J. Pharm. Pharmacol. 2000, 52: 731–761
Received November 4, 1999
Accepted March 28, 2000

Apolipoprotein A-I, Cyclodextrins and Liposomes as Potential Drugs for the Reversal of Atherosclerosis. A Review

CRISPIN R. DASS AND WENDY JESSUP*

*Johnson and Johnson Research, Box 4555, Strawberry Hills 2012 and *Cell Biology Unit, Heart Research Institute, 145 Missenden Rd, Camperdown 2050, Australia*

Abstract

Several studies have revealed that high-density lipoprotein (HDL) is the most reliable predictor for susceptibility to cardiovascular disease. Since apolipoprotein A-I (apoA-I) is the major protein of HDL, it is worthwhile evaluating the potential of this protein to reduce the lipid burden of lesions observed in the clinic. Indeed, apoA-I is used extensively in cell culture to induce cholesterol efflux. However, while there is a large body of data emanating from in-vitro and cell-culture studies with apoA-I, little animal data and scant clinical trials examining the potential of this apolipoprotein to induce cholesterol (and other lipid) efflux exists. Importantly, the effects of oxysterols, such as 7-ketocholesterol (7KC), on cholesterol and other lipid efflux by apoA-I needs to be investigated in any attempt to utilise apoA-I as an agent to stimulate efflux of lipids.

Lessons may be learnt from studies with other lipid acceptors such as cyclodextrins and phospholipid vesicles (PLVs, liposomes), by combination with other effluxing agents, by remodelling the protein structure of the apolipoprotein, or by altering the composition of the lipoprotein intended for administration in-vivo. Akin to any other drug, the usage of this apolipoprotein in a therapeutic context has to follow the traditional sequence of events, namely an evaluation of the biodistribution, safety and dose–response of the protein in animal trials in advance of clinical trials. Mass production of the apolipoprotein is now a simple process due to the advent of recombinant DNA technology.

This review also considers the potential of cyclodextrins and PLVs for use in inducing reverse cholesterol transport in-vivo. Finally, the potential of cyclodextrins as delivery agents for nucleic acid-based constructs such as oligonucleotides and plasmids is discussed.

Background on Atherosclerosis

Mammalian cells obtain cholesterol for membrane synthesis mostly via the receptor-mediated endocytosis of low-density lipoprotein (LDL; Goldstein & Brown 1974). Macrophages and vascular endothelium additionally have receptors that recognise certain modified forms (e.g. acetylated) of LDL (Stein & Stein 1979; Brown & Goldstein 1986). An important early event in atherogenesis is the subendothelial retention of atherogenic lipoproteins, including LDL (Schwenke & Carew 1989a, b; Nievelstein et al 1991), lipoprotein A (LpA) (Kreuzer et al 1994) and triglyceride-rich lipoproteins (Rapp et al 1994). Atherosclerosis-susceptible sections of the arteries are distinguished by an increased retention of lipoproteins compared with resistant regions (Schwenke & Carew 1989b). Retained lipoproteins trigger such events as lipoprotein oxidation and endothelial changes (Ross 1995), considered central to the atherogenic process (Williams & Tabas 1995).

LDL can undergo both aggregation and fusion (reviewed in Kruth 1997). These changes may cause increased retention of LDL in the atherosclerotic lesion connective-tissue matrix and enhance LDL uptake by macrophages. Mast cells may play an important role in fusion since mast-cell-derived proteases can induce fusion of LDL through proteolysis of apolipoprotein B (apoB). LDL in atherosclerotic lesions have been found to be sialic acid poor and ceramide enriched, changes which contribute to LDL aggregation. Macro-

Correspondence: C. R. Dass, Johnson and Johnson Research, Box 4555, Strawberry Hills 2012, Australia.
E-Mail: cdass@medau.jnj.com

phages accumulate around lipid cores of arteriosclerotic plaques (von Eckardstein 1996) and mobilise extracellular cholesterol deposits by uptake of lipoproteins, crystals and debris (Libby & Clinton 1993). Cholesterol-loaded macrophages are prominent features of atherosclerotic lesions, while macrophage necrosis has been proposed to play an important role in plaque destabilisation. With unesterified (free) cholesterol loading, macrophage phosphatidylcholine biosynthesis is elevated, thereby keeping the unesterified cholesterol : phospholipid ratio (UC : PL) from rising to toxic levels (Tabas et al 1996). However, with prolonged unesterified cholesterol loading, this adaptive response fails, the UC : PL ratio reaches cytotoxic levels, and macrophage death ensues. Accumulation of cholesterol esters gives the cytoplasm of macrophages the characteristic appearance referred to as foam cells.

Macrophage foam cells isolated from atherosclerotic lesions contain a large quantity of unesterified and esterified cholesterol. This results from a discrepancy between rates of uptake, synthesis, metabolism and export of cholesterol. In general, cells closely regulate their cholesterol levels by feedback down-regulation of cholesterol biosynthetic enzymes and of plasma membrane receptors for LDL (the major extracellular source of cholesterol). In addition, most cells can export excess cholesterol to certain extracellular acceptors such as HDL. The predominant protein component of HDL is apolipoprotein A-I (apoA-I), a key component for stimulation of cholesterol efflux from cells.

Accumulation of cholesteryl ester lipid droplets in cells loaded with acetylated LDL (AcLDL) involves seven steps. Lysosomal hydrolysis of cholesteryl esters is followed by transfer of cholesterol to the extralysosomal cytosol for re-esterification by acyl coenzyme A : cholesterol acyltransferase (ACAT) to form cholesteryl ester lipid droplets. Normal cholesterol efflux then requires hydrolysis of these cholesteryl esters by nCEH (non-lysosomal neutral cholesteryl ester hydrolase) to release free cholesterol. Synthesis of phosphatidylcholine and sphingomyelin for packaging of cholesterol occurs and is followed by delivery of cholesterol to the plasma membrane and desorption of cholesterol from the plasma membrane across the unstirred water layer on to an acceptor in the medium that will bind and transport cholesterol. The human macrophage model used in

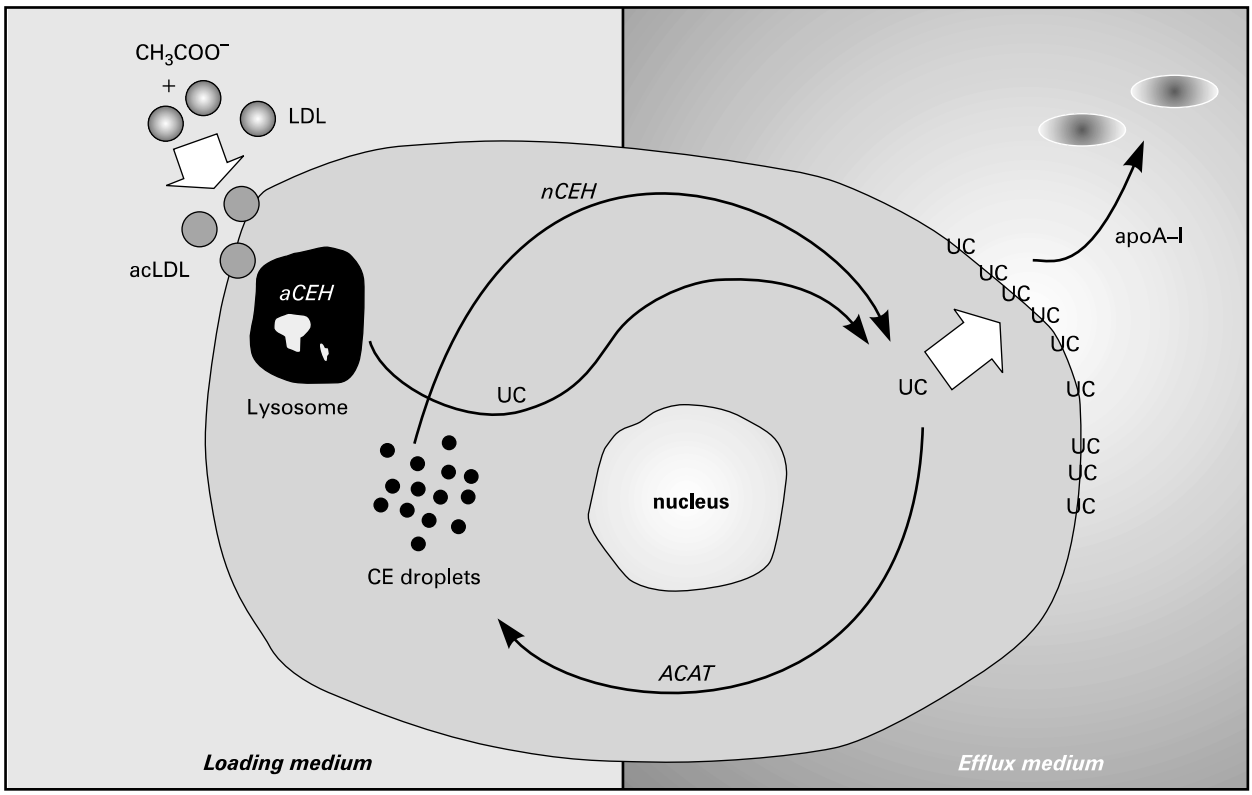

Figure 1. Cholesterol pools within a test system. aCEH, acidic cholesterol ester hydrolase; apoA-I, apolipoprotein A-I; nCEH, neutral cholesterol ester hydrolase; ACAT, acyl coenzyme A : cholesterol acyltransferase; acLDL, acetylated LDL; CE, cholesteryl ester; LDL, low-density lipoprotein; UC, unesterified cholesterol.

most laboratories for studies of cholesterol (or phospholipid, or both) efflux is shown in Figure 1.

Shift in Emphasis in Atherosclerosis Management

In the past (as is still predominantly practised), preventive management of atherosclerosis relied heavily on the administration of drugs along with dietary restrictions to induce beneficial changes in plasma cholesterol levels. While these drugs lower plasma cholesterol levels and thereby induce a slowing of the atherogenic process, or even a regression of the disease, responses to treatment vary. This may be attributed to the fact that the primary aim of these therapies is to reduce serum cholesterol while not affecting the cholesterol trapped in plaques that can occlude arteries. Thus, there is an increasing trend to assist the natural mobilisation of cholesterol from peripheral cells and promote its transfer to the liver where it is catabolised. There is also a growing trend in the attempt to reduce the lipid burden in lesions, as is manifested by a growing number of animal studies.

Lipid-burden reduction in lesions may be effected by isolated and purified natural acceptors, such as apoA-I, or synthesised artificial acceptors, such as cyclodextrins and phospholipid vesicles (PLVs). In addition, apoA-I may be mass-produced using recombinant DNA technology which involves expression of the apolipoprotein gene in bacteria such as *Escherichia coli*. There are apparently different mechanisms by which phospholipid-containing particles (such as HDL) or lipid-poor apolipoproteins (e.g. apoA-I) extract cholesterol from cells. Phospholipid-containing acceptors such as HDL particles remove cholesterol mainly by a passive process involving aqueous diffusion in which cholesterol desorbs from the plasma membrane and moves through the aqueous layer until it contacts, and is adsorbed by, the acceptor (Figure 2). This flux between the plasma membrane and acceptor is bidirectional and net transfer of cholesterol is determined by a gradient. Efflux to these acceptors may be assisted by the binding of acceptor units to select elements of the plasma membrane such as the caveolae. Lipid-free apoA-I interaction with cells involves transient binding of the apolipoprotein to the cell surface, followed by abstraction of plasma membrane cholesterol together with phospholipid as the particle dissociates from the plasma membrane. Small HDL particles containing phospholipid, apoA-I and cholesterol

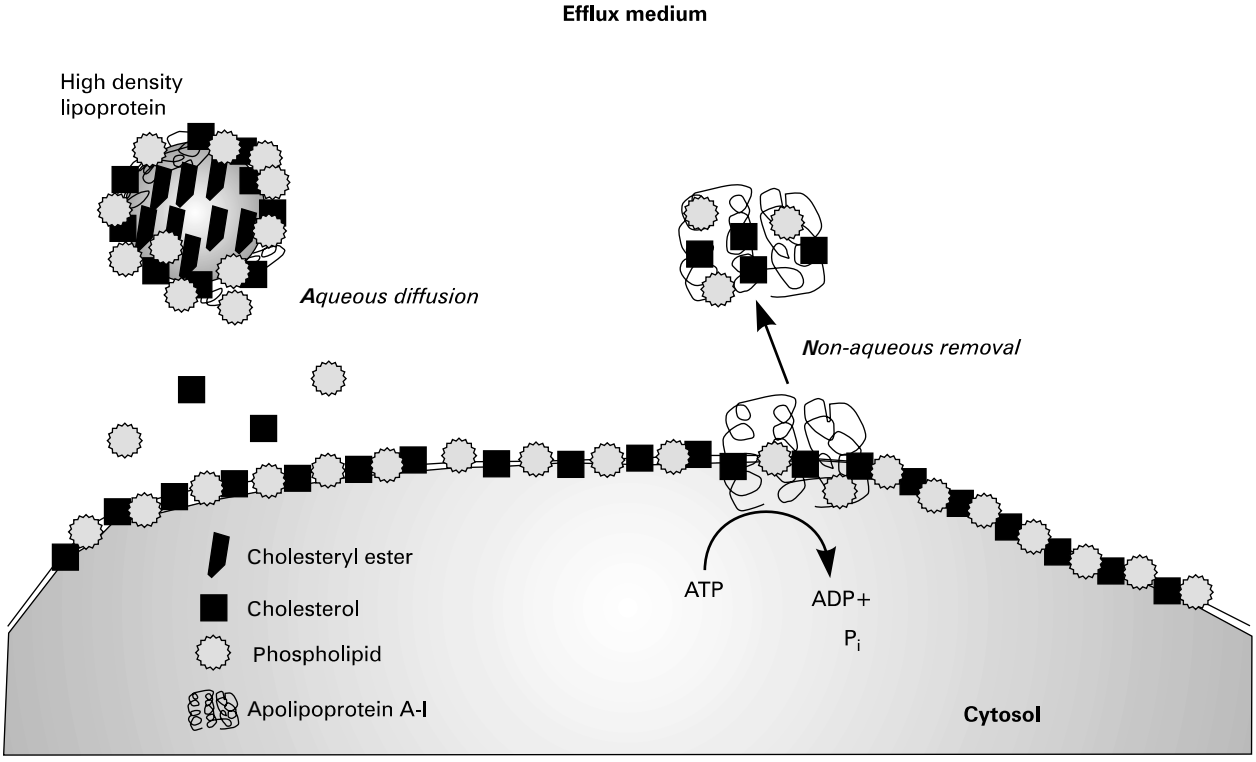

Figure 2. Mechanisms for efflux. Cholesterol diffuses across an intervening aqueous space before desorption by high-density lipoprotein while apolipoprotein A-I physically interacts with the plasma membrane to extract cholesterol.

are generated in the extracellular fluid. It seems that phospholipid association with apoA-I enables the apolipoprotein to hold on to the desorbed cholesterol molecules probably by provision of a hydrophobic environment.

