

HDL MORE THAN LDL: AN ACHIEVABLE GOAL

A Clinical Experience

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Studies from the past decade have concluded that the incidence of coronary events can be reduced by treating hyperlipidemia. Specifically, clinical trials have consistently demonstrated that by reducing the low-density lipoprotein cholesterol (LDL-C) to lower levels can reduce cardiovascular morbidity and mortality. However, changes in lipid levels have usually been small and the overall clinical benefits have been limited. Until recently, more large, placebo-controlled, randomized trials with angiographic end points showed marked lowering in the total and LDL cholesterol with complementing increase in high-density lipoprotein cholesterol (HDL-C) that caused actual retardation in the progression of coronary atherosclerosis and some even demonstrating regression. With these studies suggesting that lower is better in terms of LDL cholesterol, physicians and researchers worldwide have increased their doses of statins to achieve this goal. Intensive vs. moderate statin therapy became the subject of interest by many investigators which all proved to be very effective in lowering to a greater extent the level of LDL-C. Consequently, these investigations generated several questions that became a challenge for all medical researchers. How low would be the target for LDL-C to halt the progression of coronary atherosclerosis? Does a very low LDL-C level can actually show regression of coronary plaques? And if we achieve an LDL-C level below the existing guideline, would the battle for coronary disease stops here? How about HDL-C level? Since HDL-C work to remove LDL-C from artery walls, can HDL-C level be raised to a level even higher than LDL-C to completely clean the arteries?

Raising HDL-C level could very well be the next step in the trend of coronary atherosclerosis regression.

Absolute lowering of LDL-C

In 2002, the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) issued a guideline that the goal of risk reduction is an LDL-C level of less than 100mg/dl for persons at high risk of a major cardiovascular event.¹ Two years after, an update suggested that for patients with coronary heart disease who are very high risk, an LDL-C of less than 70mg/dl even with a pretreatment level less than 100mg/dl might be an appropriate target.² O'Keefe et al suggested that the physiologically normal range for LDL-C and also the threshold for development of atherosclerosis and consequent cardiovascular events may be as low as 50 to 70mg/dl. They further extrapolated that the LDL-C level at which the cardiovascular event rate is predicted to approach zero is 57mg/dl for primary prevention and 30mg/dl for secondary prevention.³

These published guidelines triggered several important clinical trials that investigated the effect of intensive lipid-lowering treatment in patients with coronary artery disease. The Pravastatin or Atorvastatin Evaluation and Infection Therapy – Thrombolysis in Myocardial Infarction 22 (PROVE IT-TIMI 22) trial evaluated pravastatin 40mg/d (standard therapy) as opposed

to atorvastatin 80mg/d (intensive therapy), in more than 400 patients who had recently experienced an acute coronary event. The estimated rate of all-cause mortality and major cardiovascular events after 2 years was 26.3% in the standard therapy group while 22.4% in the intensive therapy group. With an LDL-C goal of 60-70mg/dl, there was a 16% relative reduction in the incidence of unstable angina, death, heart attack, and need for revascularization in favor of the intensive regimen ($P=.005$).⁴

The Treating to New Targets (TNT) trial randomly assigned patients with stable coronary artery disease to receive atorvastatin 10mg/d or atorvastatin 80mg/d. The LDL-C levels achieved during the study period were 101mg/dl and 77mg/dl, respectively. A primary cardiovascular event occurred in 8.7% of the intensive therapy while 10.9% of the standard therapy group. The composite end point of coronary heart disease, nonfatal myocardial infarction, resuscitation after cardiac arrest, and stroke was reduced by 22% ($P<.001$) in the atorvastatin 80mg. group.⁵

The Incremental Decrease in End Points Through Aggressive Lipid Lowering (IDEAL) study randomized patients with a history of myocardial infarction and compared the effects of atorvastatin 80mg/d (intensive group) and simvastatin 20mg/d (moderate group) lipid-lowering regimens. The mean on-treatment LDL-C levels were 81mg/dl and 104mg/dl, respectively. The composite end point of coronary death, nonfatal myocardial infarction, or cardiac arrest with resuscitation was reduced by 11% ($P=.07$), and nonfatal myocardial infarction by itself was reduced by 17% ($p=.02$) in the atorvastatin 8mg group, while the total mortality rate was unchanged.⁶

These findings definitely support the conclusion that lower is better for LDL-C specifically when lowered significantly below established target levels. This generation of statin

studies did confer further advantage by lowering LDL-C to levels close to that of an infant. Although there is still no definite target level set on to how low would be the optimum level for LDL-C, there is also no definite evidence that a lower lipid concentration is not associated with lower risk. Thus, the optimal approach to risk reduction may be to implement aggressive therapy designed to provide significant absolute reductions in LDL-C levels rather than to ensure that the patient reaches a specific target. In fact, from a recent meta-analysis by the Cholesterol Treatment Trialists'(CTT) Collaborators of 14 randomized, placebo-controlled trials of statins showed that the proportional reductions in the incidence of major cardiovascular events approximated the absolute reductions in LDL-C, even in patients whose pre-treatment LDL-C level was greater than 100mg/dl.⁷

Another noteworthy study is the Reversal of Atherosclerosis with Aggressive Lipid Lowering (REVERSAL). This study evaluated intensive therapy with atorvastatin 80mg. compared with moderate-intensity therapy with pravastatin 40mg. It showed that intensive therapy with atorvastatin 80 mg/day lowered mean LDL-cholesterol to 79 mg/dL (2.04 mmol/L) and reduced disease progression. On the other hand, moderate therapy with pravastatin 40 mg/day, lowered LDL-cholesterol to only 110 mg/dL (2.85 mmol/L) and failed to retard progression of atheroma volume.⁸ Intravascular ultrasound (IVUS) is a catheter-based technique, which provides real-time high-resolution images allowing precise tomographic assessment of lumen area, plaque size, and composition of a coronary segment, and therefore provides new insights into the diagnosis of and therapy for coronary disease. Although the study did not observe statistically significant plaque regression, the use of IVUS showed promising use as a more accurate tool in measuring arterial plaques and degree of stenosis than possible with angiography.

