheral vessels as well. The move towards ever lower thresholds for intervention has gained ground with the publication of the Heart Protection Study and seems set to continue. The results of a number of trials in progress are awaited with great interest and will further refine recommendations for treatment. Have lipid guidelines come of age? Perhaps not quite yet, but definitely heading in the right direction.

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Appendix to Chapter 10

Figure 10.7 *(cont.)*Figure 10.8 *(cont.)*

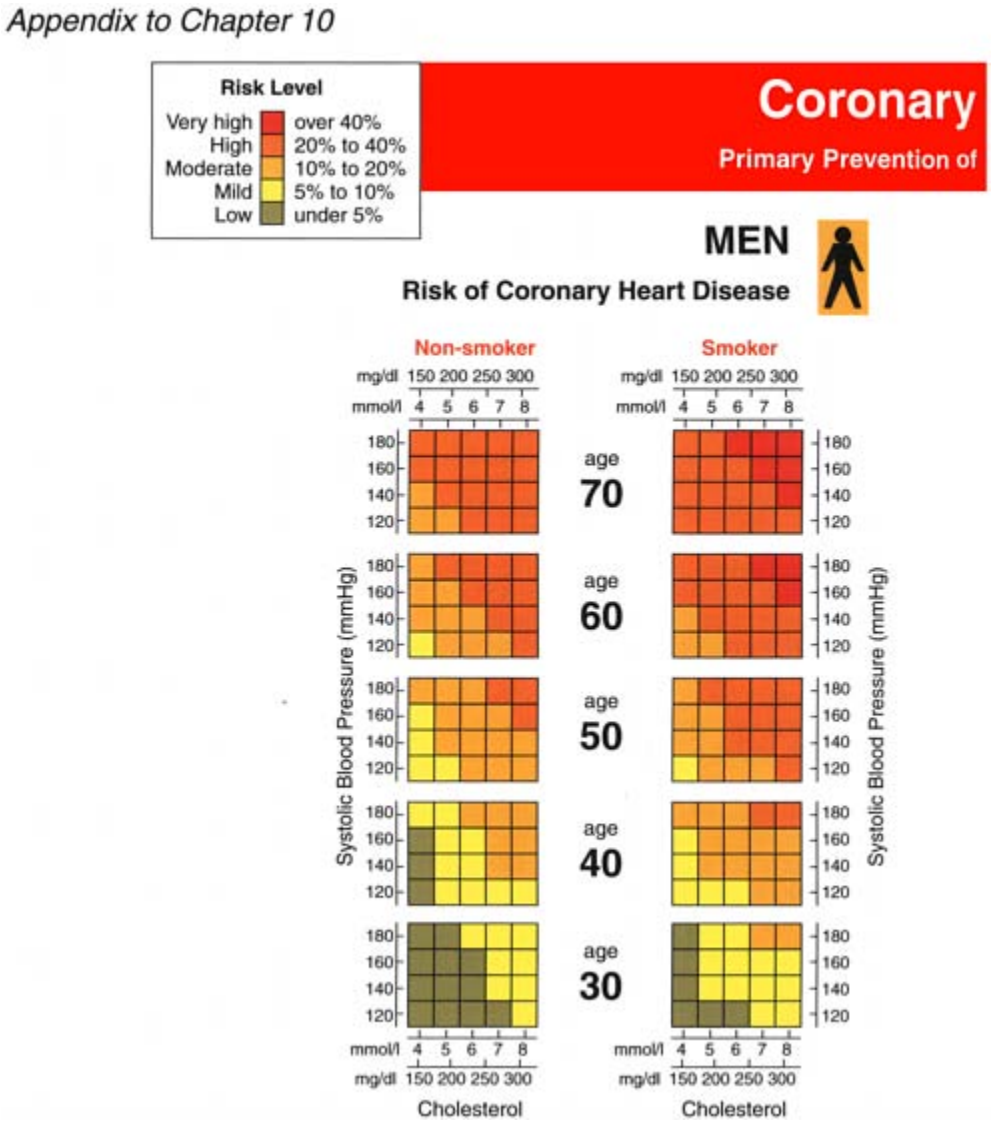
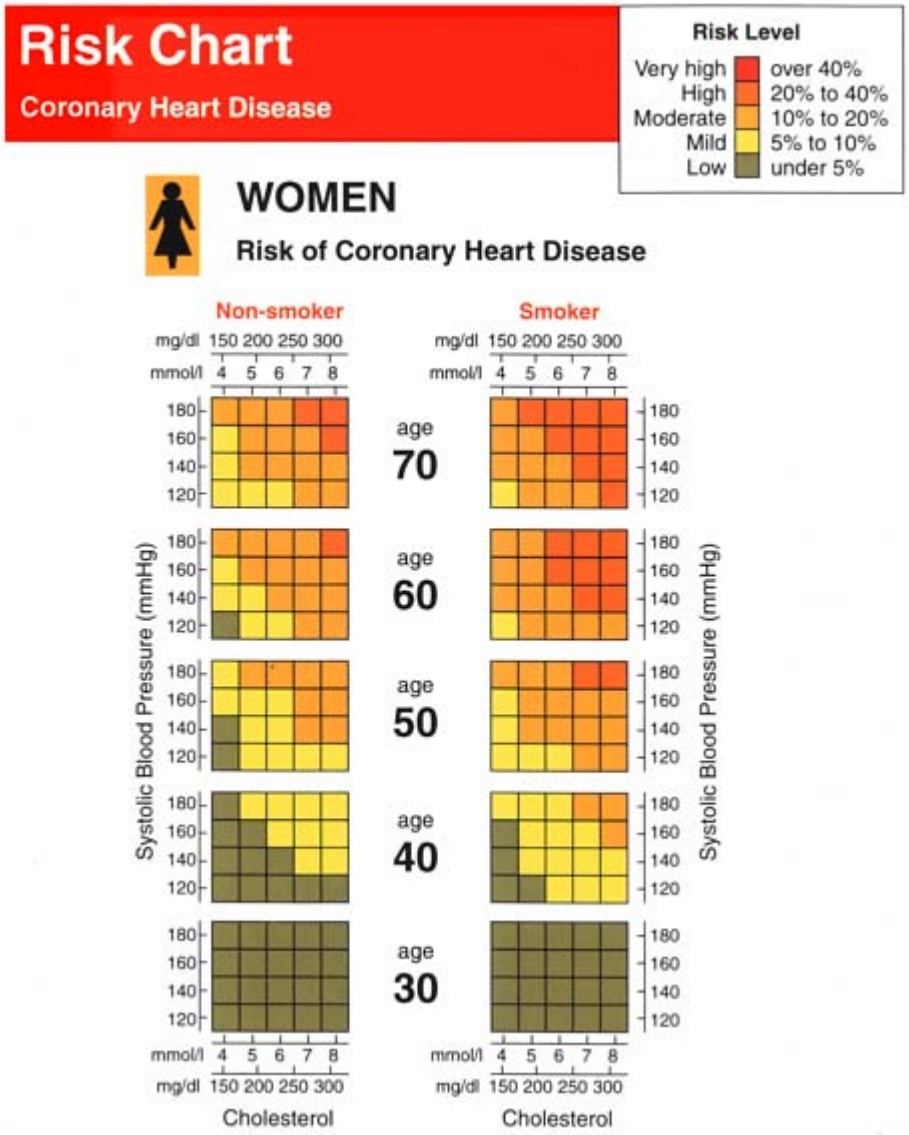


Figure 10.7

The European Societies Coronary Risk Charts. Charts are for men and women who smoke and do not smoke. Separate charts are available for men and women with diabetes (not shown). To estimate a person's CHD risk over the next ten years, find the table for their gender, smoking status and age. Within the table find the cell nearest to their systolic blood pressure and serum total cholesterol.