Apolipoproteins

Apolipoproteins are the functional components of lipoproteins. They determine the structure of the lipoprotein particles and their metabolism, commencing with the synthesis and secretion of lipoproteins from specific cells, through the enzymatic reactions and lipid transfers that lipoproteins undergo in circulation, culminating in the binding of lipoproteins to cell receptors and their eventual catabolism (Jonas et al 1993). Efflux studies with isolated lipoproteins or reconstituted lipoproteins are suited for investigations on the structure–function relationships between acceptor and efflux, while whole serum and plasma provide the opportunity to study cholesterol efflux under conditions in which all the assorted lipoproteins, enzymes and transfer factors that might influence efflux are present (Fournier et al 1996).

Apolipoproteins facilitate the removal of cell cholesterol by phospholipids (Mahlberg et al 1991; Hara & Yokoyama 1992; Mahlberg & Rothblat 1992). By promoting the removal of cholesterol from intimal macrophages, high-density lipoprotein (HDL) permits the transfer of this cell cholesterol to the liver and facilitates its excretion in bile (Goldberg et al 1991; Johnson et al 1991; Oram & Yokoyama 1996). Thus, HDL retards the progressive formation of sterol-rich lesions in the artery wall.

HDL contains two major proteins, apoA-I and apolipoprotein A-II (apoA-II), which comprise approximately 70% and 20% of the total HDL protein mass, respectively. HDL exists in human plasma in two main forms – one containing apoA-I with apoA-II (AI/AII-HDL) and the other containing apoA-I without apoA-II (AI-HDL). ApoA-I activates lecithin : cholesterol acyltransferase (LCAT), the enzyme responsible for esterification of plasma cholesterol on HDL. This catalysis promotes the maturation of HDL particles in plasma and facilitates reverse cholesterol transport by maintaining a concentration gradient for the diffusion of cellular unesterified cholesterol to HDL (Glomset 1968; Ohta et al 1992). Furthermore, cholesterol is esterified by LCAT in HDL particles generated with apoA-I but not with apoA-II (Czarnecka & Yokoyama 1995).

HDL quantified by its cholesterol or its apoA-I content, is the best single predictor of coronary artery disease (Brouillette & Anantharamaiah 1995). Results from several clinical trials of coronary artery disease risk show an inverse correlation with serum HDL levels and that rising HDL levels results in regression of atherosclerotic lesion growth and reductions in fatal and nonfatal myocardial infarctions (Badimon et al 1989, 1990). In mice, the protective effect of HDL (formulated in-vivo from human apoA-I, in transgenic mice) is primarily if not exclusively due to particles containing human apoA-I (Rubin et al 1991; Paszty et al 1994).

When cholesterol-loaded macrophages are incubated in plasma-containing medium, cholesterol moves from the cells to HDL and is then esterified by LCAT (Glomset 1968; Ho et al 1980). LCAT acts on discoidal particles, which are good substrates for LCAT, by esterifying the hydroxyl moiety of free cholesterol with an acyl chain derived from the *sn*-2 position of phosphatidylcholine, thereby forming lyso-phosphatidylcholine and cholesteryl ester (McCall et al 1995). In species that express plasma cholesterol ester transfer protein (CETP), a significant fraction of HDL cholesteryl ester is transferred to other plasma lipoproteins for further metabolism (Tall 1993), while excess cholesterol carried by HDL is returned to the liver for catabolism. ApoA-I activates LCAT in plasma thereby promoting esterification of cholesterol (Fielding & Fielding 1981). Since apoA-I has the ability to efflux phospholipids from cells as well, its capability to activate LCAT is an interesting phenomenon. Esterification reduces the likelihood of HDL-cholesterol re-entering cells (Czarnecka & Yokoyama 1995).

Apolipoprotein A-I: Structure, Preparation and In-vitro Characterisation

ApoA-I is a 243-amino-acid protein which possesses multiple tandem repeating antiparallel 22-mer amphipathic α-helices (interrupted by β-turns at proline and glycine residues), which are thought to be responsible for association of the apolipoprotein with lipids (Segrest et al 1992, 1994). The 243-amino-acid protein contains a repetitive motif of 11 amino acids found in other exchangeable apolipoproteins.

ApoA-I may be purified from human plasma or, alternatively, produced using recombinant DNA techniques in *Escherichia coli* (Bergeron et al 1997). Purified apoA-I tends to self-associate, the degree of association varying from laboratory to

laboratory. This is attributable to the chemical heterogeneity of the protein (reviewed in Brouillette & Anantharamaiah 1995). Differences in the isolation procedures utilised can produce different isoforms, and different rates of methionine oxidation can further add to the heterogeneity. ApoA-I is a chemically labile protein, probably due to its flexible or loose tertiary structure in solution (Tall et al 1976). ApoA-I exists in different polymorphic forms based on discrepancies in isoelectric points (pIs). The major isoprotein has a pI of 5·6–5·7, but several acidic forms have also been observed. Generation of the more acidic forms is due to deamidation of asparagine and glutamine residues in the apoprotein (Ghiselli et al 1985). The heterogeneity of the particles formed post-efflux from macrophages highlights the polymorphic and flexible nature of the apolipoprotein (unpublished results).

One factor compounding apoA-I heterogeneity is the susceptibility of two of the three methionine residues (Met-112 and Met-148, but not Met-86) present on apoA-I to oxidation (von Eckardstein et al 1990). Interestingly, while oxidation seemingly does not affect LCAT activation, it alters immunorecognition of apoA-I which suggests an attenuation in conformation of the apolipoprotein on HDL (Marcel et al 1989), and alters the efflux capacity of apoA-I (Panzenböck et al, unpublished results).

It has been demonstrated that short peptides mimicking the secondary structure of apoA-I are able to imitate its ability to remove cholesterol from cells (Davidson et al 1994; Mendez et al 1994; Yancey et al 1995), suggesting that a non-specific mechanism appears to be involved. On the other hand, specific sequences of apoA-I have been implicated in its ability to efflux cholesterol (Banka et al 1994; Sviridov et al 1996a, b), denoting a more specific, possibly receptor-mediated action. The 223–243 region of apoA-I has been suggested to be most likely involved in the formation of apoA-I-lipid complexes (Sviridov et al 1996a, b).

Importantly, Sviridov et al (1996a) found that two regions within the apoA-I lipoprotein are involved in phospholipid-specific binding. This was also noted in an earlier study (Palgunachari et al 1996). In addition, monoclonal antibodies recognising the epitopic region apoA-I-(140–150) specifically inhibited the ability of apoA-I-containing lipoproteins to promote efflux of intracellular (but not plasma membrane) cholesterol (Sviridov et al 1996b). The carboxy (−COOH) terminus of apoA-I is important for lipid binding (Gillotte et al 1996; Laccotripe et al 1997), while the terminal amphipathic helices 44–65 and 220–241 are the most important for the initial binding of apoA-I to lipids, and hence in effluxing lipids from plasma membranes (Palgunachari et al 1996).

The presence of amphipathic helical segments in acceptor particles is necessary for efficient efflux of cholesterol from cells (Davidson et al 1994). These helical segments appear to interact with the cell surface and facilitate efflux of unesterified cholesterol by influencing the lipid packing properties of the membrane. Furthermore, the conformation of the helical segments can influence the ability of extracellular acceptors to either sequester or retain cholesterol. By interacting with the cell surface, these helical segments decrease the distance which a cholesterol molecule must traverse to be incorporated into the acceptor particle (Rothblat et al 1992).

Interestingly, while efficient apoA-I-mediated cholesterol efflux from mouse L-cell fibroblasts is dependent upon the presence of α-helical segments, Gillotte et al (1996) have shown that complexes formulated from mutated segments had a similar capacity for cholesterol efflux. By incorporating more molecules of protein in reconstituted HDL (rHDL) particles containing apoA-I deletion mutants, the number of helical segments were kept similar to particles made from intact apoA-I. A decreased phospholipid/apoA-I ratio is taken to be indicative of a reduced capacity of the apoprotein to carry phospholipid (Frank et al 1997). This results in the formation of complexes with 3 apoA-I molecules (Lp3A-I) with dimyristoylphosphatidylcholine (DMPC) and even Lp4A-I at higher POPC/apoA-I ratios.

Using 3 deletion mutants (corresponding to deletion of apoA-I residues 100–143, 122–165 and 144–186), Frank et al (1997) demonstrated that amphipathic α-helices within residues 100–186 are directly involved in interacting with phospholipids. The orientation of these helices are believed to be parallel to the phospholipid acyl chain (Brasseur et al 1990; Wald et al 1990). The helical region 100–121 is seemingly more important for the stabilisation of the lipid–apoprotein complex formed, whereas helices within residues 122–186 appear to be critical to the initial rates of association of apoA-I with DMPC. The elimination of helix 100–121 may annul an important tertiary structure in apoA-I that stabilises the conformation and association to phospholipids that otherwise occur as a result of hydrogen bonds and salt bridges. The central domain of 100–186 is believed to play an essential role in maintaining the plasticity of apoA-I and in its ability to form different classes of HDL particles.

Apolipoprotein A-I and Efflux Studies with Cultured Cells

Before in-vivo administration of apoA-I as an agent for stimulating reverse cholesterol transport, its potential has been documented in numerous cell culture studies with various types of cells under variant conditions. Cell culture studies commonly examine the efficacy of these apolipoproteins in effluxing cholesterol (and other lipids) and also provide some data for toxicity. In addition, results from cell culture studies are derived relatively more quickly than results from in-vivo studies. Synthetic peptides containing only one (18A) or two (37pA) amphipathic helical segments mimicking those present in apoA-I stimulated cholesterol efflux from both mouse macrophages and L-cells in a manner similar to lipid-free apoA-I (Yancey et al 1995). The order of efflux efficiency (determined as the mass concentration at which half-maximal efflux was attained, EC50) was apoA-I > 37pA > 18A. This indicated that acceptor efficiency was related to the number of amphipathic helical segments per molecule.

When the helical content of 18A was increased by neutralising the charge at the ends of peptide Ac-18A-NH_2, there was a notable increase in the efficiency for cholesterol efflux (EC50 of 18A = 17 $\mu g\,mL^{-1}$ while that for Ac-18A-NH_2 = 6 $\mu g\,mL^{-1}$ (Yancey et al 1995). Furthermore, when the amphipathicity of the helix in 18A was reduced by scrambling the amino acid sequence, hence reducing its lipid affinity, cholesterol and phospholipid efflux were not stimulated. The efficiency of cholesterol efflux by the peptides followed the ordering of their lipid affinities: 37pA > Ac-18A-NH_2 > 18A. This order was also relevant to phospholipid efflux.

Movement of cholesterol molecules from the plasma membrane to acceptor lipoprotein involves an aqueous diffusion mechanism in which the cholesterol desorbs from the membrane and is incorporated into the acceptor after traversing the intervening aqueous phase (Phillips et al 1987; Mendez & Uint 1996; Atger et al 1997; Figure 2). Net movement of cholesterol is determined by the processes of influx and efflux, and, largely, on the sterol esterification catalysed by LCAT. Although efflux is not dependent on specific binding of HDL to the cell surface, interaction of HDL with cell membrane can mediate translocation of internal pools of cholesterol to the plasma membrane.

A second mechanism suggested for HDL-mediated removal of cholesterol is that of apolipoprotein dissociating from the HDL particles and interacting with cell-surface binding sites (Oram & Yokoyama 1996). Cell-surface binding sites that recognise HDL apolipoproteins are responsible for the removal of cell cholesterol by the alternative (non-aqueous) mechanism. Loading of cells with cholesterol increases the number of these binding sites (Oram et al 1983, 1987; Schmitz et al 1985) and increases apolipoprotein-mediated cholesterol and phospholipid efflux (Oram et al 1983; Johnson et al 1988; Bielicki et al 1992). Binding of peptides or apolipoproteins to these sites results in removal of lipids from cells (Savion & Kotev-Emeth 1993; Mendez et al 1994). Trypsin treatment of mouse peritoneal macrophages and rat aorta smooth muscle cells results in complete inhibition of cholesterol and phospholipid efflux by apoA-I (Li et al 1995). In contrast, trypsin treatment of cells had only a slight effect on HDL-mediated efflux of cholesterol.

Apolipoprotein-mediated cholesterol removal from microsomal membranes (Nunez & Swaney 1984), from Chinese hamster ovary (CHO) cells (Forte et al 1993), fibroblasts (Bielicki et al 1992; Asztalos et al 1997), endothelial cells (Savion & Kotev-Emeth 1993), Fu5AH rat hepatoma cells (Fournier et al 1996) and macrophages (Hara & Yokoyama 1991; Yancey et al 1995) requires phospholipid association with the protein (Davidson et al 1995a; Forte et al 1995; Sviridov et al 1996b). ApoA-I, in the absence of serum, facilitates cholesterol efflux from certain types of cells, including mouse peritoneal macrophages (MPMs; Gelissen et al 1996) and human monocyte-derived macrophages (HMDMs; Dass et al 1999a), probably by effluxing and associating with phospholipids from the plasma membrane (Yancey et al 1995).