Achieving Regression

In two of the most important angiographic trials in modern medicine, the works of Dr. David Blankenhorn and Dr. Greg Brown showed, for the first time, that heart disease can be reversed. Dr. Blankenhorn's Cholesterol Lowering Atherosclerosis Study (CLAS) reported in the Journal of the American Medical Association in 1987 is a prospective, placebo-controlled, angiographic trial designed to test the hypothesis that aggressive lowering of LDL cholesterol with concomitant increase in HDL cholesterol will reverse or retard the atherosclerotic process. Specifically, CLAS was designed to determine whether combined therapy with colestipol plus niacin will produce clinically significant change in coronary, carotid, and femoral artery atherosclerosis and coronary bypass graft lesions. To this purpose, 188 nonsmoking men aged 40-59 years with progressive atherosclerosis and previous coronary bypass surgery were randomized to diet plus drug or diet plus placebo. The study has demonstrated beneficial effect by showing plaque regression or reversal in almost 20 percent of these patients and arresting progression or advancement of arterial plaque in another 40 percent.⁹

In 1990, Dr. Brown's study, FATS or Familial Atherosclerosis Treatment Study achieved significant regression in patients treated with lovastatin (Mevacor), the first statin drug. The trial was placebo controlled, and the drugs were a combination of placebo-colestipol (conventional therapy), niacin-colestipol, or lovastatin-colestipol. A total of 146 men aged 62 years or younger with apolipoprotein B levels 125 mg/dl or greater, established coronary artery disease, and family history of vascular disease were assessed for changes in severity of stenosis of the proximal coronary arteries. The study showed that in men with coronary artery disease who were at high risk for cardiovascular events, intensive lipid-lowering therapy reduced the frequency of progression of

coronary lesions, increased the frequency of regression, and reduced the incidence of cardiovascular events. Although not an original end point of the study, clinical events (death, myocardial infarction, revascularization for worsening angina) occurred in 10 (19%) of 52 patients who received conventional therapy compared with 2 (4.2%) of 48 who received niacin-colestipol therapy. Lipid and lipoprotein variables that correlated significantly with a change in severity of proximal stenosis were apolipoprotein B, LDL, HDL, and apolipoprotein A-I.¹⁰

Ten years later, a follow-up of FATS compared patients who were treated with aggressive triple-drug therapy with a statin, niacin, and bile acid sequestrant, with a group receiving usual care. Cardiovascular events and total mortality were substantially reduced in the triple-therapy group compared with the usual-care group (both $p \leq 0.05$). Although small and not randomized, the results suggest a benefit from aggressive combination therapy in patients with low HDL.¹¹

The most recent drug study that has achieved significant regression in most cardiac patients is the ASTEROID or A Study to Evaluate the Effect of Rosuvastatin on Intravascular Ultrasound Derived Coronary Atheroma Burden trial. This was published in the JAMA by Dr. Steven Nissen of the Cleveland Clinic. They utilized serial studies using IVUS which enables assessment of the progression or regression of coronary atherosclerosis by visualizing the occurrence of atheroma in the vessel wall.¹² A previous study using IVUS carried out by Dr. Nissen and colleagues, the Reversal of Atherosclerosis with Aggressive Lipid Lowering (REVERSAL), showed that atorvastatin was able to prevent progression of atherosclerosis as evaluated by intravascular ultrasound. As opposed to retarding or halting progression of disease, however, regression has not been convincingly

demonstrated in this study.⁸ ASTEROID is the first large IVUS study that showed regression in atherosclerosis.¹²

ASTEROID was an open-label, single-arm, blinded endpoint trial designed to study the effect of rosuvastatin 40 mg daily in 507 patients who had undergone coronary angiography and who had evidence of CAD. A total of 349 patients had evaluable IVUS examinations both at baseline and after 2 years of treatment. The plaque volume in the target coronary artery was measured at the initial catheterization and again after 2 years of treatment and IVUS imaging was used to measure the effect on the change in plaque volume compared with the baseline value for the target vessel. The primary efficacy end points were change in percent atheroma volume (PAV) in the entire target vessel, and change in total atheroma volume (TAV) in the most diseased 10-mm segment of the target vessel. The secondary efficacy end points were percent reduction in TAV in the entire target vessel, and percent change from baseline in lipid and lipoprotein levels.¹²

The treatment reduced PAV for the entire vessel by -0.79% ($p < 0.001$ vs. baseline), reduced TAV in the most diseased 10-mm subsegment by -9.1% ($p < 0.001$ vs. baseline), and normalized TAV by -6.8% ($p < 0.001$ vs placebo). For the primary efficacy parameter of change in PAV, 64% of patients showed regression of atheroma volume; for the second primary efficacy parameter, 78% of patients demonstrated regression in the most diseased 10-mm segment of the target vessel. The impact of treatment on atherosclerosis regression was seen across a range of subgroups, including men and women, older and younger patients, and those with LDL-C levels above and below the mean. LDL-C was reduced from 130.4 mg/dL at baseline to 60.8 mg/dL by the end of the study, a mean reduction of 53.2% ($p < 0.001$). Treatment also increased HDL-C from 43.1 mg/dL at baseline to 49.0 mg/dL by the end of the study, a mean increase of 14.7% ($p < 0.001$).¹²

Dr. Nissen claimed that ASTEROID is the first IVUS efficacy trial to show unequivocal evidence of disease regression. Moreover, the reductions in LDL-C levels achieved in this study were so far the lowest values ever observed in a statin/atherosclerosis progression trial, and the magnitude of the elevation in HDL-C levels also surpassed that seen in previous statin trials.^{12,13}

ASTEROID provides us with further scientific evidence to support the concept that lower is likely better for LDL-C levels. Further, by showing that actual plaque regression can be achieved, the enthusiasm for lowering LDL-C to low levels (~60mg/dl) as achieved in this trial is bound to increase.¹⁴

Safety of Intensive Statin Therapy

Statins are first-line therapy for reducing LDL-C levels. They are the most effective and practical class of drugs that can reduce LDL-C levels to established and lower target levels in patients at high risk for atherosclerotic cardiovascular disease (ASCVD).^{1,2} These agents are being used in millions of high-risk people worldwide. Many others receive statins for primary prevention. The total number can only be expected to rise with time. Although favorable results from a large number of controlled clinical trials underpin the benefits of statin therapy, it is not surprising that the safety of statins has received much attention.¹⁵

The safety profile of statins becomes a particularly important issue when aggressive therapy is under consideration. In general, the statins have an excellent safety record.¹ Controlled trials and clinical practice have demonstrated that the frequency of clinically significant side effects are quite low. Safety concerns have focused primarily on the effects on the liver and skeletal muscle.