Risk Chart
Coronary Heart Disease
Risk Level
Very high over 40%
High 20% to 40%
Moderate 10% to 20%
Mild 5% to 10%
Low under 5%
WOMEN
Risk of Coronary Heart Disease
Non-smoker
Smoker
mg/dl 150 200 250 300
mmol/l 4 5 6 7 8
age 70
age 60
age 50
age 40
age 30
180
160
140
120
Systolic Blood Pressure (mmHg)
Cholesterol

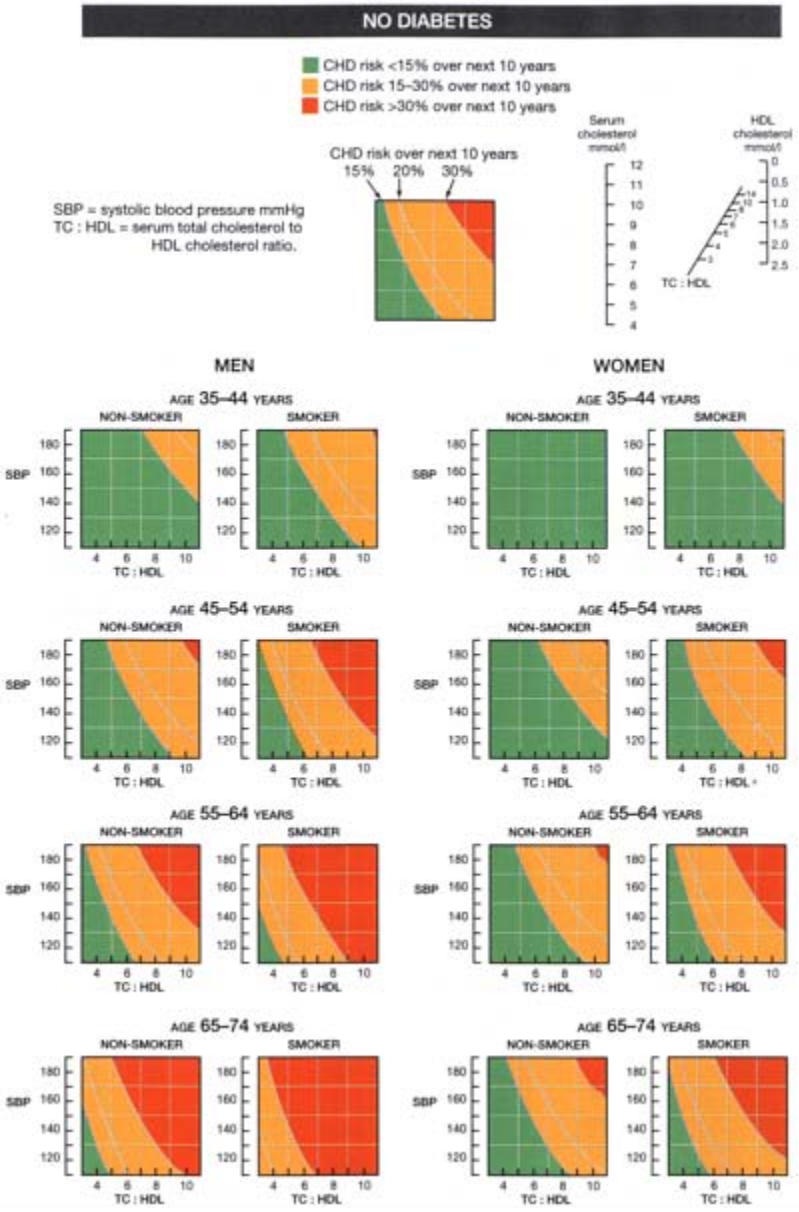
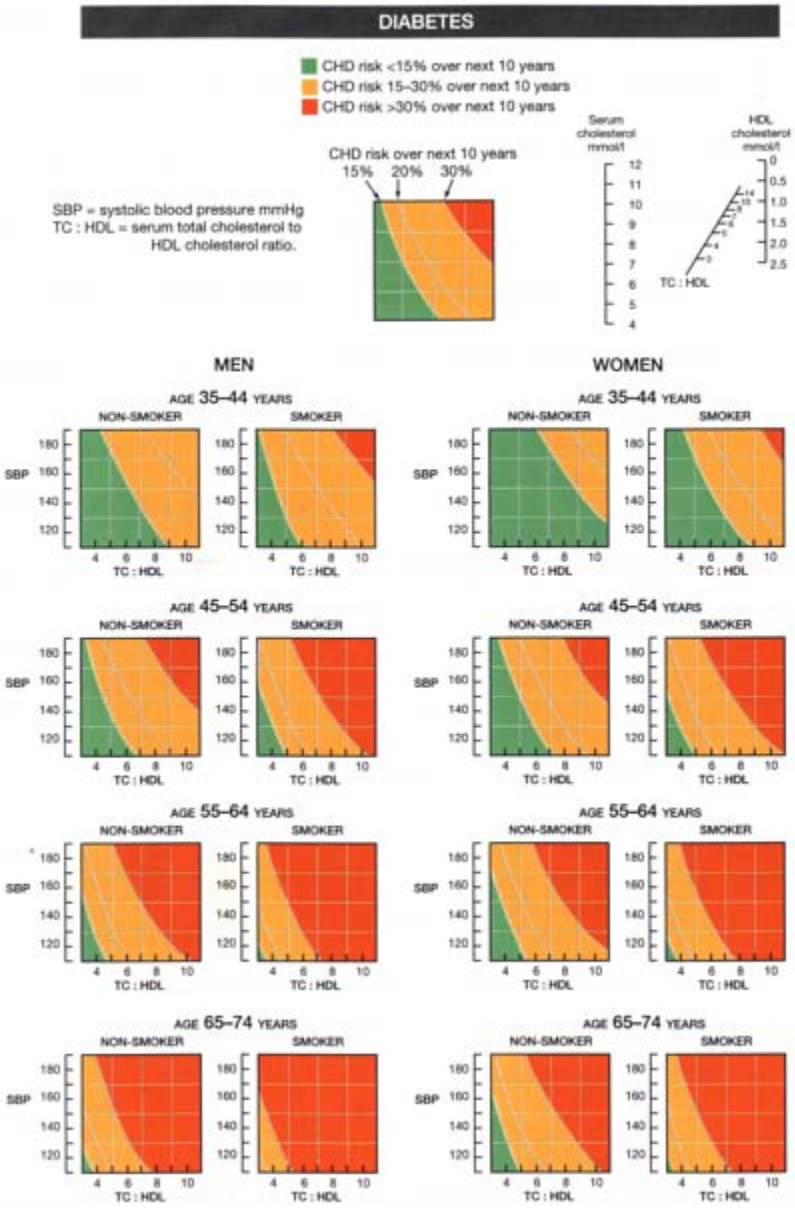


Figure 10.8

Joint British Societies Coronary Risk Prediction Charts. Charts are for men and women with and without diabetes who smoke or do not smoke. To estimate a person's CHD risk over the next 10 years, find the table for their gender, diabetic status, age and smoking habit, then enter their systolic blood pressure and total cholesterol: HDL cholesterol ratio.


DIABETES
CHD risk <15% over next 10 years
CHD risk 15–30% over next 10 years
CHD risk >30% over next 10 years
CHD risk over next 10 years
15% 20% 30%
SBP = systolic blood pressure mmHg
TC : HDL = serum total cholesterol to HDL cholesterol ratio.
Serum cholesterol mmol/l
HDL cholesterol mmol/l
TC : HDL
MEN
WOMEN
AGE 35–44 YEARS
AGE 45–54 YEARS
AGE 55–64 YEARS
AGE 65–74 YEARS
NON-SMOKER
SMOKER
SBP
TC : HDL

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Note: page numbers in *italics* refer to figures and tables