ApoA-I forms similarly-sized discs with various phospholipids (Davidson et al 1995b). However, the conformation of apoA-I varies among the particles. The differences in conformation of apoA-I are consistent with phospholipid fluidity influencing the interaction between amphipathic α-helical segments and phospholipid acyl chains (Davidson et al 1995b). Cholesterol efflux increases with the fluidity of acceptor particles (Davidson et al 1995a). For phosphatidylcholine, the shorter and more saturated the fatty-acid chains, the more effectively cholesterol efflux is promoted by rHDL and phosphatidylcholine vesicles.

Apolipoprotein-mediated efflux of cellular cholesterol is reduced or absent in cells not enriched with cholesterol (Bielicki et al 1992; Mendez & Uint 1996). Mendez (1997) demonstrated that efflux with apolipoprotein is observed in normal fibroblasts upon enrichment with cholesterol and is dependent on metabolic energy (i.e., an active

process). A functional Golgi apparatus is required for cholesterol removal by apolipoproteins (Mendez & Uint 1996). In contrast, efflux with lipid-containing non-apolipoprotein acceptors, regardless of cholesterol loading, is not an active process. Thus, cholesterol removal by these lipid acceptors occurs by the above-mentioned aqueous diffusion process (Phillips et al 1980).

Events leading to particle assembly and their relationship to cholesterol efflux, however, have not been fully elucidated (Mendez 1997). Cholesterol efflux has been suggested to vary as a result of differences in the capacity of apoA-I to form complexes with phospholipids responsible for removal of cellular cholesterol (Sviridov et al 1996b). Presumably, the phospholipid layer on the lipoprotein surface serves as a region that can solubilise cholesterol, and HDL plays an important role in efflux, being a small phospholipid-rich lipoprotein (Jian et al 1997). Lipid-free apoA-I promotes efflux of phospholipid and cholesterol from cholesterol-enriched cells (Saito et al 1997; Dass et al 1999a; Gelissen et al 1999; Wilson et al 2000). One study suggests that efflux of phospholipid and unesterified cholesterol occurs simultaneously (Gillotte
et al 1998). Gillotte and colleagues (1998) used human skin fibroblasts to demonstrate that these molecules were desorbed together, in what they called microsolubilisation of the membrane. Furthermore, the authors postulated both effluxes to be linked due to the similar effect of apoA-I concentration on both effluxes. It is important to bear in mind the confounding factors that predominate in cell culture efflux studies (listed in Table 1).

Table 1. Variables relevant to cell culture studies examining apolipoprotein A-I-mediated efflux.

Isolation and purification techniques used to obtain apolipoprotein A-I
Source of apolipoprotein A-I; mammalian or bacterially expressed
Physical state of apolipoprotein A-I; lipid composition
Age of apolipoprotein A-I preparation
Cell type
Donor-dependent differences in cells
Cell density
Cell maturity (passage number and age in culture)
Whether cells were loaded with lipids and content of cellular lipids
Treatment of cells with other agents, example thioglycolate for MPMs
Concentration of apolipoprotein A-I in efflux medium
Components in efflux medium
Duration of efflux
Efflux evaluated as mass cholesterol (phospholipids) or radiolabelled lipid

Physiology of ApoA-I

Following its dissociation from HDL, several potential fates exist for lipid-free apoA-I. Firstly, apoA-I may be excreted via the kidneys (Horowitz et al 1992). Secondly, it may enter the interstitial space where it has the capacity to recruit phospholipids and cholesterol from plasma membranes thus forming nascent HDL particles (Bielecki et al 1992; Hara & Yokoyama 1992; Forte et al 1995; Li et al 1995). Thirdly, lipid-free apoA-I can be re-incorporated into pre-existing HDL particles that are increasing in size due to an accumulation of core cholesteryl esters generated by LCAT (Liang et al 1995). Finally, apoA-I can form the nucleus of new HDL particles by combining with phospholipids and unesterified cholesterol released from triglyceride-rich lipoproteins undergoing lipolysis (Musliner et al 1991; Clay et al 1992). Whereas lipid-free apoA-I may derive from the kidneys, studies performed in rats reveal that free apoA-I is rapidly catabolised in this organ (Glass et al 1983; Ponsin et al 1986). Newly synthesised apoA-I molecules are recycled between their free and lipid-bound forms and are used multiple times to mobilise lipids from tissues before eventually being cleared from plasma (Oram & Yokoyama 1996).

In humans, apoA-I is synthesised predominantly in the liver and small intestine (Zannis et al 1982). The primary translation product is a preproprotein, 267 amino acids long, which is 24 amino acids larger than the major circulating protein (Gordon et al 1983). An 18-amino-acid peptide is cleaved intracellularly during translocation in the RER (rough endoplasmic reticulum) to proapoA-I, which contains a hexapeptide amino-terminal extension. However, the location and mechanism by which proapoA-I is processed is unclear.

A small amount (5–10%) of lipid-free apoA-I-like particle exists in human plasma (Neary & Gowland 1987; Neary et al 1991; Asztalos & Roheim 1995; Liang et al 1995). The low concentration is attributed to its fast rate of turnover and its possible incorporation into other HDL fractions. It has been shown that CETP-mediated transfer of cholesteryl ester from HDL to very-low-density lipoprotein (VLDL) and LDL reduces the size of HDL as well as resulting in the desorption of apoA-I from HDL (Liang et al 1995). This results in a pool of essentially lipid-free apoA-I. This small particle should be filtered through the capillaries more than pre-β1 particles, which are at least twice as large. This free apoA-1 also contributes to the formation of pre-β1 by interacting with plasma membranes of the peripheral cells and accepting cholesterol and phospholipid (Hara & Yokoyama 1992).

The pre-β-migrating apoA-I lipoprotein fraction has a molecular weight of approx. 70 000 Da (Castro & Fielding 1988) although recent estimates state it to be 60 000 Da (Kunitake et al 1992). This particle may be a particularly important factor for reverse cholesterol transport (Fielding & Fielding 1980; Barbaras et al 1987; Castro & Fielding 1988; Francone et al 1989; Davidson et al 1995b). Transfer of carried cholesterol from this particle to a higher molecular weight pre-β apoA-I species, to LDL and to the α-migrating apoA-I that makes up 96% of total apoA-I in plasma, occurs (Castro & Fielding 1988). However, it is not known whether the smaller particles are converted to the larger particles along with their cholesterol loads. Using MPMs, Hara & Yokoyama (1992) demonstrated that pre-β-HDL-like particles transferred their cholesterol and phospholipid loads to microemulsions made from phosphatidylcholine and triolein. The half-life of the particle was estimated to be less than 10 min.

Pre-β HDL particles are present in the plasma of dogs (Lefevre et al 1988), monkeys (Castle et al 1991) and mice (Ishida et al 1990). Particles resembling pre-β have been found in the plasma of patients with LCAT deficiency (Soutar et al 1982; Chen et al 1984), in the plasma of patients undergoing chronic haemodialysis (Gebhardt et al 1984), and in the medium of HepG2 cells (McCall et al 1988). The plasma concentration of these particles can range from negligible amounts in patients who have undergone small-bowel resection (Kunitake et al 1992) to more than 50% of apoA-I mass in the plasma of hypertriglyceridaemic patients (Ishida et al 1987; Neary & Gowland 1987). The average concentration of pre-β HDL is about 5% of the apoA-I mass in plasma of normolipidaemic individuals (Ishida et al 1987; Neary & Gowland 1987). Importantly, lipid-poor apoA-I fractions have been found in human aorta (Heideman & Hoff 1982).

Kunitake et al (1992) revealed that when human plasma was incubated at 37°C for 2 h, apoA-I in the pre-β mobility form disappeared. This shift was subsequently blocked by the addition of the ACAT inhibitors, 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) and menthol. When α-HDL was incubated with cholesterol-loaded human skin fibroblasts, particle mobility was not altered.

When Fu5AH hepatoma cells were incubated with normal human serum previously incubated with either DMPC or bovine brain sphingomyelin (BBSM), the efflux capacity of serum was enhanced by approximately 50% at saturating levels of phospholipid (Jian et al 1997). After lipid modification, the sizes of HDL_2 and HDL_3 increased, while LDL size was increased only after DMPC modification. The result of this study indicated that part of the exogenous phospholipid is directly inserted into pre-existing HDL and part of it forms new lipid–protein complexes with the apolipoproteins that dissociate from HDL. DMPC is selected for such studies as it forms lipoproteins when incubated with apolipoproteins at its phase-transition temperature of 24°C (Pownall et al 1978). DMPC MLVs, when present in media without serum, are very inefficient in accepting cholesterol (Fournier et al 1996). Efflux increases significantly when serum is added to the cells.

In contrast, following a study using lipid vesicular bilayers, Saito et al (1997) stated that the capacity of apoA-I to bind to the bilayer surface is modulated by headgroup space rather than the acyl chain fluidity of the lipid. The authors suggested that cholesterol affects the bilayer surface so as to increase the amount of apoA-I binding to bilayers. It has also been demonstrated that SPM is elevated in early cholesterol acceptors (pre-β-HDL and γ-migrating apoE-containing lipoprotein) suggesting that sphingomyelin might enhance the ability of these particles to promote cholesterol efflux (Huang et al 1994; Fielding & Fielding 1995a).

Cholesterol efflux reaches a plateau phase after a certain period of incubation of apoA-I, and a plateau is approached when HDL or trypsin-modified (75% protein not digested) HDL is incubated with cholesterol-enriched fibroblasts (Mendez 1997). This is in contrast to that observed with phospholipid vesicles (PLVs), where efflux increases with increasing vesicle concentration. However, HDL is significantly more effective at decreasing cellular cholesterol esters, like lipid-free apoA-I, but unlike PLVs.

Apolipoprotein-mediated cellular cholesterol efflux is linked to mobilisation of cholesterol from an intracellular pool by ACAT to a second pool from which pre-β-HDL is generated (Li et al 1997). The pre-β-HDL particle is generated in response to apolipoprotein–cell interaction and subsequent intracellular signalling. Binding of apolipoprotein to the cell surface is essential for assembly of the pre-β-HDL with cellular phospholipid, and intracellular cholesterol mobilisation is required for enrichment of the pre-β-HDL with cholesterol. These actions are largely independent of the diffusion-mediated nonspecific efflux of cellular cholesterol.

Small pre-β particles are the preferred substrate for LCAT which is responsible for cholesterol esterification in plasma (Jauhiainen et al 1993). This, plus the fact that pre-β particles are implicated strongly in reverse cholesterol transport,

makes it necessary for attempts to understand the mechanisms that regulate the size of HDL particles. The range of HDL particles observed in plasma is a result of the interaction of several factors present in plasma such as the phospholipid transfer protein (PLTP; Jauhiainen et al 1993).

Nascent HDL particles are discoidal and comprise a phosphatidylcholine bilayer and a protein shell which shields the hydrophobic lipid tails from the aqueous surrounds (Phillips et al 1998). As it circulates through the body, HDL collects unesterified cholesterol which is stored in the lipid bilayer. LCAT allows a gradient of unesterified cholesterol to be formed when it catalyses amphipathic unesterified cholesterol into hydrophobic cholesteryl esters which collect among the lipid tails. This transforms the discs into spherical (spheroidal) structures in which a hydrophobic core of cholesteryl esters is shielded by lipid and protein (Tansey et al 1997). At this stage, unesterified cholesterol collection stops and the mature HDL particle is recognised and taken up by the liver. A preliminary model of the HDL particle has been proposed (Phillips et al 1997): a circular disc of 160 lipids forming a bilayer, with two apoA-I molecules and 6000 water molecules; a 46 000-atom moiety in total.

Support for Use of Apolipoprotein A-I as a Drug: In-vivo Studies

HDL plasma fraction inhibits the development of fatty streaks and lipid deposition in the aortic wall of cholesterol-fed rabbits (Badimon et al 1989). The $1{\cdot}063-1{\cdot}250\,g\,mL^{-1}$ (HDL–VHDL) significantly reduced lipid deposition in aortas of cholesterol-fed rabbits (Badimon et al 1990). This fraction contains HDL_2, HDL_3 and very-high-density lipoprotein (VHDL). Transgenic mice with high apoA-I and HDL levels in the plasma were significantly protected from the development of fatty-streak lesions (Rubin et al 1991).

Over-expression of apolipoprotein A-I in transgenic mice prevents diet-induced atherosclerosis (Rubin et al 1991) and can reduce the extent of atherosclerosis in apoE knockout mice (Paszty et al 1994). Table 2 lists the types of studies that have been performed in-vivo with apoA-I-containing particles; Table 3 lists some of the confounding

Table 2. In-vivo studies with apolipoprotein A-I.

Composition	Species	Dosage	Major effects	Reference
Lipid-free apoA-I	Human	$1{\cdot}25-10\,mg\,kg^{-1}\,h^{-1}$ over 5 h	Inconclusive on efflux of C, FCR of free apoA-I reduced by PLs	Nanjee et al 1996a
Lipid-free apoA-I	Human	$25\,mg\,kg^{-1}$ bolus	Similar kinetics in plasma to slow infusion	Nanjee et al 1996a
ApoA-I/PC discs	Human	$6{\cdot}25-10\,mg\,kg^{-1}\,h^{-1}$ over 4 h	Increase in HDL-C over 24 h, efflux/esterification of HDL-C	Nanjee et al 1996b
ProapoA-I/PC complexes	Human	1·6 g bolus	20% increase in HDL-C after 24 h	Carlson 1995
ProapoA-I/PC complexes	Human	4 g bolus	40% increase in neutral sterol excretion, over 14 days of administration	Ericksson et al 1995
ApoA-I/PC complexes	Rabbit	0·2 mg bolus	FCR of apoA-I depends on charge, lipid composition and conformation of protein	Braschi et al 1999
Rec. apoA-I M/PC complexes	Rabbit	40 mg on alternate days over 10 days	Reduction in post-injury intimal thickening dependent on timing of infusion	Soma et al 1995
Rec. apoA-I M/PC complexes	Rabbit	40 mg on alternate days over 10 days	Reduced post-injury intimal thickening, reduced macrophage content in intima	Ameli et al 1994
ApoA-I/PC complexes	Rabbit	NA	FCR of apoA-I/PC > apoHDL/PC	Koizumi et al 1988
Rec. apoA-I M/PC	Rat	$20\,mg\,kg^{-1}$ daily over 4–10 days	Delay in time to thrombus formation, inhibition of platelet aggregation, reduction in weight of thrombus	Li et al 1999
Lipid-free apoA-I	Rat	NA	Rapid metabolism of protein in kidney	Ponsin et al 1986
Lipid-free apoA-I	Rat	0·5 mg bolus	Rapid metabolism in kidney	Glass et al 1983

ApoA-I, apolipoprotein A-I; FCR, fractional catabolic rate; HDL, high-density-lipoprotein; NA, not available; PC, phosphatidylcholine; PL, phospholipid; rec. apoA-I, recombinant apoA-I.