Six statins currently are available by prescription in the United States. In order of increasing LDL-lowering potency per milligram of drug, they are fluvastatin, lovastatin, pravastatin, simvastatin, atorvastatin, and rosuvastatin. Growing evidence from clinical trials supports the concept that to reduce ASCVD events in high-risk patients, “the lower, the better” applies to LDL levels.^{2,5} To achieve very low LDL levels, higher doses of statins often are required. Pharmaceutical companies naturally promote their own statins as being superior to others. One way to do this is to be able to claim greater LDL-lowering potency, both per milligram and absolutely. Attempts to achieve greater LDL lowering with increasing doses of statin, though a goal both in the highly competitive marketplace of the “statin wars” and in the clinic, ultimately will be limited by toxicity. Historically, this has taken the form of myotoxicity.¹⁵

The connection between statin therapy and adverse reactions of the skeletal muscle is well established. Since the withdrawal of cerivastatin from the United States in 2001 due to an excessive rate of rhabdomyolysis compared with other statins, increasing scrutiny has been placed upon the safety of statins and other lipid lowering agents.¹⁶ Between 2% and 7% of patients report myalgia, defined as proximal or diffuse muscle pain, tenderness, and/or weakness.^{17,18} Myopathy—muscle pain, tenderness, and weakness accompanied by elevations of creatine kinase (CK) of more than 10 times the upper limit of normal (ULN)—is much more rare, affecting only 0.01% to 0.5% of patients treated with statin monotherapy.^{17,18} Myopathy has been reported with all currently marketed statins, particularly at higher doses. The risk of adverse events such as myopathy and rhabdomyolysis increases when statins are used together with agents that share a common metabolic pathway.^{18,19} Atorvastatin, simvastatin, and lovastatin are metabolized primarily by the cytochrome P-450 (CYP) 3A4 pathway. The 3A4 isoenzyme is also the major

pathway for the metabolism of many other drugs and has been implicated in the development of statin-drug interactions.^{18,20} Clinically important drug interactions have been reported when the statins that are metabolized through the CYP 3A4 pathway are combined with fibrates (particularly gemfibrozil), cyclosporine, macrolide antibiotics, digoxin, azole antifungals, protease inhibitors, and warfarin.^{18,19,21} In a patient population that requires polypharmacy, the potential for drug interactions with these agents suggests the need for caution because elevated serum statin concentrations are associated with increased myotoxicity.^{18,19} Rosuvastatin has a lower potential for CYP drug interactions since it is metabolized to a minimal extent via the CYP 2C9 and 2C19 iso-enzymes, rather than CYP 3A4,20 resulting in a somewhat different drug-interaction profile. Coadministration with azole antifungals, erythromycin, fenofibrate, or digoxin does not significantly alter the pharmacokinetic profile of rosuvastatin, although coadministration with cyclosporine increases plasma concentrations of rosuvastatin, and when both agents are indicated, rosuvastatin should be administered at the lowest dose.^{18,22}

Side effects of statins tend to be dose related. Most side effects, including myopathy, disappear on withdrawal of the medication. Even with severe myopathy, most patients survive with supportive measures, although fatalities occasionally occur.¹⁵ In rare cases, myopathy may lead to rhabdomyolysis, characterized by profound CK elevations, muscle necrosis, and renal failure secondary to myoglobinuria.^{17,18} Rhabdomyolysis is potentially fatal (approximately 0.15 fatal case per 1 million statin prescriptions).^{18,23} An estimated 50% of all cases of statin-related rhabdomyolysis may be attributable to a drug-drug interaction.^{18,19} Concomitant fibrate therapy, which in itself is associated with some risk of myopathy, appears to raise the risk.^{16,18} The risk appears significantly greater when gemfibrozil, as opposed to fenofibrate, is combined with a statin.

Progression from myopathy to rhabdomyolysis can almost always be reversed by early diagnosis and treatment of symptomatic elevations of CK levels with adequate hydration and cessation of suspect drugs.^{18,19}

In 2002, the American Heart Association (AHA), American College of Cardiology (ACC), and National Heart, Lung, and Blood Institute (NHLBI) issued an advisory on the safety of statins and their use in clinical practice. Most important was the identification of certain types of patients who are at higher risk for severe myopathy. The following conditions were listed as carrying higher myopathy risk that require either avoidance of statins or their use in lower doses: advanced age (especially >80 years) (women more than men), small body frame and frailty, multisystem disease (eg, chronic renal failure, especially if caused by diabetes), perioperative periods, multiple medications (especially gemfibrozil, cyclosporine, azole antifungals, itraconazole and ketoconazole, macrolide antibiotics, erythromycin and clarithromycin, HIV protease inhibitors, the antidepressant nefazodone, and verapamil), consumption of large quantities of grapefruit juice (usually >1 quart per day), and alcohol abuse (which independently predisposes to myopathy). A number of the cited interacting drugs are, in fact, problems when taken with certain statins, and the products are now thoroughly labeled in this regard. Other drugs may be added to this list, so physicians should consult the package inserts of the drugs they prescribe. If these guidelines⁷ are followed, the risk for severe myopathy accompanying statin therapy should be substantially reduced.^{15,16}

Elevated hepatic transaminase levels have been reported in 0.5% to 2.0% of statin-treated patients and are dose dependent.^{1,16,18} However, there is little or no evidence that statins cause progressive liver disease.³ Elevated transaminase levels have not been linked with hepatotoxicity^{18,24,25} and are reversible with dose

reduction or discontinuation of therapy.^{16,18} Progression to liver failure due to statin therapy is exceedingly rare, if it ever occurs.^{1,16,18} No evidence exists that statins aggravate existing hepatic disease with long-term transaminase elevations due to hepatitis B or C.¹⁶ Nevertheless, persistent elevations in transaminases can be perplexing and cholestasis and active liver disease are still contraindications to statin therapy.¹

In summary, statin therapy effectively lowers LDL and has an overall excellent safety profile in clinical trials. However, the use of high-dose statin therapy also entails greater risk of adverse events, such as myopathy and liver function test abnormalities, and this must be carefully weighed against the potential benefit for each patient. Alternative approaches must be rediscovered and utilized such as targeting high-density lipoproteins and triglycerides which may offer yet another option for coronary heart disease prevention in high-risk patients.²⁶

Pleiotropic Effects of Statins

The advent of statin therapy has revolutionized the ability of the clinician to manage patients at risk for the development of an ischemic event due to dyslipidemia. Large-scale clinical trials involving thousands of patients in both primary and secondary prevention have clearly demonstrated that statin therapy will reduce cardiovascular mortality across a broad spectrum of patient subgroups. Additionally, in adequately powered trials, total mortality has been successfully decreased by the use of statin therapy.²⁷ However, the overall benefits observed with statins appear to be greater than what might be expected from changes in lipid levels alone, suggesting effects beyond cholesterol lowering. Indeed, recent studies indicate that some of the cholesterol-independent or "pleiotropic" effects of statins involve improving endothelial function, enhancing the stability of atherosclerotic plaques, decreasing

oxidative stress and inflammation, and inhibiting the thrombogenic response.²⁸

The causal theory of pharmacologic benefit reiterates the lipid hypothesis, which states that dyslipidemia is central to the process of atherosclerosis and the clinical benefit which accrues from statin therapy is a function of the degree of lipid lowering. The noncausal theory supports the premise that clinical benefits are related primarily to pleiotropic effects of statins (endothelial function, inflammation, coagulation and plaque vulnerability) as being the major modulators of clinical benefit.²⁷ Many of these pleiotropic effects are mediated by inhibition of isoprenoids, which serve as lipid attachments for intracellular signaling molecules. In particular, inhibition of small GTP-binding proteins, Rho, Ras, and Rac, whose proper membrane localization and function are dependent on isoprenylation, may play an important role in mediating the pleiotropic effects of statins.²⁸

In the recent REVERSAL trial, high-dose atorvastatin appeared to halt disease progression compared to more moderate lipid lowering.^{8,29} Moreover, ASTEROID, presented at ACC '06, suggests that high-dose rosuvastatin actually produces plaque regression. The plaque stabilization effects of high-dose statin therapy are consistent with the biological effects of statins on inflammation, endothelial function, and coagulation.^{29,30}

In late 2005, an analysis of data from PROVE IT-TIMI 22 showed that intensive therapy using atorvastatin 80 mg was associated with a significant reduction in the composite clinical endpoint of death, myocardial infarction, or rehospitalization for recurrent acute coronary syndrome as early as 30 days after the acute event compared with standard-dose pravastatin (40 mg).^{29,31} A trend in favor of intensive therapy was evident at 15 days after randomization. Similar results were reported at about the same time for

patients in the ASCOT-LLA study, where there was a trend for benefit from atorvastatin as early as 30 days following randomization, with significant benefits observed at 90 days, 180 days, one year, two years, and then maintained through the termination of the trial (3.3 years).^{29,32} These very early benefits of intensive statin therapy are in sharp contrast with the Program on the Surgical Control of Hyperlipidemias (POSCH) trial, in which ileal bypass was associated with a dramatic early reduction in low-density lipoprotein (LDL) cholesterol but no early reduction in clinical events.^{29,33}

The potential mechanism that might explain these early effects of statin therapy is that statins inhibit HMG-CoA reductase, which is responsible for the reduction in circulating LDL cholesterol beginning one to two weeks after therapy initiation. Statins also inhibit HMG-CoA reductase within endothelial cells, vascular smooth muscle cells, and inflammatory cells (the monocyte/macrophage system), which affects important signaling pathways. In cell culture, animal, and clinical studies, these effects appear within hours after statin administration and may be dose dependent.^{29,30,34}

The vascular inflammatory response is a complex process that leads to thrombus formation, angiogenesis, neointimal thickening, and atherosclerosis (12). Markers of inflammation such as C-reactive protein, IL-6, TNF- α , and monocyte-chemotactic protein-1 (MCP-1) have, in varying degrees, been proposed as CVD risk factors.^{35,36} Recent evidence indicates that statins decrease C-reactive protein levels in just 6 wk of treatment, independent of LDL cholesterol reduction, and suggests that statins possess anti-inflammatory actions. The PROVE IT-TIMI 22 authors cited median C-reactive protein (CRP) level at 30-days (1.6 mg/l in the atorvastatin group compared with 2.3 mg/l in the standard therapy arm; $p < 0.001$) as supporting greater early anti-inflammatory pleiotropic effects with intensive statin

therapy.^{29,39,40} Another marker of inflammation was evaluated in the MIRACL trial, which evaluated intensive atorvastatin therapy given early after ACS. Patients in the placebo group with initial levels of soluble CD40 ligand (sCD40-L) above the 90th percentile had a higher risk of recurrent events compared with patients with sCD40-L concentrations at or below the 90th percentile. However, the increased risk associated with high initial sCD40-L was completely abrogated by treatment with atorvastatin.²⁹

Endothelial dysfunction has relevance to the pathogenesis, progression and prognosis of a wide spectrum of cardiovascular diseases. It is characterized by reduced bioavailability of nitric oxide (NO), LDL-oxidation in the vascular wall and the vascular inflammatory response. All these pathological processes fundamental to the development and progression of endothelial dysfunction, are modulated by increased vascular oxidative stress in dyslipidemia.⁴¹ Statins have been shown to improve endothelial dysfunction by increasing nitric oxide bioavailability as well as by reducing LDL oxidation and vascular inflammatory response.⁴² These pleiotropic effects of statins may be relevant based on reduced cellular concentrations of important and biologically active intermediates that influence endothelial phenotype. Statins directly enhance expression, phosphorylation state, and activity of the endothelial isoform of nitric oxide (NO) synthase, and CRP reduces NO synthase expression, suggesting a mechanism by which statins may specifically protect against the adverse effects of inflammation on the vasculature.²⁹

There have been inconsistencies in the evidence that it is all about the lipids. We now know from the literature that there are other things that statins do. For example, trials have found that statins substantially reduce the risk for stroke, which is more consistent with their hypothesized antithrombotic effects than with their LDL-lowering effects (high LDL levels are not a major

independent risk factor for stroke). We're also starting to think of statins as something that might prevent colon cancer, and that's not related to cholesterol either. In addition, a recent large statin trial conducted in patients receiving dialysis found no substantial benefit despite reductions of 42% in LDL cholesterol levels, suggesting that even dramatic reductions are not always associated with clinically significant lowering of cardiovascular risk.⁴³

For clinicians and patients, this issue is much more than an academic debate. Compared with empirically treating patients at high cardiovascular risk with statin doses similar to those used in clinical trials, titrating lipid therapy to recommended LDL cholesterol goals entails considerably greater clinical complexity by using intensive doses of statins.⁴³ As mentioned earlier, the use of high-dose statin therapy entails greater risk of adverse events such as myopathy and liver function test abnormalities which must be carefully weighed against the potential benefit for each patient.²⁶ In clinical practice, fewer than half of those receiving high doses of statins have achieved LDL cholesterol levels less than 1.81mmol/L (<70 mg/dL) and one of the reasons for the inadequate lipid management include adverse events.¹⁸ To help patients reach the recommended goals, clinicians use combination therapy. In many combination-drug-therapy regimens, statin doses might be lowered with an additional drug. If the cardiovascular benefit is not with the achieved LDL-C target or if the benefit rests with some of the other pleiotropic effects of statins, clinicians might be underdosing patients at risk for cardiovascular disease events.⁴³

Studies have consistently shown that HDL-C not only bring about beneficial effects by reverse cholesterol transport - moving cholesterol from artery wall back to the liver for excretion, but also it has anti-inflammatory, anti-oxidant, anti-thrombotic, and nitric-oxide inducing properties. Sounds familiar? These are the very same

pleiotropic effects of statin therapy. If we can reduce our dose of statins through combination therapy and consequently elevate the level of HDL-C, it is as if we are treating patients with high intensity statins with all its benefit but without the possible adverse effects. Also, clinicians will not worry about underdosing patients with statins.