Table 3. Variables relevant to in-vivo studies with apolipoprotein A-I.

Isolation and purification techniques used to obtain apolipo protein A-I
Source of apolipoprotein A-I; mammalian or bacterially expressed
Physical state of apolipoprotein A-I; lipid composition, charge and size
Age of apolipoprotein A-I preparation
Animal species
Age of animals
Composition of animal diet: cholesterol-enriched or standard
Quantity of apolipoprotein A-I administered
Type of administration: bolus or infusion
Study duration

factors (variables) that make a comparison of results from different in-vivo studies difficult.

Exogenously administered HDL can prevent (Badimon et al 1989) or regress (Badimon et al 1990) experimentally-induced atherosclerotic plaques in rabbits. In rabbits whose peripheral tissues are loaded with cholesterol by intravenous injection of acLDL or native human LDL, changes in HDL (increase in cholesterol content), signifying cholesterol efflux from cells, mirrors those observed in-vitro. Complexes of apoA-I and phosphatidylcholine or free apoA-I promote efflux of unesterified cholesterol from various cells in-vitro including arterial smooth muscle cells (Stein & Stein 1973; Stein et al 1975), the mouse monocyte/macrophage cell line J774A.1 (Westman et al 1993), resident MPMs (Gelissen et al 1996; Kritharides et al 1996), human skin fibroblasts (Asztalos et al 1997) and HMDMs (Westman et al 1998; Dass et al 1999a; Panzenböck et al, unpublished data).

Delipidated HDL (apoHDL) complexed to phosphatidylcholine has been noted to promote efflux of cholesterol from perfused rabbit aortas in the presence of LCAT activity (Koizumi et al 1988). Injection of these complexes into hyperlipidaemic rabbits decreased plasma cholesterol but increased HDL cholesterol (Koizumi et al 1988). Plasma total phospholipids were increased after injection with the apoHDL/phosphatidylcholine discs. It was shown that radiolabelled phosphatidylcholine that was delivered complexed with apoA-I was used by LCAT in esterification of free cholesterol, with esters being detected in both plasma and HDL. A list of in-vivo studies looking at administration of either free or complexed apoA-I is given in Table 2.

The most feasible route of administration of apoA-I or its complexes may be that of intravenous injection. This would allow the drug entities to gain access to the diseased site in the arteries quickly, as well as to the peripheral cells from where cholesterol exchange may take place. If practical, an intra-arterial route may also be considered as this would provide a direct and rapid exposure of the lesions to the therapeutic lipoproteins. The possibility of delivering these apoA-I particles with sustained-release devices such as miniosmotic pumps needs examination. Finally, the success of these particles in reducing lipid burdens in-vivo would largely be dependent on the state of the lesions, that is, whether they are developing immature soft burdens or mature calcified deposits.

ApoA-I Particles: Importance of Size

It has been suggested that efflux may be independently affected by the size and composition of HDL particles, as well as the properties of apoA-I (Sviridov et al 1996b). However, when discoidal reconstituted HDL (rHDL) particles are compared on the basis of their phospholipid content, parameters such as size, composition and apoA-I conformation do not significantly alter the ability of these particles to promote cholesterol efflux (Davidson et al 1994, 1995a, b). However, apoA-I conformation correlates with the ability of apoA-I in rHDL to activate LCAT (Jonas et al 1989). Sparks et al (1992) have shown that an increase occurs in rHDL major diameter (for discs) with rising cholesterol content.

Addition of 2 molecules of cholesterol to discoidal rHDL significantly increases the stability of apoA-I molecules to denaturation by quanidinium hydrochloride (Sparks et al 1993). However, addition of 6 or more molecules has the opposite effect, significantly reducing the stability of the helical segments of apoA-I. The lipoprotein's ability to form complexes with phospholipids is impaired if cholesterol is present in the medium (Sparks et al 1993). Addition of cholesterol or a reduction in disc major diameter appears to displace apoA-I from the phospholipid bilayer and thereby induces a reorganisation of the helical segments into a more compact structure. Sphingomyelin has been noted to stabilise rHDL particles (Swaney 1983). This has been attributed to the ability of sphingomyelin to enhance apoA-I–phospholipid interactions.

Zhao et al (1996c) found that among lipoprotein A-I particles containing two, three or four apoA-I per particle, lipoprotein A-I containing four apoA-Is were better than those with three, which in turn were better than those with two apoA-Is per rHDL. Lipid-free recombinant apoA-I, as well as purified plasma apoA-I, incubated with CHO cells, is able to assemble nascent HDL particles by recruiting

phospholipids and cholesterol from the cell membrane (Forte et al 1993, 1995). In a study using mouse macrophages and L-cells, Yancey et al (1995) postulated that lipid release from these cells occurred with phospholipid efflux preceding that of cholesterol. This suggests that apoA-I first interacts with the cell to form protein–phospholipid complexes, which in turn are able to accept unesterified cholesterol. Furthermore, efflux of unesterified cholesterol from mouse L-cell fibroblasts and rat Fu5AH hepatoma cells depends on the amount of phospholipid present in the acceptor particle (Davidson et al 1995a). Phospholipid association with apoA-I is also important in enhancing efflux of unesterified cholesterol from MPMs (Gelissen et al 1999).

Nascent apoA-I products formed have diameters of approximately 7·3, 9·0 and 11·0 nm, and formation proceeds in a step-wise fashion where smaller particles are precursors for formation of larger ones (Forte et al 1993). The most common products formed by mixing phospholipids with apolipoproteins are lipid-bilayer discs surrounded by apolipoproteins, which adopt highly α-helical structures (Pownall et al 1987).

While these particles have been analysed on polyacrylamide gels of 8–25% concentration gradient (Frank et al 1997; Tansey et al 1997), gels of up to 40% polyacrylamide are required to run the relatively small particles containing apoA-I to equilibrium. This run to equilibrium is not possible with percentages of up to 35% polyacrylamide, since particles travel off the gels. Particles of known Stokes' diameters are also run alongside samples containing apoA-I to enable estimation of particle sizes. These proteins are usually thyroglobulin (17·0 nm), ferritin (12·2 nm), lactate dehydrogenase (8·1 nm) and BSA (7·1 nm).

For particles prepared with apoA-I, the number of apoA-I molecules/particle is two or more, depending on the ratio of phospholipid to apoA-I. Within each of these particle classes, subclasses of particles of differing lipid levels and sizes can be observed (Nichols et al 1983; Zorich et al 1987). The subclasses are thought to be formed from different apolipoprotein conformations.

Subsequent studies carried out by Forte et al (1996) have, however, revealed that the 9·0- and 11·0-nm particles formed smaller particles upon reincubation with cells. Yet, when the 7·3-nm particles were incubated with additional small quantities of lipid-free apoA-I, larger discoidal complexes were observed. This suggests that the formation of larger particles may be driven by the availability of lipid-free apoA-I and is consistent with previous findings (Forte et al 1993, 1995). The number of apoA-Is per HDL particle increases with size, with 7·3 nm having two, and 9·0 nm containing three, while the 11·0-nm particles contain four molecules of apoA-I (Forte et al 1996). Jonas et al (1989) have shown that Lp2A-I ranges from 7·7 nm (7·2 nm by electron microscopy) to 9·6 nm (9·8 nm) in diameter, while Lp3A-I is 10·9 nm (11·7 nm). Such discrepancy between electron microscopy and GGE (gradient gel electrophoresis) may be due to the staining and preparation of particles before electron microscopy is performed and an inherent degree of uncertainty with GGE.

Another study has revealed that small rHDL particles arise from larger particles of the same class by depletion of phospholipids (Jonas et al 1988, 1989, 1993). Jonas et al (1993) noted that rHDL sizes increase with increasing proportions of phosphatidylcholine to apolipoprotein and with increasing molecular weight of the apolipoprotein. At low palmitoyl-oleoyl phosphatidylcholine (POPC) : apoA-I ratios (Zhao et al 1996d), Lp1A-I are small in size (approximately 5–6 nm), with only one molecule of apoA-I (LpA-I). At a ratio above 11 : 1, LpA-I form well defined complexes containing two apoA-I molecules (Lp2A-I) and are 7·6–7·7 nm in size.

In a recent study with THP-1 macrophage foam cells, Bielicki et al (1999) demonstrated that apoA-I depleted cholesteryl esters within cells by 50% following 24 h of efflux at a concentration of $10\,\mu g\,mL^{-1}$. This reduction was accompanied by an unesterified cholesterol : phospholipid mole ratio of nascent HDL in the medium of 0·80 (±0·15) while complexes isolated from macrophages had an ratio of 0·59 (±0·08). Seventy-percent more nascent HDL were formed from exposing apoA-I to foam cells rather than with macrophage cells. A majority (85%) of particles (7·4, 9·0 and 11·0 nm diameter) from both foam cells and macrophages possessed apoA-I without apoE. In contrast, apoA-I/apoE particles were 13–16 nm in diameter, while a smaller proportion of apoE (only)-containing particles were 19–20 nm.

Small HDL particles serve as intermediate shuttles ferrying cholesterol from plasma membrane to larger HDL particles that function as reservoirs (sinks) where cholesterol is converted to cholesteryl esters (Johnson et al 1991; Fielding & Fielding 1995b; Fournier et al 1996; Rodrigueza et al 1997a). Certain apolipoproteins in their free form interact with cholesterol-loaded macrophages to generate HDL-like particles, and as a result reduce intracellularly-accumulated cholesteryl esters (Hara & Yokoyama 1991). Cholesteryl esters accumulated in macrophages are almost depleted by exposure of cells to HDL solution repeatedly

(Brown et al 1980). This may suggest a more active mechanism for cholesterol removal by HDL from macrophages.

Such a mechanism has also been proposed for albumin, which is a small-diameter molecule, making it an attractive candidate as an initial acceptor for the diffusional (non-specific) component of efflux (Fielding & Fielding 1995a). The small size of the early acceptors of cholesterol may favour insertion of cholesterol. Their small size and greater diffusion coefficient would accelerate their entry into the unstirred water layer surrounding cells or to receptor sites on the membrane surface at rates greater than larger particles (Castro & Fielding 1988).

The Importance of Phospholipids in the Ability of ApoA-I to Efflux Cholesterol

The increased ability of apoA-I to remove cell unesterified cholesterol has been attributed to a change in electrostatic properties of the LpA-I particles (Zhao et al 1996d). Reducing the net negative charge on an HDL particle (by accepting phospholipid molecules) may increase the collisional frequency and rate of transfer of unesterified cholesterol from the plasma membrane. Increased negative charge on an HDL particle is suggested to repel negatively-charged residues on apoA-I. Thus, not only does phospholipid binding reduce such charges, but it also may stabilise, via this neutralisation, the structure of apoA-I molecules. In addition, a neutral HDL particle is anticipated to approach the negatively-charged plasma membrane more easily than a negatively-charged one. Sialic acid residues on the plasma membrane would present some of this anionic hindrance.

It has been demonstrated that either apoA-I or proapoA-I reconstituted discoidal complexes (RDC; phosphatidylcholine plus apoA-I) have an equal ability to sequester cholesterol from laden HMDMs (Westman et al 1995). HMDM cellular cholesterol content may be reduced significantly with as low as $20\,\mu g\,mL^{-1}$ proapoA-I/phosphatidylcholine RDCs. At $200\,\mu g/mL$, significant decreases in both free and esterified cholesterol were seen. In fact, these indices declined for both apoA-I and proapoA-I RDCs dose-dependently at a concentration of approximately $50-100\,\mu g\,mL^{-1}$.

Cell-derived cholesterol is rapidly transferred to small lipid-poor HDL, which contains only apoA-I (LpA-I) and has pre-β1 electrophoretic mobility, and then is transferred from pre-β1 HDL to other lipoproteins in the following order: pre-β2 HDL, α-HDL and finally LDL (Fielding & Fielding 1995). The amount of the pre-β HDL particles may increase in the plasma of hyperlipidaemic individuals (Ishida et al 1987). Interestingly, it has been suggested that discoidal rHDL complexes of pre-β moiety consist of either partially or fully-delipidated apoA-I, while those of α-moiety are actually complexes of apoA-I with phospholipids (Koizumi et al 1988).

Upon association with phospholipids, apoA-I conformation changes substantially (Jonas et al 1989; Bergeron et al 1995). Within each of these particle-size classes, subclasses arise, probably as a result of differing lipoprotein conformations (Jonas et al 1989). Incorporation of few phospholipid molecules into sonicated LpA-I directly affects epitope exposure in a central region of apoA-I (Zhao et al 1996d). This region has been proposed to be a cholesterol-binding region (Sparks et al 1993; Bergeron et al 1995) and to be involved in efflux of unesterified cholesterol (Banka et al 1994; Sviridov et al 1996b).

The differential effects of phospholipids on unesterified cholesterol efflux to sonicated and discoidal LpA-I may be related to alterations in the α-helical thermodynamic stability on the two types of complexes. On sonicated LpA-I particles, apoA-I has a thermodynamic stability less than that of lipid-free apoA-I (Sparks et al 1995a). Thus, on these lipoproteins, apoA-I would rather be in a lipid-free state. Addition of small amounts of unesterified cholesterol to these particles increases the stability of apoA-I (Sparks et al 1993) and thus these particles would retain unesterified cholesterol since it is thermodynamically feasible to do so. However, discoidal LpA-I are generally more stable in comparison to lipid-free apoA-I. This may cause these particles to be metabolised slowly while allowing these particles to store more lipids.