Raising HDL-C: The new target

Some patients with concomitant medical conditions or potential drug interactions are not suitable candidates for high-dose statin therapy because of the excessive risk of side effects.²⁶ If we can lower the doses of statins that we use in clinical settings and still produce a significant drop in LDL-C by incorporating other complementary drugs, sensible diet and exercise, it would be phenomenal since we are getting rid of the possible adverse effects of high-dose statins. Although studies have shown that only a few number of patients in aggressive statin trials experienced untoward effects of the drugs, it is still not absolutely risk-free. Additionally, those people who experienced these effects may very well be the ones who are high risk for a cardiac event and would require most of a lower LDL-C. Even though there is clear evidence that more aggressive LDL lowering with statins provides greater risk reduction in most high-risk patients, approaches targeting HDL-C should provide a therapeutic alternative to high-dose statin monotherapy.²⁶

It has been fifty years since the initial report that patients presenting with a myocardial infarction had lower levels of the lipoprotein fraction that demonstrated α -migrating mobility on gel electrophoresis, which was later identified as HDL-C.⁴⁴ A number of large population studies have reported that the incidence of clinical coronary heart disease was inversely correlated with plasma levels of HDL-C.^{45,46} For every 1 mg/dL increase in plasma HDL-C, the incidence of

clinical events declines by 2-3%. In fact, in the Framingham Heart Study, plasma HDL-C was found to be the strongest biochemical predictor of clinical events⁴⁶. Also, in clinical trials that test the impact of lipid lowering therapies, HDL-C proves to be an important predictor of event rates.⁴⁷ When the HDL-C level is raised by drug therapy, coronary atherosclerosis is decreased and coronary heart disease events are lessened. Increases in HDL-C are in fact independently correlated with coronary angiographic and clinical benefit. HDL-C is thought to bring about its beneficial effects by stimulating the removal of cholesterol from cells in the vascular wall. The cholesterol is taken up by HDL-C and shuttled in part to the liver for excretion in the bile.⁴⁸

Although multiple lines of evidence have consistently shown that HDL-C protect against coronary heart disease, raising HDL-C levels as an approach to reducing heart disease risk has not really been fully explored.⁴⁹ In 2003, a study that appeared in the Journal of the American Medical Association, shook the medical community as it may cause a “paradigm-shift” in the treatment of coronary heart disease. The ApoA-I Milano trial, led by Dr. Steven Nissen, involved 57 acute coronary syndrome patients in a randomized, prospective, double-blind, multicenter clinical trial conducted from November 2001 to March 2003. All patients had experienced an acute coronary syndrome, either unstable angina (severe chest pain) or heart attack. Each patient had a single coronary artery examined using IVUS within two weeks following the cardiac event. The procedure is performed along with cardiac catheterization. Patients were randomized to three treatment groups — placebo, low dose or high dose of intravenous recombinant ApoA-I Milano phospholipid complex. This is an unusual variant of APOA-1, the main constituent of HDL-C.⁵⁰ The Milano variant was discovered by Dr. Cesare Sirtori and Dr. Guido Francheschini (University of Milan, Italy) in a group of related Italians who all had unusually low HDL levels but no sign of

cardiovascular disease.⁵¹

The study drug was administered as a weekly intravenous infusion for a total of five weeks. Patients underwent IVUS of the originally imaged coronary artery for a second time within two weeks following the final infusion. The second images then were compared with baseline images to compare plaque levels. Researchers found a statistically significant change in volume of the thickened, fatty lining inside the arteries of patients who had received either the high or the low dose of intravenous recombinant ApoA-I Milano/phospholipid complex. With just five weekly infusions, it has produced a modest but significant 4% regression of coronary atherosclerosis in 47 patients.⁵⁰ This was the first compelling evidence that regression in atherosclerosis can be done and that HDL-C has the lead role in achieving this goal.

This study is particularly important in that it suggests that raising HDL may be the key to atherosclerosis regression, which has remained elusive with interventions that lower LDL. Few months after the ApoA-1 Milano Trial, another IVUS study was conducted by Dr. Nissen. The REVERSAL study showed that the highest doses of statins that reduced LDL the most halted the progression of atherosclerosis but did not induce regression.⁸ Nissen said that we should not be surprised and that in interpreting these results we should considering the roles of both LDL-C and HDL-C. LDL-C is the culprit behind the accumulation of atheroma, but it does not have any role in removing cholesterol, which is the job of HDL-C. We have to target both LDL and HDL. He explained further that we are not going to abandon LDL and the statins, but we need to add in HDL-raising therapy. With LDL lowering we have been stuck with a 35% reduction in event rates. That is wonderful if you are one of those 35%, but it's not so great if you are one of the other 65%. They need something else. something else is probably HDL-raising treatment.⁴⁹

Two years after the REVERSAL trial, Dr. Nissen and his fellow investigators published another phenomenal study, the ASTEROID trial. This is the most recent and the first largest IVUS study that have shown atherosclerosis regression. The study showed that the use of high-intensity statin therapy, with the intention of achieving very low levels of LDL cholesterol, can regress coronary atherosclerosis.¹² They claimed that the 53.2% reduction in LDL-C level achieved in this study were the lowest ever achieved in a statin trial. But more importantly, the accompanying 15% increase in HDL-C holds promise that HDL-modifying therapy in concert with LDL-C lowering therapy may achieve even greater amounts of plaque regression.¹⁴ Dr. John Kastelein of the Academic Medical Center in Amsterdam speculated that if we can get a 50% to 60% reduction in LDL and a 50% to 60% increase in HDL, we are no longer talking about the 20% to 30% reduction in heart disease seen with the statins, but a 70% reduction. There is really a potential to reduce coronary events by 80% to 90% by combining potent LDL-lowering and HDL-raising therapies.⁴⁹

HDL-C more than LDL-C: Our Experience

Fifty-six patients presented themselves for cardiac consultation during 2005 and 2006. After measurement of lipid profile and consultation, combination drug regimens including low-dose statins, niacin, bile acid sequestrants and fibrates together with lifestyle modification through diet and exercise were prescribed. In a follow-up visit the measurements were repeated.

Of the 56 patients, 26 (46.4%) were male and 30 (53.6%) were female. By race they were: African American (12.5%), Asian (17.9%), Caucasian (64.3%), and Hispanic (5.4%), roughly paralleling the make-up of the racial distribution of the practice. Also, 46.4% indicated they exercise and 17.9% were smokers.

The following table reports the average for the measurement indicated along with the number of patients on which it was taken and the

probability that the difference could have occurred by chance.

Table 1 – Average of Measurement at Initial and Follow-up Visit as Well as Statistical Test for Difference

Average of Measure:	Initial Visit	Follow-Up Visit	Number of Patients	Significance
Total Cholesterol	210.21	131.09	56	.000
LDL	123.45	46.23	56	.000
HDL	58.79	67.89	56	.003
Triglycerides	139.71	84.88	56	.000
Percent LDL	58.02%	34.79%	56	.000
Percent HDL	28.08%	52.04%	56	.000
Percent Triglycerides	13.20%	13.17%	56	.983
LDL/HDL	2.20	.69	56	.000
Total Cholesterol/HDL	3.79	1.96	56	.000
I/HDL	.018	.016	56	.003
LDL IIIab	19.57	22.48	52	.033
LDL IVb	3.72	5.01	52	.032
HDL IIb	20.69	26.78	54	.000
APO B	100.11	54.09	47	.000
APO A1	169.37	165.95	41	.680
APO B/APO A1	.63	.33	40	.000
Homocysteine	10.54	12.31	47	.089
Lipo PLA2	164.13	130.87	31	.008

Clearly, Total Cholesterol was lowered from 210.21 to 131.09 and this difference was statistically significant (p=.000). Similarly, and more importantly, LDL was lowered from 123.45 to 46.23 while HDL was increased from 58.79 to 67.89. The amount of Triglycerides was lowered from 139.71 to 84.88 but when total cholesterol is divided into the percent in each of the three categories a revealing picture emerges. Although the percent of total cholesterol that is LDL was reduced from 58.02% to 34.79% and the percent that is HDL was increased from 28.08% to 52.04% - both statistically significant changes at the (p=.000) levels – the percent triglycerides did not change either statistically or even noticeably.

Of all the remaining measures, every one except APO A1 and Homocysteine changed significantly at the .05 level, most were clearly below that and Homocysteine approached significance at the .10 level (p=.089). Note that APO A1 dropped but it was not statistically significant; also note, however, that because the drop in APO B was significant, the APO B to APO A1 ratio did drop significantly (p=.000).

Finally note that the results are derived from a fairly small sample of 56 individuals and the strong statistical significance achieved came from the powerful changes and not simply from the power of the test.

The New Challenge

When ASTEROID was published, the medical community was astonished by its impact when it claimed that heart disease can now be readily regressed by a high-dose statin as shown by IVUS imaging on atherosclerosis-laden arteries. Many speculated that this could lead to a ‘paradigm shift’ that could very well change the practice of cardiology in its treatment of coronary artery disease. Clearly, the search for new guidelines have been geared towards regression of atherosclerosis and reversal of heart disease.

Since ASTEROID Trial is being considered the landmark of heart disease reversal studies, we can’t help but compare our data to this study. Clearly, our numbers are far better than ASTEROID in both decreasing LDL-C and increasing HDL-C. Also, we have increased HDL-C levels more than LDL-C levels, something that has never been achieved before. Furthermore, we’ve done it through combination therapy with low-dose statin and lifestyle modification through diet and exercise.

Behind every great study are limitations. And these limitations poses new challenges that motivates thinkers to search for new knowledge that can substantiate and complement the existing idea. One of the limitation of these studies that need to be addressed is the need for randomization. The standard of cardiology research is a prospective randomized, placebo-controlled clinical trial. Since both studies were not controlled by a placebo or by a comparator, the magnitude of the benefit that we’re seeing is not interpretable.

By showing that actual plaque regression can be achieved with low levels of LDL-C (~60mg/dl) and modest increase in HDL-C (15%), surely a very low LDL-C (>50mg/dl) and a greater magnitude of increase in HDL-C which surpasses

the level of LDL-C can duplicate this regression in arterial plaques. However, we must remember that these studies were not event-driven. There were no clinically meaningful endpoints. Hence, it is unlikely that these results will change clinical practice or guidelines significantly other than to endorse strongly the option of lowering LDL-C to less than 60mg/dl and increasing HDL-C to more than 50mg/dl and to raise the question of whether this optional goal should become a recommended target.¹⁴ Therefore, future studies will be challenged as we investigate whether there is any benefit, in terms of hard cardiovascular end points, with really low levels of LDL-C accompanied by maximal increases in HDL-C.

Furthermore, to obtain the effect on morbidity and mortality in any cardiovascular prevention trial, it requires sample size in the range of 10,000 to 20,000 patients and follow-up of at least 5-7 years. In both ASTEROID and our experience, though sample size were very much limited, it showed strong statistical significance that were achieved from the powerful changes in lipid profiles. It is therefore up to future investigators who would follow the footsteps of these studies to utilize large sample sizes and establish reliable results to prove that what we have accomplished are achievable in every patient.

Other available avenues to pursue are the ways to manipulate particle sizes of both LDL-C and HDL-C and the need for a more non-invasive test to follow-up patients with significant plaques. LDL particle size decreases with increasing density. Smaller, denser LDL particles seem more atherogenic than the larger, lighter particles, based on the experimental findings that smaller LDL particles are more susceptible for oxidation *in vitro*, have lower binding affinity for the LDL receptors and lower catabolic rate, have a higher concentration of polyunsaturated fatty acids, and potentially interact more easily with proteoglycans of the arterial wall. Clinical studies have shown that a smaller LDL subfraction profile is associated

with an increased risk of heart disease, even when total cholesterol level is only slightly raised. Although LDL particle size is genetically determined, its phenotypic expression may also be affected by environmental factors such as drugs, diet, obesity, exercise or disease. Factors that shift the LDL subfractions profile towards larger particles may reduce the risk of heart disease.⁵² Likewise, increasing the size of HDL would confer additional benefits.