Initial rates of unesterified cholesterol efflux from human skin fibroblasts during the first five minutes are similar for both types of particles but efflux to discoidal LpA-I increases more rapidly over time compared with sonicated LpA-I (Zhao et al 1996d). The superior ability of discoidal LpA-I to efflux may also be due to a larger lipid interfacial surface area allowing more contact area for releasing and binding cholesterol molecules from plasma membranes (Phillips et al 1987; Davidson et al 1995).

Variation in the initial POPC/apoA-I ratio is associated with a 1-nm size variation of the complexes (Frank et al 1997). As the ratio of DOPC/apoA-I decreases, particles of a smaller diameter are formed. Preliminary results from Frank et al (1997) have revealed that at high POPC/apoA-I molar ratios, the complexes formed are more heterogeneous. It would be interesting to find out

what happens when cholesterol is included in this mixture.

ApoA-1 has been postulated to contain a lipophilic hinge domain that is capable of looping out of the lipoprotein particle (Segrest et al 1992). Such a domain may anchor the particle within 0·5–3 nm of the cell surface, thereby decreasing the distance for effluxing unesterified cholesterol molecules (Davidson et al 1994). Furthermore, the layer of water between two lipid surfaces in close proximity should be less polar than bulk phase water. Such an environment is thought to facilitate the transfer of hydrophobic molecules across this space (Rothblat et al 1992).

Liposomal Acceptors: Preparation and In-vitro Characterisation

The second class of lipid acceptors are liposomes (also called phospholipid vesicles, PLVs). These vesicles are synthesised via simple procedures in the laboratory or are available commercially. Advantages of these agents include non-toxicity, economical production and the ability to tailor-make reagents according to the intended specific use (Table 4). Due to their large size ($\geq$100 nm), conventional liposomes are confined mainly within the vasculature. Other cholesterol acceptors, smaller in size and thus capable of accessing the interstitium and plasma membranes, are believed to be partners of liposomes in mobilisation of stored peripheral cholesterol. Thus, it has been proposed that two specific particle interactions (shuttling and remodelling) take place between HDL and PLVs in promoting the mobilisation of cellular cholesterol (Rodrigueza et al 1997a). As intravenously delivered PLVs are cleared predominantly by the liver, it has been postulated that PLVs enhance the ability of HDL to promote reverse cholesterol transport (Rodrigueza et al 1993, 1997a).

Table 4. Advantages of using liposomes.

Formulated from relatively inexpensive chemicals
Prepared using non-hazardous protocols
Can be made from a wide array of molecular components and thus may possess various features
May be formulated using relatively simple methods
Drug may be bound without chemical modification on/within/surrounded by the lipid bilayer
Can be prepared with a relatively high drug : lipid ratio
Non-cytotoxic and biodegradable
Small size and lipidic nature allows extravasation into lesion sites
Size alteration enables control over clearance rates from the bloodstream
Chemical composition may be exploited to enhance circulation times in-vivo
Restrict access of carried materials to the external milieu providing protection against degradation in-vivo

Phospholipids self-assemble into spherical bilayers called vesicles or liposomes when hydrated in aqueous buffer (New 1994). These vesicles are commonly sized using polycarbonate filters with pore sizes depending on the diameter required for the vesicles (Dass 1998; Dass et al 1999b, 2000). Liposomes, like microspheres, may be specialised by the addition of various ligands or chemicals on (or into) its bilayer coat, allowing them to better perform a given task (reviewed in Dass et al 1997a,b). When delivered intravenously at sufficient doses, the vesicles retain their structure in the bloodstream and have been found to extract cholesterol from peripheral tissues and lipoproteins (Rodrigueza et al 1997a). Thus, these liposomes act as sinks, storing cholesterol mobilised from both peripheral cells and the blood vessel wall.

Results from earlier studies using infusions of phospholipids must be treated with caution since the preparations were not characterised properly and their cholesterol-mobilising abilities were not monitored appropriately (Friedman et al 1957). The mechanisms behind the removal of cholesterol reserves in cells by PLVs were proposed over a decade ago, but only recently have they been proven (Rodrigueza et al 1997a,b).

Use of Liposome Acceptors in Cell Culture

Perhaps the most important finding from the Rodrigueza et al (1998) study was that, theoretically, it is possible to reduce years of cholesterol accumulation within weeks with phospholipid infusions. This removal may be further enhanced with the aid of agents such as lipid-free apoA-I or cyclodextrins. However, several incipient problems may hinder efficient use of these vesicles: PLVs (100 nm in diameter) alone are inefficient in removing cholesterol from cultured cells (Davidson et al 1995; Rodrigueza et al 1997a; Jessup & Kritharides, unpublished results) and PLVs (100 nm) are considered too large to penetrate the vasculature and enter the interstitium to accept cholesterol from peripheral cells or extracellular deposits (due to cell lysis) located deep within lesions. In contrast, HDL particles are small enough to gain access to peripheral cells and are also found to be located throughout plaques (Stein & Stein 1979). They are also capable of removing sufficient amounts of cholesterol from cells, including macrophages (Gelissen et al 1996; Dass et al 1999a).

The rate of spontaneous transfer of cholesterol between membranes is a function of the degree of saturation of the phospholipid fatty acyl chains and the sphingomyelin content of the membrane (Phillips et al 1987; Bittman 1988). It is also a function of the curvature of the surface from which desorption occurs (McLean & Phillips 1984; Fugler et al 1985). Furthermore, the rate of release of cholesterol from donor vesicles and transfer to acceptor vesicles is sensitive to factors that modify the aqueous-phase solubility of cholesterol, like the presence of chaotropic salts (Clejan & Bittman 1984) and polar water-miscible organic solvents (Bruckdorfer & Sherry 1984).

Large unilamellar vesicles (LUVs, diameter $\geq$ 1 μm) made from POPC were also noted to strip HDL particles off unesterified cellular cholesterol (Rodrigueza et al 1997). Apart from removing cholesterol from HDL particles, the LUVs donate phospholipid to the particles allowing them to efflux more cholesterol from Fu5AH cells (Rodrigueza et al 1997a).

It has been demonstrated that PLVs are unable to deplete cholesteryl ester mass from MPMs under conditions where HDL depletes 75% of the cholesteryl esters (Ho et al 1980). Phospholipid liposomes can remove free cholesterol from cholesterol-laden cells but these vesicles have limited capacity to deplete the intracellular pool of cholesterol available for esterification by ACAT unless apolipoproteins are also present (Mendez et al 1994). However, as mentioned above (aqueous diffusion model), other studies have revealed that cholesterol removal in cultured cells depends on desorption from the plasma membrane and is not affected by HDL apolipoproteins (Karlin et al 1987; Mahlberg & Rothblat 1992; Rothblat et al 1992). For instance, removal of free cholesterol from cells was effected by PLVs in the absence of apolipoproteins (Rothblat & Phillips 1982) and by phospholipid–albumin complexes (Bartholow & Geyer 1982). These studies suggest an apolipoprotein-independent pathway of cholesterol efflux. Furthermore, emulsions of phospholipids and triglycerides have been shown to deplete intracellular cholesterol (as esters) from macrophages during long incubations (Hara & Yokoyama 1992).

When incubated with Fu5AH rat hepatoma cells (Rodrigueza et al 1997), LUVs made of POPC at 1 mg mL^{-1} phospholipid, effluxed approximately 20% of unesterified cellular cholesterol label and mass in a slow continuous fashion (half-time for unesterified cholesterol efflux, approximately 50 h). In contrast, HDL_3 at 25 μg mL^{-1} effluxed approximately 15% unesterified cellular cholesterol label with no effect on mass ($t_{\frac{1}{2}} = 4$ h). However, when both LUVs and HDL_3 were incubated with cells simultaneously, over 90% of unesterified cholesterol label and approximately 50% of mass ($t_{\frac{1}{2}} = 4$ h) were extracted.

Unesterified cholesterol release $t_{\frac{1}{2}}$ values from cells by rHDL, small unilamellar vesicle (SUV, diameter $\leq$ 200 nm) and large unilamellar vesicle (LUV) particles are approximately 15, 45 and 235 min, respectively (reviewed in Davidson et al 1995). Thus, there is a general correlation between unesterified cholesterol efflux capacity and acceptor size, with small particles being more effective than larger ones when compared on the basis of phospholipid composition. LDL, which has a diameter of approximately 25 nm (similar to SUVs), exhibits similar capacity to efflux unesterified cholesterol from mouse L-cell fibroblasts and rat Fu5AH hepatoma cells (Davidson et al 1995b). However, correlation between size and unesterified cholesterol efflux capability does not extend beyond 70 nm and below 12 nm diameters. The frequency of collisions between dissolved unesterified cholesterol molecules and acceptor particles is inversely proportional to the particle radius and directly proportional to the number of acceptor particles present (Davidson et al 1994).

In-vivo Administration of Liposomes

Intravenous infusion of phospholipids into animals with experimentally-induced atheroma favourably influences the atheroma (Friedman et al 1957; Adams et al 1967) but the effect is limited by the rapidity with which phospholipid vesicles are cleared from plasma (Dewailly et al 1976). In contrast, it has been noted that apoA-I/phosphatidylcholine complexes are cleared from plasma at a slower rate than phosphatidylcholine alone (Malmendier et al 1983). The ability of phospholipids to act as acceptors of cholesterol from cells in culture was demonstrated seminally by Burns & Rothblat (1969). Repeat doses of phospholipid reverses atherosclerosis in a variety of animal models. Infusion of polyunsaturated phospholipids into rabbits on a cholesterol-rich diet reduces lipid levels in the liver (Adams et al 1967).

Not all PLV therapy may be beneficial for prevention of atherogenesis. It has been demonstrated in rabbits that administration of SUVs suppresses LDL receptor and HMG-CoA reductase mRNA expression (Rodrigueza et al 1997b) while increasing the content of apoB-rich atherogenic lipoproteins (Williams & Scanu 1986; Rodrigueza et al 1997b). Similarly, an increase in plasma LDL level is seen when human subjects are administered

apoA-I/phospholipid discs (Kuivenhoven et al 1996).

For cholesterol mobilisation in mice, liquid crystalline LUVs were found to be best (Rodrigueza et al 1993). These PLVs delivered effluxed cholesterol to the liver at a rate of 0·5 mol cholesterol/mol phospholipid injected. These vesicles are easy to prepare and are non-toxic even at high doses. LUVs made from egg phosphatidylcholine possess a large surface area for acceptance of cholesterol in-vivo. In addition, these vesicles have longer circulating times than multilamellar vesicles (MLVs) or even SUVs (Rodrigueza et al 1993); in rabbits (Rodrigueza et al 1998), the circulating half-life was 30 h. MLVs are rapidly cleared from the circulation due to their heterogeneous size distribution (Semple et al 1998), while SUVs are unstable as a result of their highly-strained curved bilayer conformations (Parente & Lenze 1984; Scherphof & Morselt 1984). Importantly, SUVs do not cause an elevation of plasma LDL (Rodrigueza et al 1997b).

The injection of PLVs (114 nm average diameter) at 300 mg kg^{-1} via the marginal ear vein into New Zealand White rabbits resulted in a 2·5-fold increase in plasma total cholesterol levels in animals on a cholesterol-enriched (0·5%) diet and animals maintained on a standard diet (Rodrigueza et al 1998). This rise was due solely to unesterified cholesterol levels rising in the plasma while cholesteryl ester levels remained constant, as noted previously (Rodrigueza et al 1997b). Akin to an earlier observation in mice (Rodrigueza et al 1993), the lipoprotein pool of cholesterol equilibrated with the PLVs in rabbits (Rodrigueza et al 1998). The authors state that this dose is higher than is presently used in liposomal drug delivery but is below the level of lipid given clinically to patients receiving daily parenteral nutrition in the form of Intralipid.

Rodrigueza et al (1998) estimated that about 4 g of cholesterol was mobilised from peripheral tissues in the rabbits. These authors found that ten injections each of 300 mg kg^{-1} phospholipid at ten-day intervals did not cause an alteration in the clearance profiles in either diet-enriched or standard-chow rabbits. Non-toxicity was also demonstrated in mice (Rodrigueza et al 1993). Importantly, erythrocyte cholesterol levels remained constant at 150 nmol 10^{-9} cells during the treatment with PLVs, similar to the previous murine study (Rodrigueza et al 1993). Thus, these vesicles would be more beneficial for use than certain cationic liposomes which cause haemolysis of erythrocytes (reviewed in Dass & Burton 1999). However, other sites should have been monitored, such as the organs of the mononuclear phagocytic system, namely the liver and spleen, which avidly extract vesicles from the bloodstream (reviewed in Dass et al 1997d). There have been murine studies where 80% of the liposomal load was cleared by the liver (Rodrigueza et al 1993). Lipids from vesicles are converted into bile acids in the liver and excreted (Roerdink et al 1981).

Liposome clearance in-vivo depends predominantly on the coating of the vesicular surface with proteins in serum. These proteins include albumin, complement and related proteins, immunoglobulins, fibronectin, C-reactive protein and β2-glycoprotein I (reviewed in Semple et al 1998). Liposomes are also targeted by apolipoproteins such as A-I, A-II, A-IV, B, C and E (Guo et al 1980; Williams & Scanu 1986). Interaction of vesicles with apolipoproteins results in uptake by macrophages due to the opsonising effect of the apolipoprotein binding. Apolipoproteins have also been implicated in the uptake of liposomes by hepatocytes via apoB and apoE receptors (Williams et al 1984; Bisgaier et al 1989).

Lesions in PLV-treated rabbits harboured less lipid deposits and demonstrated reduced plaque thickening compared with animals receiving carrier medium (saline) only (Rodrigueza et al 1998). The arch and thoracic regions of PLV-treated rabbits exhibited moderate reductions in intimal : medial width ratios, while no changes were evident in the abdominal aorta. The thoracic lesions were lipid-rich, softer and more malleable to the touch compared with those in the arch. Clinically, softer, less-stable plaques rich in macrophage foam cells appear to be prone to rupture.

Inhibition of Reverse Cholesterol Transport by Oxysterols

There is now increasing evidence that oxidised products of cholesterol (oxysterols) contribute to the process of atherogenesis (reviewed in Brown & Jessup 1999). The major oxysterol present in lesions and foam cells is 7-ketocholesterol, which is produced by non-enzymatic mechanisms. Relative to cholesterol, 7-ketocholesterol is present at levels of 0·3% and 1·1% in human carotid atherosclerotic lesions and human macrophage foam cells isolated from advanced lesions, respectively (reviewed in Brown & Jessup 1999). The oxidative precursor of 7-ketocholesterol, 7-hydroperoxycholesterol, has also been detected in human atherosclerotic lesions (Brown et al 1997). This suggests that at least some of the 7-ketocholesterol present in lesions is synthesised in-situ, since dietary 7-hydroperoxycholesterol is not expected

to survive transport from the intestine to the circulation.

Oxysterols may contribute to the generation of the highly thrombogenic necrotic core of advanced atherosclerotic plaques (Guyton et al 1990). Oxysterols accumulate in human macrophage foam cells derived from atherosclerotic plaques (Mattsson-Hulten et al 1996) and have the potential to affect reverse cholesterol transport to an extracellular acceptor (Gelissen et al 1996). Mouse peritoneal macrophages loaded with copper-oxidised low-density lipoprotein (OxLDL) were less able to transport intracellular cholesterol to apoA-I than were cells loaded with acetylated LDL (AcLDL). Interestingly, much less 7-ketocholesterol was effluxed in both absolute and proportional terms. Similar findings have been recorded with HMDM foam cells (Dass et al 1999a; Jessup et al 1999; Wilson et al 2000).

Approximately 1% of the Western diet consists of oxidised cholesterol (van de Bovenkamp et al 1988). Major dietary oxysterols are 7-ketocholesterol (also called 7-oxocholesterol), 7αOH, 7βOH and cholesterol-α- and -β-epoxide (reviewed in Brown & Jessup 1999). Foods rich in cholesterol such as eggs and dairy and meat products are major dietary sources, especially those foods which are heated in air or stored for lengthy periods. For instance, 7-ketocholesterol was found to be the dominant oxidation product in butter and dairy spread (1·3 and 5·7 $\mu g\, g^{-1}$ milk lipid, respectively) after 13 weeks storage at 4°C (Nielsen et al 1996). These oxysterols are absorbed in the intestine and are initially transported in chylomicrons (Vine et al 1997). In plasma, oxysterols are esterified in similar proportions to cholesterol (Szedlacsek et al 1995).

Rooney et al (1986) found that 7-ketocholesterol orders phosphate groups by reducing their thermal mobility to a degree equivalent to the low-temperature packing lattice of pure dipalmitoylphosphatidylcholine (DPPC) bilayers. This was taken to suggest general interactions between the 7-position group of the oxysterol and the head groups of the DPPC. In addition to the phosphate groups, 7-ketocholesterol is noted to increase the packing order of ester groups in the bilayer interface region. In summary, these results could indicate that the partial intercalation of the sterol nucleus into the bilayer results in a steric hinderance of the phospholipid headgroup motions by 7-ketocholesterol. For instance, cholesterol oxides have been noted to accumulate in, and cause membrane damage associated with, cataracts (Girao et al 1998). 7-Ketocholesterol was found to be the second major oxysterol (4·2 mmol/mol cholesterol) after 7-β-hydroxycholesterol (7·3 mmol/mol cholesterol) present in human cataracts.

Evaluating the ability of an *Escherichia coli* signal peptide (LamB) to bind to bilayers containing combinations of neutral lipids of egg phosphatidylcholine, sphingomyelin, cholesterol, ketocholesterol, and nitroxide-containing phospholipid, Voglino et al (1998) found that binding was nearly completely inhibited in bilayers composed of phospholipids with a nitroxide moiety at the 7 position in one of their acyl chains, or in egg phosphatidylcholine containing equimolar ketocholesterol. The authors proposed that the incorporation of nitroxide or ketone groups into the HC region near the lipid headgroup increases the effective width of the hydrophilic interfacial region, thus preventing some of the hydrophobic amino acids in the α-helix from reaching the nonpolar HC centre. This is believed to reduce the free energy of partitioning and inhibiting peptide binding. Thus, peptide-lipid interactions are heavily dependent on interfacial dipoles. This may also be the way by which 7-ketocholesterol inhibits apoA-I from interacting with the plasma membrane.

There is a deficient induction by apoA-I of cholesterol efflux from macrophages loaded with oxLDL (Kritharides et al 1995). There is also an accompanying marked impairment of the release of 7-ketocholesterol, the major oxysterol in these cells. Recent studies confirm that intracellular accumulation of oxysterols in macrophages can impair cholesterol efflux (Gelissen et al 1996; Dass et al 1999a). 7-Ketocholesterol-supplemented LDL that is acetylated and taken up by MPMs results in most of the cellular 7-ketocholesterol being esterified, indicating that 7-ketocholesterol, thus delivered, acts as a substrate for ACAT (Gelissen et al 1996). In this study, the 7-ketocholesterol content of cells was 50% of total cellular sterol in the form of 7-ketocholesterol (both esterified 7KE and unesterified 7-ketocholesterol), more than 50% of its 7-ketocholesterol content being in an unesterified form. Unesterified cholesterol efflux, from MPMs loaded with 7-ketocholesterol-AcLDL to apoA-I, was impaired in cells containing > 50 nmol 7-ketocholesterol/mg cell protein in contrast to cells loaded with oxysterol-free acetylated LDL. In fact, efflux of 7-ketocholesterol was inversely proportional to the quantity of 7-ketocholesterol in MPMs. In contrast, inhibition of cholesterol efflux by apoA-I from HMDMs commences at a 7-ketocholesterol cellular content of 5% or 5 nmol/mg cell protein of total 7-ketocholesterol (unpublished results). Figure 3 depicts the various functions of 7-ketocholesterol, most of which constitute ways by which 7-ketocholesterol may contribute to the process of atherogenesis.

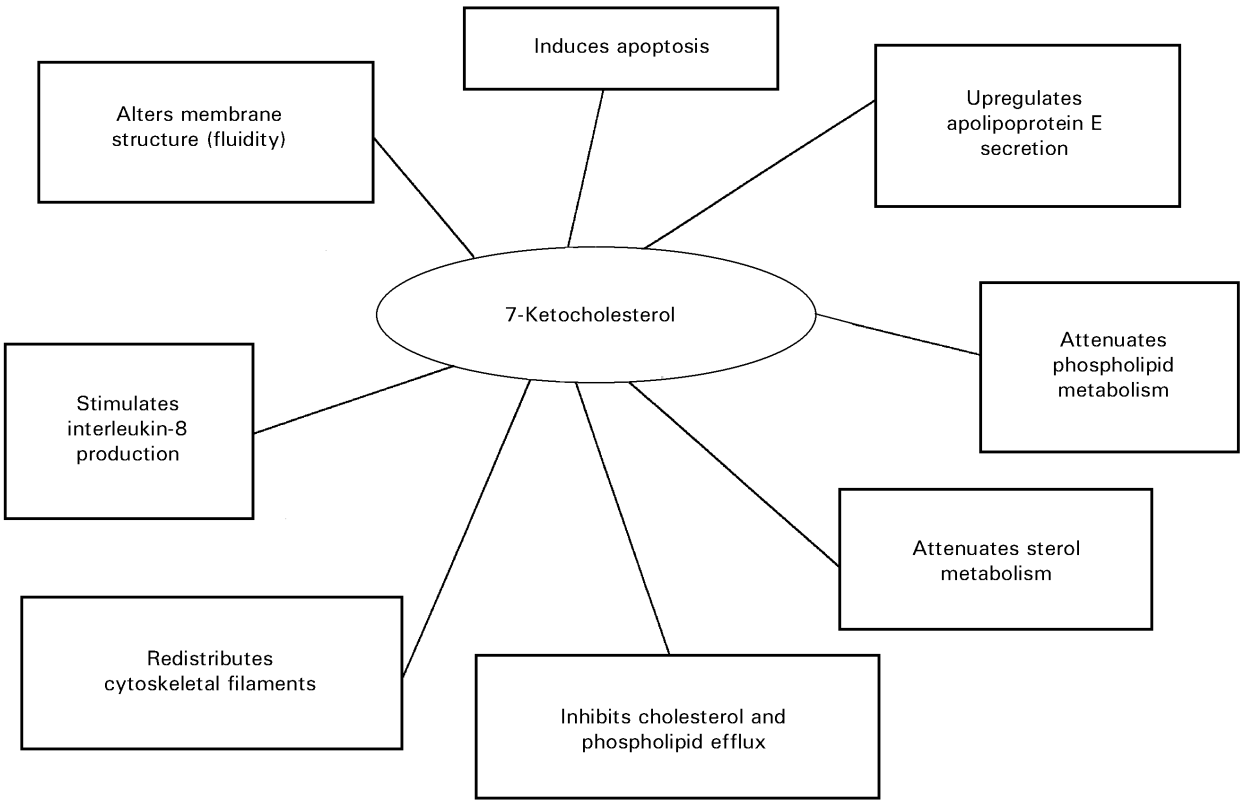

Figure 3. Effects of 7-ketocholesterol on mammalian cells. All these effects are potentially pro-atherogenic, except the upregulation of apolipoprotein E secretion which serves to remove cholesterol from cells.

Selective efflux of 7-ketocholesterol from MPMs has been attained by use of hydroxypropyl-β-cyclodextrin (hpβCD), a cyclic sugar polymer, at a concentration of $0-5\,mg\,mL^{-1}$ (Kritharides et al 1996). Efflux of 7-ketocholesterol was concentration- and time-dependent and the rate of removal from cells was 50-fold that achieved with lipid-free apoA-I. Intracellular free 7-ketocholesterol and 7KEs decreased simultaneously with an increase of 7-ketocholesterol in the medium. Physical solubilisation of 7-ketocholesterol with hpβCD was greater than that achieved with unesterified cholesterol. Efflux of other oxysterols was also enhanced with hpβCD. However, while this selective efflux occurs in HMDMs as well, efflux differential is less than seen with MPMs (unpublished results). Interestingly, cholesterol efflux with other cyclodextrins such as methylated β-cyclodextrin (meβCD), trimethylated β-cyclodextrin (trimeβCD) and carboxyethylated β-cyclodextrin (ceβCD) are inhibited when cells are loaded with 7-ketocholesterol.

Background to Cyclodextrins

Cyclodextrins are oligosaccharides that are produced by an enzymatic modification of starch. β-Cyclodextrin contains seven glucopyranosyl (glucosyl) units linked in a ring formation in the $\alpha(1-4)$ position (Duchene 1987). Hydrogen and glycoside oxygen atoms are oriented toward the pore, thereby rendering it hydrophobic. β-cyclodextrin has the capacity to form inclusion complexes with various molecules, especially bile acids and sterols (Riottot et al 1993; Klein et al 1995). Such binding hinders the absorption of bile acids and sterols from the small intestine. Host-guest bonds include H-bonds and hydrophobic or ionic interactions between the cyclodextrin cavity and the guest molecule (Bolyan & Ganzler 1998).

Apart from β-cyclodextrin, there exist α- and γ-cyclodextrins which possess six and eight α-(1–4)-glucopyranose units. All forms of cyclodextrins may contain additional functional groups which modify the hydrophilic/hydrophobic properties of the parent molecule (Szejtli 1988). Cyclodextrins have been utilised for delivery of pharmacologically active hydrophobic compounds within polar environments. Some of the therapeutic agents so complexed include testosterone (Muller & Albers 1991; Salehian et al 1995), oestradiol (Brewster et al 1988), β-sitosterol, the main dietary phytosterol (Awad et al 1996), and the antiepileptic drug

carbamazepine (Brewster et al 1991, 1997). Apart from drug molecules, cyclodextrins have also been used to host sterols such as cholesterol and 27-hydroxycholesterol (Winegar et al 1996).

Chemically-modified β-cyclodextrins, such as 2-hydroxypropyl β-cyclodextrin (2hpβCD) and methylated β-cyclodextrin (meβCD), have enhanced features with regards to solubility, complex formation and toxicity. β-cyclodextrins have a high affinity for sterols compared with other lipids, making these second generation compounds more effective in modifying cholesterol metabolism in-vivo (Riottot et al 1993).

While β-cyclodextrin is not hydrolysed by salivary or pancreatic α-amylase (Suzuki & Sato 1985), it is fermented in the large intestine by resident microflora in animals (Suzuki & Sato 1985; Levrat et al 1994) and humans (Antenucci & Palmer 1984; Flourie et al 1993) to glucose and oligosaccharides. They are known to be negligibly hydrolysed in, and slightly absorbed from, the stomach and small intestine (Andersen et al 1983; Antenucci & Palmer 1984; Flourie et al 1993). Following oral administration of β-cyclodextrin in humans, only traces are recovered in stools (Flourie et al 1993).

By an entrapment of bile acids in the ileum, β-cyclodextrin increases their cecal pool, an effect that is directly proportional to the dietary level of β-cyclodextrin (Favier et al 1995; Ferezou et al 1997). Also, the solubility of bile acids in the large intestine remains low despite degradation of β-cyclodextrin, which may represent a mechanism of limiting bile-acid salvage from the large intestine. In pigs, inclusion of 5 or 10% β-cyclodextrin in a cholesterol-enriched diet produced a dose-dependent increase in faecal elimination of bile acids (Ferezou et al 1997).

β-Cyclodextrins form stable inclusion complexes with cholesterol by incorporating it into their hydrophobic cavity. In the rat, β-cyclodextrin is able to decrease low density lipoprotein (LDL) cholesterol with minimal effect on HDL cholesterol (Favier et al 1995). Such a mechanism could lead to a lower LDL cholesterol level in species in which plasma cholesterol is loaded principally on LDL. However, a marked reduction in rat plasma HDL1 and LDL cholesterols as well as apolipoprotein B and E concentrations were noted by Moundras et al (1994) when animals were fed β-cyclodextrin.