While IVUS used by the ASTEROID investigators shows promising potential to be used as a routine diagnostic procedure to measure atherosclerosis, its invasive nature warrants time and that IVUS plaque-measurement is too cumbersome as it takes a technician a full day to calculate one patient's plaque volume from videotaped images. We need faster and simpler-to-use methods to spot dangerous, inflamed plaque so patients can be treated before they get heart attacks. The CT angiography is a much less invasive and more patient-friendly procedure that can diagnose coronary artery disease in less time. Also, studies have indicated that it is likely plaque composition and not the degree of stenosis that drives adverse coronary events. The traditional catheter angiography can only show the extent of artery narrowing while CT angiography can differentiate soft unstable plaque from hard stable plaque. Something no other diagnostic procedure can do.

The idea that coronary artery disease can be cured is now getting towards being a reality when we consider what has already been achieved. ASTEROID has demonstrated clearly the regression of atherosclerosis in arteries by decreasing LDL-C to a level lower than established target and increasing HDL-C. Our experience, on the other hand, decreased LDL-C to very low levels and raising HDL-C higher than LDL-C. Several important research questions that will likely impact our management of atherosclerotic arterial disease in a significant way

are being raised. Was it the LDL-C decrease, the HDL-C increase, the percent changes in these lipids, or the LDL-C/HDL-C ratio that led to plaque regression?¹⁴ What is the implication of raising HDL-C more than LDL-C? Would regression be achieved faster with higher HDL-C? Answering these questions could very well be the next step in achieving absolute reduction in ASCVD and we could be living in a world with zero coronary event.

References:

1. Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. *Circulation*. 2002; 106:3143-3421.
2. Grundy SM, Cleeman JI, Bairey Merz CN, et al, Coordinating Committee of the National Cholesterol Education Program. Implications of Recent Clinical Trials for the National Cholesterol Education Program Adult Treatment Panel III guidelines. *Circulation*. 2004;110:227-239
3. O'Keefe JH, Cordain L, Harris WH, Moe RM, Vogel R. Optimal low density lipoprotein is 50-70mg/dl: lower is better and physiologically normal. *J. Am. Coll. Cardiology*. 2004;43:2142-2146.
4. Cannon CP, Braunwald E, McCabe CH, et al, Pravastatin or Atorvastatin Evaluation and Infection Therapy – Thrombolysis in Myocardial Infarction 22 Investigators. Intensive versus moderate lipid lowering with statins after acute coronary syndromes. *N. Engl. J. Med*. 2004;350:1495-1504.
5. LaRosa JC, Grundy SM, Waters DD, et al. Treating to New Targets (TNT) Investigators. Intensive lipid lowering with atorvastatin in patients with stable coronary disease. *N. Engl. J. Med*. 2005 Apr 7; 352:1425-1435.
6. Pedersen TR, Faergeman O, Kastelein JJ, et al, Incremental Decrease in End Points Through Aggressive Lipid Lowering (IDEAL) Study Group. High-dose atorvastatin vs usual-dose simvastatin for secondary prevention after myocardial infarction: the IDEAL study: a randomized controlled trial. *JAMA*. 2005;294:2437-2445.
7. Cholesterol Treatment Trialists' (CTT) Collaborators. Efficacy and safety of cholesterol-lowering treatment: prospective meta-analysis of data from 90,056 participants in 14 randomized trials of statins. *Lancet*. 2005;366:1267-1278.
8. Nissen SE, Nicholas SJ, Sipahi I, Libby P, Raichlen JS,