In addition, β-cyclodextrin has an interesting lipid-lowering effect especially on plasma triglycerides (Favier et al 1995). This favours hypocholesterolaemia (Riottot et al 1993; Levrat et al 1994). β-Cyclodextrin is fermented mainly to propionate in the large intestine (Levrat et al 1994; Moundras et al 1994). The three different forms of cyclodextrins (α, β and γ) have been used to alter the composition of erythrocyte membranes (Klein et al 1995) while β-cyclodextrin has been noted to selectively extract cholesterol from plasma membranes (Neufeld et al 1996).

Cholesterol Efflux Mediated by Cyclodextrins

Cholesterol efflux by the available cyclodextrins differs. Cholesterol can be extracted from cell membranes by cyclodextrins (Kilsdonk et al 1995; Klein et al 1995). However, in a study utilising monolayer membranes consisting of either cholesterol alone or in association with either DPPC or *N*-palmitoylsphingomyelin, α-cyclodextrin was incapable of inducing efflux while efflux with γ-cyclodextrin was lesser than with β-cyclodextrin (Ohvo & Slotte 1996). These discrepancies are attributed to a smaller cavity in α-cyclodextrins and a cavity that is less hydrophobic in γ-cyclodextrins.

This study (Ohvo & Slotte 1996) also highlighted the fact that desorption of cholesterol from a membrane of both cholesterol and phospholipid was slower than that from a membrane of purely cholesterol. Furthermore, desorption was more retarded from sphingomyelin monolayers than from phosphatidylcholine monolayers. Depletion of plasma membrane sphingomyelin greatly accelerates cyclodextrin-mediated cholesterol removal (Neufeld et al 1996). Cholesterol interfacial packing is tighter in sphingomyelin than in phosphatidylcholine bilayers. Both inter- and intramolecular hydrogen bonding is possible in sphingomyelin bilayers in contrast to phosphatidylcholine bilayers where less extensive intermolecular and no intramolecular hydrogen bonding is possible. An intermolecular hydrogen bond has been thought to contribute to the high affinity of sphingomyelin for cholesterol (Boggs 1987).

Cyclodextrin loses its ability to suppress esterification (by preventing internalisation of plasma membrane cholesterol to organelles) when plasma membrane sphingomyelin is depleted (Neufeld et al 1996). This has been attributed to a diversion of lysosomal cholesterol away from the plasma membrane without compromising its ability to reach the endoplasmic reticulum when plasma membrane sphingomyelin is depleted. At high cyclodextrin concentrations (10–100 mM), cyclodextrin per-se can trap cholesterol and serve as a sink (Kilsdonk et al 1995; Yancey et al 1996). Atger et al (1997) noted that although addition of cyclodextrins to serum enhanced the exchange of cholesterol between cells and serum, only the

combination of cyclodextrin and LUVs shifted the equilibrium and produced net clearance.

Using J774 mouse macrophage cells, Atger et al (1997) noted that efflux of cholesterol with various cyclodextrins were as follows: methyl-β-CD > β-cyclodextrin > 2OHβCD > carboxymethyl-β-CD > tetradecasulphated-β-CD. A more extensive study examining the efflux potentials of various cyclodextrins with HMDMs has been performed (unpublished results) and elucidates the ranking to be similar. Cyclodextrin specifically targets removal of cholesterol from the cell surface (Neufeld et al 1996). In our laboratory, the ability of cyclodextrin to remove free cholesterol while not removing the cholesteryl ester stores have been noted in both MPMs and HMDMs (unpublished results). Conversely, incubation of cyclodextrin–cholesterol complexes with cells depleted of plasma-membrane cholesterol leads to a restoration of plasma-membrane cholesterol (Klein et al 1995).

In several cell types (Neufeld et al 1996; Yancey et al 1996), biexponential kinetics of cellular cholesterol efflux were observed, suggesting the presence of two kinetic pools of cholesterol: a fast pool ($t_{\frac{1}{2}}$ = approximately 19–23 s; Yancey et al 1996) and a slow pool ($t_{\frac{1}{2}}$ = 15–30 min). Cell cholesterol content was altered by incubating cells with cyclodextrins complexed with increasing levels of cholesterol. The number of pools remained at two, but the size of the fast pool increased. After depleting cells of the fast pool, this pool was replaced in its entirety following a 40-min recovery interval. Yancey et al (1996) also deduced that cholesterol in the slow efflux pool is exchangeable with the fast pool. Movement of cholesterol from the slow to the fast pool appeared to follow first-order kinetics with a $t_{\frac{1}{2}}$ of between 20–30 min.

Similar results have been noted with apoA-I-mediated efflux from human skin fibroblasts (Zhao et al 1996a). A rapid efflux phase is observed during the first 15 min, which reaches a plateau up to approximately 30 min. This precedes a second efflux phase which lasted 30–60 min in that study. The biphasic efflux may be due to a transition of intracellular pools of unesterified cholesterol to the plasma membrane for efflux (thus making up the second phase of efflux). However, this biphasic nature of unesterified cholesterol efflux is not present in smooth muscle and endothelial cells (Zhao et al 1996a). It has been suggested that the primary phase represents the release of plasma membrane unesterified cholesterol into the medium, while the second phase would signify the collisional properties or the ability of the lipoprotein particle to bind and retain unesterified cholesterol.

Cyclodextrins, being able to release and retain unesterified cholesterol better than lipoproteins, are able to reduce the first efflux phase time significantly. This is attributed to the hydrophobic cavity of the cyclodextrins as well as the smaller diameter of these particles. With lipoproteins, the unesterified cholesterol released across the distance between the plasma membrane and the acceptor, determines the time taken for unesterified cholesterol to be retained by the acceptors. However, the second phase is similar for both types of acceptors since this phase is predominantly influenced by the ability of the acceptor to actively accept unesterified cholesterol. At this stage, both lipoproteins and cyclodextrins are close enough to accept unesterified cholesterol at similar rates.

In our laboratory, it has been noticed that the removal of plasma membrane unesterified cholesterol from HMDMs by trimeβCD either alone or in combination with lipid-free apoA-I results in the macrophages assuming more pseudopodia in comparison with those incubated with BSA or A-I alone (unpublished results). It may be speculated that the removal of unesterified cholesterol (and possibly phospholipids) from the plasma membrane causes a relaxing of the membrane structure, thus stimulating pseudopodial development. In fact, Irie & Uekama (1997) state that both phosphatidylcholine and sphingomyelin are removed by cyclodextrins from the outer half of the plasma membrane bilayer, leading to the formation of stomatocytes due to the inward bending of the plasma membrane. The authors also claim that proteins are removed from the membranes, probably as an after-effect of the gradual loss of lipids that normally anchor membrane-bound proteins in place.

The macrophages may also be responding to a cytotoxic phenomenon such as FC (free cholesterol) depletion of plasma membrane FC by attempting to relocate from the assault. Similar results have been shown to occur in rabbit alveolar macrophages where freshly isolated alveolar macrophages, when incubated with bronchoalveolar lavage fluid (BALF) containing high amounts of phospholipids, did not migrate as much as when the alveolar macrophages were incubated in surfactant-free medium for 24 h (Tanaka et al 1997). Interestingly, the inhibition of migration by a fraction of the BALF enriched in phospholipids, was reversed by normal serum, but not by heat-inactivated serum. This may be the result of the action of LCAT, catalysing the addition of lecithin to unesterified cholesterol on serum-dwelling apolipoproteins.

It has been proposed that, in-vivo, the retention of cholesterol in the sink would be greater if cholesterol was first converted to cholesteryl esters by

LCAT activity (Atger et al 1997). This would enable the HDL particles to internalise the esters, thereby freeing the acceptor for desorption of more cholesterol from cells (Stein & Stein 1973). The efficiency of an acceptor is linked partially to its ability to readily diffuse through the unstirred water layer and cell glycocalyx and concentrate at the cell surface (Phillips et al 1987; Davidson et al 1995b). In addition, small acceptors will diffuse to the cell surface more rapidly than larger particles (Davidson et al 1995b).

Cyclodextrins have diameters around 1·5 nm (compared to 7–12 nm for HDL particles) and as such, can diffuse more readily through the unstirred water layer and also filter more readily through the glycocalyx that normally restricts access of larger particles from contact areas on the plasma membrane (Davidson et al 1995b). Cyclodextrins are also quoted as having diameters less than 1 nm (von Eckardstein 1996). Thus, this small acceptor can overcome cell-specific differences in cholesterol efflux (Kilsdonk et al 1995). In fact, β-cyclodextrin has been noted to function as a cholesterol shuttle catalysing the exchange of cholesterol between cells and serum lipoproteins (Atger et al 1997). The rate-limiting step in the aqueous diffusion pathway of cholesterol efflux would seem to be the release of sterol from the surface of the donor particle. Van der Waals interactions with phospholipids in the donor membrane and aqueous-phase solubility are important factors that determine the ease with which sterols undergo intermembrane exchange.

Hydroxypropyl-β-cyclodextrin, with the aid of POPC LUVs, promotes approximately 40% of cellular cholesterol efflux within 2 h from Fu5AH rat hepatoma cells (Rodrigueza et al 1997b). LUVs efflux only 1·5% and cyclodextrin only 2% over the same time-period. Cyclodextrins are able to remove cholesterol from cells efficiently due to their ability to reduce the activation energy for cholesterol efflux from 20 kcal mol^{-1} (required for movement of cell cholesterol to phospholipid acceptors) to 7–9 kcal mol^{-1} (Yancey et al 1996). This difference has been attributed to the need for cholesterol molecules in the plasma membrane to desorb completely into the aqueous phase before being absorbed by HDL particles or phospholipid liposomes. This is in contrast to the direct incorporation into the hydrophobic cavity of the cyclodextrin molecule without the necessity for travelling through an intermediate aqueous phase (Phillips et al 1987; Yancey et al 1996).

Hydroxyalkylated cyclodextrins such as hydroxypropyl-β-cyclodextrin (hpβCD): are $>$ 50-fold more soluble in water than native precursors, and hence do not precipitate lipoproteins; unlike their native cousins, are non-toxic to animals (Olivier et al 1991; Riottot et al 1993; Ferezou et al 1997) including humans (Salehian et al 1995); and solubilise both cholesterol and 7-ketocholesterol (Kritharides et al 1996). Kritharides et al (1996) proved that hpβCD removes 7-ketocholesterol from MPMs loaded with OxLDL more effectively than apoA-I and solubilises and removes 7-ketocholesterol preferentially compared with cholesterol. However, such a mechanism has not been proven to exist in HMDMs with hpβCD (unpublished results). Other cyclodextrins such as methylβCD and trimethylβCD are capable of removing substantial quantities of cholesterol while again failing to increase efflux of 7-ketocholesterol from these cells.

It is interesting to ponder why there is preference between two such closely related sterols as cholesterol and 7-ketocholesterol. It has been postulated that cyclodextrin complex guest molecules based on their chiral isomerism as well as positional isomerism (Janini et al 1996). Thus, they are now being used in chromatography as stationary phases bonded to a solid support or as mobile-phase additives to separate isomers (Bressolle et al 1996; Knoche et al 1996). Thus, structural differences between cholesterol and its oxidised cousin 7-ketocholesterol, may be responsible for the discrepancy in efflux. In any case, when the same HMDM cells are loaded with more 7-ketocholesterol, a significant quantity of 7-ketocholesterol efflux occurs with certain cyclodextrins (unpublished results).

Apart from removing cellular cholesterol, cyclodextrins have been employed to monitor monolayers (Ohvo & Slotte 1996) and the intracellular movement of cholesterol in cultured cells (Neufeld et al 1996), as well as loading cholesterol into mammalian cells (Klein et al 1995; Christian et al 1997). Loading of cholesterol with either LDL or modified LDL results in distribution of cholesterol among all intracellular cholesterol pools (Brown & Goldstein 1986; Huang et al 1993; Fielding & Fielding 1995a). With cyclodextrin loading, cholesterol is presumably delivered to the plasma membrane only. In addition, the cholesterol thus delivered is able to be metabolised by ACAT. Finally, the preparation of cyclodextrin/cholesterol complexes is fast and simple, and can also be used for introduction of sterols other than cholesterol.

Exposure of HepG2 cells to 0·5% 2hpβCD stimulates cholesterol efflux within minutes and increases cholesterol synthesis over a 1–2-day period (Peluso & Dixon 1997). Administration of the cyclodextrin over 15 days increases unesterified cellular cholesterol content by 25–41%, decreases

the triglyceride content by 59%, and increases apoB secretion 3- to 4-fold, whilst the cholesteryl ester content remains the same. In contrast, when cyclodextrin-complexed cholesterol ($20\,\mu g\,mL^{-1}$) is delivered to cells, followed by a 1-day equilibration period (without cholesterol), unesterified cellular cholesterol increases by 76% and cholesteryl ester content increases 10-fold, while apoB secretion is not affected. The authors postulate that cyclodextrin administration (0·5%) increases cellular cholesterol content and flux in different cellular compartments, thereby protecting nascent apoB from proteolysis, and thus facilitating apoB secretion.

Cyclodextrins as Drugs and Drug-delivery Vehicles

Perhaps the major obstacle to in-vivo efficacy of cyclodextrin therapy is the localisation of the effects of the cyclodextrin molecules to the injured site. Cyclodextrins, being relatively small in size compared with PLVs or non-adsorbing to surfaces such as that which occurs with apoA-I, may be targeted to lesions (in the context of atherosclerosis) by the use of double-balloon catheters or mini-osmotic pumps. While mini-osmotic pumps deliver drugs at a constant rate and are available at various flow rates, their efficiency is dependent on several factors, including temperature (Walker et al 1998). In arteries clogged with lipid deposits, localised delivery with these pumps presents a challenge, since an invasive approach into the artery may prove technically difficult. Additionally, delivery into the artery transports the molecules from the injured site. Side-effects may become a problem, but not so much as with just intravascular (systemic) delivery. However, administration via these pumps ensures a constant supply of cyclodextrin molecules for bathing the lesion site.