- Ballantyne CM, Davignon J, Erbel R, Fruchart JC, Brodie B, Grines CL, DeMaria AN, for REVERSAL Investigators. Effect of intensive compared with moderate lipid-lowering therapy on progression of coronary atherosclerosis: a randomized controlled trial. *JAMA*. 2004; 291:1071-1080.
9. Blankenhorn DH, Nessim SA, Johnson RL, et al. Beneficial effects of combined colestipol-niacin therapy on coronary atherosclerosis and coronary venous bypass grafts. *JAMA*. 1987;257:3233-3240.
10. Brown G, Albers JJ, Fisher LD, et al. Regression of coronary artery disease as a result of intensive lipid-lowering therapy in men with high levels of apolipoprotein B. *N Engl J Med*. 1990;323:1289-1298.
11. Brown BG, Brockenbrough A, Zhao X-Q, Dowdy AA, Monick EA, Huss Frechette EE, Poulin DC, Rocha AL. Very intensive lipid therapy with lovastatin, niacin, and colestipol for prevention of death and myocardial infarction: a 10-year Familial Atherosclerosis Treatment Study (FATS) follow-up. *Circulation* 1998;98:I-635.
12. Nissen SE, Nicholls SJ, Sipahi I, et al, ASTEROID Investigators. Effect of very high-intensity statin therapy on regression of coronary atherosclerosis: the ASTEROID trial. *JAMA*. 2006 Apr 5;295:1556-1565. Epub 2006 Mar 13.
13. Sipahi I, Nichols SJ, Tuzcu EM, Nissen SE. Coronary Atherosclerosis can regress with very intensive statin therapy: Interpreting the ASTEROID trial. *Cleveland Clinic Journal of Medicine*. Oct. 2006. Vol.73:Num10. 937-944.
14. Nambi V. A Commentary on The Asteroid Trial. *Baylor College of Medicine, Houston, Texas*. 2006.
15. Grundy, SM. The Issue of Statin Safety. Where do We Stand?. *Circulation*. 2005. Print ISSN: 0009-7322. Online ISSN: 1524-4539.
16. Pasternak RC, Smith SC Jr, Bairey-Merz CN, Grundy SM, Cleeman JI, Lenfant C. ACC/AHA/NHLBI clinical advisory on the use and safety of statins. *Jour Am Coll Cardiol*. 2002;40:567-572.
17. Jacobson TA. Combination lipid-lowering therapy with statins: safety issues in the postcerivastatin era. *Expert Opin Drug Saf*. 2003;2:269-286.
18. Jacobson TA. The Safety of Aggressive Statin Therapy: How Much Can Low-Density Lipoprotein Cholesterol Be Lowered?. *Mayo Clin Proc*. September 2006;81(9):1225-1231.
19. Ballantyne CM, Corsini A, Davidson MH, et al. Risk for myopathy with statin therapy in high-risk patients. *Arch Intern Med*. 2003;163:553-564.
20. Rosenson RS. Current overview of statin-induced myopathy. *Am J Med*. 2004;116:408-416.
21. Davidson MH. Combination therapy for dyslipidemia: safety and regulatory considerations. *Am J Cardiol*. 2002;90(suppl 2):50-60.
22. Cheng JWM. Rosuvastatin in the management of hyperlipidemia. *Clin Ther*. 2004;26:1368-1387.
23. Staffa JA, Chang J, Green L. Cerivastatin and reports of fatal rhabdomyolysis [letter]. *N Engl J Med*. 2002;346:539-540.
24. Vuppalanchi R, Teal E, Chalasani N. Patients with elevated baseline liver enzymes do not have higher frequency of hepatotoxicity from lovastatin than those with normal baseline liver enzymes. *Am J Med Sci*. 2005;329:62-65.
25. Chalasani N, Aljadhey H, Kesterson J, Murray MD, Hall SD. Patients with elevated liver enzymes are not at higher risk for statin hepatotoxicity. *Gastroenterology*. 2004;126:1287-1292.
26. Sabharwal AK, Boord JB. Low-density lipoprotein reduction: is the risk worth the benefit?. *Curr Atheroscler Rep*. 2006 Jan;8(1):19-25.
27. Liao JK, Laufs U. Pleiotropic Effects of Statins. *Annual Review of Pharmacology and Toxicology*. February 2005. Vol. 45:89-118.
28. Farmer JA. Pleiotropic effects of statins. *Curr Atheroscler Rep*. 2000 May;2(3):208-17.
29. Naccarella GV. Do Statins Have Effects Beyond Lipid-Lowering?. *American College of Cardiology's Cardiosource*. March 2006.
30. Ray KK, Cannon CP. Pathological changes in acute coronary syndromes: the role of statin therapy in the modulation of inflammation, endothelial function and coagulation. *J Thromb Thrombolysis*. 2004;:89-101.
31. Ray KK, Cannon CP, McCabe CH, et al. PROVE IT-TIMI 22 Investigators. Early and late benefits of high-dose atorvastatin in patients with acute coronary syndromes: results from the PROVE IT-TIMI 22 trial. *J Am Coll Cardiol* 2005;:1405-10.
32. Sever PS, Poulter NR, Dahlof B, Wedel H; Anglo-Scandinavian Cardiac Outcomes Trial Investigators. Different time course for prevention of coronary and stroke events by atorvastatin in the Anglo-Scandinavian Cardiac Outcomes Trial-Lipid-Lowering Arm (ASCOT-LLA). *Am J Cardiol*. 2005;5A:39F-44F.
33. Buchwald H, Varco RL, Matts JP, et al. Effect of partial ileal bypass surgery on mortality and morbidity from coronary heart disease in patients with hypercholesterolemia. Report of the Program on the Surgical Control of the Hyperlipidemias (POSCH). *N Engl J Med* 1990;:946-55.
34. Ray KK, Cannon CP. Early time to benefit with intensive statin treatment: could it be the pleiotropic effects? *Am J Cardiol* 2005;5A:54F-60F.
35. McFarlane SI, Muniyappa R, Francisco R, Sowers JR. Pleiotropic Effects of Statins: Lipid Reduction and Beyond. *The Journal of Clinical Endocrinology & Metabolism*. 2002. 87(4):1451-1458
36. Tedgui A, Mallat Z. 2001 Antiinflammatory mechanisms in the vascular wall. *Circulation Res* 88:877-887

37. Jialal I, Stein D, Balis D, Grundy SM, Adams-Huet B, Devaraj S. Effect of hydroxymethyl glutaryl coenzyme a reductase inhibitor therapy on high sensitive C-reactive protein levels. *Circulation*. 2001. 103:1933–1935
38. Albert MA, Danielson E, Rifai N, Ridker PM, PRINCE Investigators. Effect of statin therapy on C-reactive protein levels: the pravastatin inflammation/CRP evaluation (PRINCE): a randomized trial and cohort study. *JAMA*. 2001. 286:64–70
39. Nissen SE, Tuzcu EM, Schoenhagen P, et al. Statin therapy, LDL cholesterol, C-reactive protein, and coronary artery disease. *N Engl J Med* 2005;:29-38.
40. Ridker PM, Cannon CP, Morrow D, et al. C-reactive protein levels and outcomes after statin therapy. *N Engl J Med* 2005;:20-8.
41. Fenster BE, Tsao PS, Rockson SG. Endothelial dysfunction-clinical strategies for treating oxidant stress. *Am Heart J* 2003;146:218-26.
42. Larose E, Ganz P. Statins and Endothelial dysfunction. *Semin Vasc Med*. 2004 Nov;4(4):333-46.
43. Hayward RA, Hofer TP, Vijan S. Narrative Review: Lack of Evidence for Recommended Low-Density Lipoprotein Treatment Targets: A Solvable Problem. *Annals of Internal Medicine*. 2006;145:520-530.
44. Barr DP, Russ EM, Eder HA. Protein-lipid relationships in human plasma. II. In atherosclerosis and related conditions. *Am J Med* 1951; 11:480–493.
45. Assmann G, Schulte H, von Eckardstein A, Huang Y. High-density lipoprotein cholesterol as a predictor of coronary heart disease risk. The PROCAM experience and pathophysiological implications for reverse cholesterol transport. *Atherosclerosis* 1996; 124(Suppl):S11–S20.
46. Gordon DJ, Probstfield JL, Garrison RJ, et al. High-density lipoprotein cholesterol and cardiovascular disease. Four prospective American studies. *Circulation* 1989; 79:8–15.
47. Cutri BA, Hime NJ, Nicholls SJ. High-density lipoproteins: an emerging target in the prevention of cardiovascular disease. *Cell Research*. 2006. **16**: 799–808.
48. Sacks FM. Clinical Usefulness of HDL Cholesterol as a Target to Lower Risk of Coronary Heart Disease. *British Journal of Cardiology*. September 2003.
49. Hughes S. HDL Cholesterol: The next target in the battle against heart disease. *Heartwire*. April 2004.
50. Sirtori C., Francheschini G. A-IMilano apoprotein. Decreased high density lipoprotein cholesterol levels with significant lipoprotein modifications and without clinical atherosclerosis in an Italian family. 1980 Nov; 66(5):892-900.
51. Nissen SE, Tsunoda T, Tuzcu EM. Effect of recombinant ApoA-I Milano on coronary atherosclerosis in patients with acute coronary syndromes: a randomized controlled trial. *JAMA*. 2003;290:2292-2300.
52. Ramjan I, Maxwell S, et al. Particle size: the key to the atherogenic lipoprotein? *Q J Med* 1994; 87: 709-720