Mini-osmotic pumps ensure the following important objectives of drug delivery to be met: precise delivery of the active agent to the target site; controlled (sustained) release of the active agent at the target site; minimisation of dilution of the agent in the general systemic circulation; minimisation of metabolism of the drug molecules in non-target sites; minimisation of active agent reaching non-target tissues; and capability of delivering lower doses of the active agent in-vivo. In comparison, double-balloon catheters limit the drug to the injured site for a certain period of time, thus allowing the cyclodextrin molecules to complex the lipid molecules. Once the balloons are deflated, return of flow to normal levels should ensure that the cyclodextrin molecules are carried away from the body for eradication.

Perhaps the most recent usage of cyclodextrins lies in the ability of these agents to deliver genotherapeutic agents such as plasmids, viral vectors and antisense constructs. Cyclodextrins may also be used to enhance delivery of genetic constructs to cells in culture or in-vivo. A recent study by Croyle et al (1998) has demonstrated that certain β-cyclodextrins enhance the ability of adenoviral vectors to transduce differentiated Caco-2 cells (a human colorectal carcinoma cell line). In addition, Croyle et al (1998) proved that co-delivery of the vectors did not affect functionality of the construct since they noted expression of the delivered gene in the rat jejunum. Such a novel gene therapy approach may be used to deliver anti-atherosclerotic genes such as those listed in Table 5.

In addition to delivery of plasmids, cyclodextrins may also be used to enhance intracellular delivery

Table 5. Potential genes for genotherapy against atherosclerosis.

Gene	Mechanism of action
Vascular endothelial growth factor	Stimulates proliferation of endothelium and increases vascular permeability
Fibroblast growth factor	Stimulates endothelium, smooth muscle cells and fibroblasts
Nitric oxide synthase	Produces nitric oxide, proven to be an endothelial mitogen in some models
Angiopoietin-1	Mediates recruitment of smooth muscle cells to vessel walls
Apolipoprotein A-I	Mediates reverse cholesterol transport
Apolipoprotein E	Mediates reverse cholesterol transport
Lecithin: cholesterol acyltransferase	Increases serum HDL cholesterol concentration
C-type natriuretic protein	Has antimitogenic properties and inhibits smooth muscle cells migration
Growth arrest homeobox (gax)	Expression rapidly down-regulated when blood vessels are subjected to balloon injury
Tissue inhibitor of metalloproteinase 1	Inhibits smooth muscle cell migration and proliferation
Hepatocyte growth factor	Potential contribution to protection/repair of vascular endothelial cells
Very-low-density lipoprotein receptor	Decrease in plasma cholesterol levels
Fas ligand	Inhibits neointimal formation in injured vessels
p21	Reduces intimal thickening in injured arteries
Retinoblastoma	Reduces intimal thickening in injured arteries
Thymidine kinase	Reduces intimal thickening in injured arteries

of antisense molecules (Habus et al 1995; Zhao et al 1996a). Cyclodextrins should enable the avoidance of two fundamental problems of antisense delivery in-vivo: firstly, the limited ability of oligonucleotides to extravasate from the bloodstream and penetrate cellular membranes and secondly, the high degree of susceptibility of the antisense constructs to endonucleases which rapidly degrade the oligomers (reviewed in Walker et al 1997). In most cases, degradation of the phosphodiester bonds between nucleotides renders the oligonucleotides useless for therapy.

In fact, the in-vitro stability of antisense molecules may be increased by binding to cyclodextrins such as hpβCD (Cserhati et al 1996). Whether this correlates to an increased resistance against endonucleases in cultured cells and ultimately in-vivo remains to be seen. Cyclodextrins such as dimeβCD and monoaminoβCD (maβCD) interact to some extent with short (2-mer) phosphorothioate oligonucleotides (Bolyan & Ganzler 1998). Results suggest that the amino group on the maβCD has a stabilising effect on the cyclodextrin–oligonucleotide complex via ionic interaction. However, the authors did not determine how this interaction occurred: whether the antisense constructs were treated as guest molecules or whether several cyclodextrin molecules interacted with one antisense construct. When the ionic strength of the solvent is increased, the electrostatic attraction between the amine and phosphate groups of the antisense molecules are impaired, as expected for a nucleic-acid carrier operating on an ion-exchange basis (Dass et al 1996; 1997d; Dass 1998). The effect of cyclodextrins on longer antisense molecules needs to be evaluated.

Two- to three-fold increase in the cellular uptake of antisense constructs by hydroxyalkylated β-cyclodextrins has been noted in human T-cell leukaemia H9 cells (Zhao et al 1995). The increase in antisense uptake observed with the cyclodextrin molecules was due to an increase in uptake and not just increased binding to the cellular surfaces. Stimulation of the immune system by antisense constructs is quite common and it is believed that such stimulation may be reduced by complexation of the antisense strands with cyclodextrins. In fact, Zhao et al (1996b) proved that cyclodextrins reduced the induction of elevated platelet counts by antisense strands in-vivo. Certain cyclodextrins may also modulate the intracellular distribution or activity of antisense molecules (Abdou et al 1997). Antisense constructs that may be used for reversal of atherosclerosis are listed in Table 6.

Thus, a simultaneous delivery of cyclodextrins and antisense molecules may enhance entry into cells. In addition to enhancing membrane penetration, cyclodextrin molecules should stabilise the antisense molecules by preventing their hybridisation in solution. Such base-pairing would decrease the effectiveness of the oligonucleotides. The small size of cyclodextrin molecules should allow their incorporation into pumps, unlike liposome–antisense complexes which are bulky. In addition, the concentration of commercially available cationic liposomes is too low (up to $2\,mg\,mL^{-1}$) to allow a therapeutic quantity of antisense constructs to be delivered to tumours in animal models. In contrast, cyclodextrins such as hpβCD, ceβCD, trimeβCD and meβCD may be concentrated up to >100, 1000, 200 and $>750\,mg\,mL^{-1}$ in water, respectively (Cyclolab 1995, 1999). In the context of atherosclerosis therapy, delivery of genetic constructs to diseased sites using cyclodextrins not only attempts to treat lesion-prone sites at the genetic level, but also aims to achieve reduction of the lipid burden of the already-existing lesions.

Importantly, however, cyclodextrins are incapable of stimulating hydrolysis of cholesteryl esters within foam cells, a finding that has significant bearing when an agent is chosen for reducing lipid burden in-vivo. Furthermore, cyclodextrins have been found incapable of effluxing phospholipids, an observation that is debatable. These deficiencies are also inherent with PLV-based acceptors. While these features are also possessed by apoA-I, the mass of cholesterol effluxed by this natural acceptor molecule is significantly lower than that

Table 6. Potential antisense constructs against atherosclerosis.

Target gene	Expected therapeutic outcome
Angiotensin II	Prevention of myointimal proliferation after injury
Matrix metalloproteinase enzymes	Inhibition of smooth muscle cell migration and proliferation
c-myc	Inhibition of smooth muscle cell proliferation
c-myb	Reduction in intimal thickening in injured arteries
ras	Reduction in intimal thickening in injured arteries
cdk2 kinase	Reduction in intimal thickening in injured arteries
Macrophage colony stimulating factor	Prevention of recruitment of monocytes and macrophages to vessel wall
Cholesteryl ester transfer protein	Decrease in plasma low density and very-low-density lipoprotein cholesterol

achieved with cyclodextrins in culture. Perhaps a combination of these three types of acceptors, or a combination of the features of these into a newer-generation acceptor, may constitute a pharmacological agent that is capable of significantly reducing the lipid burden of lesions. Such an acceptor must be physiologically safe and be capable of increasing cholesterol desorption into the bloodstream and eventual removal through the bile in an effort to stimulate reverse cholesterol transport in atherosclerotic-prone individuals.

Toxicity of Cyclodextrins

If cyclodextrins are to be used in-vivo, their safety has to be monitored initially in cell culture and eventually in animals before being studied clinically. Since the interaction of cyclodextrins with the plasma membranes may be the initial site of cellular damage, the incubation of erythrocytes with these agents may be useful as a bioindicator. It is believed that phospholipid association occurs best with the small cavities of α-cyclodextrins while β-cyclodextrins are ideal for binding cholesterol (Irie & Uekama 1997).

Both dimeβCD and trimeβCD are among the most powerful solubilising agents available for most lipophilic compounds (reviewed in Cyclolab 1999). These derivatives are cytotoxic, causing tissue irritation and haemolysis in a dose-dependent manner. This behaviour has been directly attributed to the ability of meβCDs to sequester cholesterol from cells. Thus, meβCDs are rarely used as drug carriers except in low doses in nasal drug delivery and in ophthalmic (eye) formulations.

However, this cytotoxicity may be put to therapeutic use. It is known that meβCDs exhibit the greatest affinity for plasma membrane lipids, especially towards cholesterol, thus attenuating membrane fluidity (Cyclolab 1999). This can perturb the action of membrane-based glycoproteins (which act to restrict drug permeability and result in poor intracellular drug accumulation). Sequestration of membrane cholesterol by meβCDs causes alteration of membrane fluidity and compromises the action of glycoproteins present on the plasma membrane. This fact is quite important in cancer therapy where certain glycoproteins such as P-glycoprotein and the multidrug resistance protein (MRP) are known to actively efflux drugs from aberrant cells. It was noted that lipophilic drugs were especially affected.

When β-cyclodextrin was administered to Fischer 344 rats in their diets at concentrations of 0, 2·5 and 5% for more than 2 years, dose-dependent effects on growth were observed in both sexes (Toyoda et al 1997). However, no effects on survival rates and mean survival times were noted. This again may be attributed to the effect cyclodextrins have on plasma membrane cholesterol and their apparent lack of effect on cell cycling. Interestingly, the dosages given were about 340- to 400-fold higher than the current human daily intake of the cyclodextrin as a food additive and from pharmaceutical usage. No carcinogenic effects were noted, as was the case for γ-cyclodextrin (Waalkens-Berendsen et al 1998).

Thus, differences in the toxicity of cyclodextrins have been noted between cells in culture and in-vivo studies. It has been noted that when the cavity of a cyclodextrin molecule is occupied, it is not cytotoxic (Irie & Uekama 1997). The best approach would be to firstly expose the cyclodextrin, judged by in-vitro tests to be the best for a certain task, to a variety of mammalian cells in culture. The next step would be to monitor its cytotoxicity in ethically acceptable animal studies, deriving important data on the dosage that is safe to use in-vivo. Select organs such as the kidneys would have to be routinely monitored, since cyclodextrins are removed and concentrated in the proximal convoluted tubule. The formation of cytocidal microcrystals in the lysosomes of epithelial cells in the renal proximal tubule due to association of cyclodextrin molecules with endogenous lipids has been noted (Frijlink et al 1991).

While γ-cyclodextrins may be administered at a high dose without toxicity in animal studies (reviewed in Irie & Uekama 1997; Til & Bar 1998), β-cyclodextrins are the most toxic, as a direct result of the ability of β-cyclodextrin molecules to bind with lipids. Unfortunately, recent attempts to curtail the toxic effects of β-cyclodextrins by various chemical modifications have led to little success despite increasing aqueous solubility. Nevertheless, introducing hydroxypropyl groups into β-cyclodextrin molecules has enabled the administration of higher doses of cyclodextrins in-vivo. For instance, intravenous doses of $40\,g\,kg^{-1}$ in rabbits (Irie et al 1992), $10\,g\,kg^{-1}$ in monkeys (Brewster et al 1989) and 30 g (total) in humans (Carpenter et al 1995) have been administered with no adverse effects. Nevertheless, caution has to be taken since hpβ-CD has been documented to induce pulmonary oedema in dogs and cause occasional distress or agitation in rabbits (Carpenter et al 1995). It may also cause a breakdown of intracellular organelles such as the perturbation of mitochondrial membrane phospholipids and fatty acids (Sun & Gilboe 1994).

Humans have been noted to tolerate high doses of hpβCD as long as the daily dose is $< 16\,g$ orally

(reviewed in Irie & Uekama 1997). Above this dose, soft stools and diarrhoea are encountered. If the data points to the safety of a certain cyclodextrin, then it may be studied in the clinic. Otherwise, the cyclodextrin may be altered chemically to dilute its toxic effects, or delivered with other agents or by devices that limit its exposure to the intended diseased site and avoid or minimise its contact with healthy tissues. Nasal delivery of cyclodextrins (Marttin et al 1998) is becoming an increasing trend providing yet another feasible route of administration.

Conclusion

Since apolipoprotein A-I (apoA-I) is the major protein of HDL (the most reliable predictor for susceptibility to cardiovascular disease development), it is worthwhile evaluating the potential of this protein to reduce lipid burdens of lesions observed in the clinic. This apolipoprotein has been used extensively to promote cholesterol efflux from a variety of cultured cells from different mammalian species. However, little animal and scant clinical trials examining the potential of this apolipoprotein to induce cholesterol (or other lipid) efflux has been performed. Importantly, the inhibitory effects of oxysterols on cholesterol and other lipid efflux by apoA-I needs to be examined in any attempt to utilise apoA-I as an agent to stimulate efflux of lipids, since recent studies highlight the toxicity of these sterol derivatives in-vivo. For usage of apoA-I as a drug molecule, important lessons may be learnt from an examination of other lipid acceptors such as cyclodextrins and phospholipid vesicles, by combining apoA-I with other effluxing agents such as phospholipid/apoA-I discs, by remodelling the protein structure of the apolipoprotein or by altering the composition of the lipoprotein to be administered in-vivo. The issue of barriers posed to drug delivery and efficacy for novel lipid acceptors have to be addressed by the combined efforts from such fields as physiology, cell biology, membrane research, pharmacy and molecular biology. Needless to say, the usage of this apolipoprotein in a therapeutic context has to be monitored in biodistribution, safety and dose–response studies in well-controlled animal trials before its introduction into clinical trials.

Acknowledgements

The support of members of the Cell Biology and Clinical Research Units of the Heart Research Institute are acknowledged.

References

Abdou, S., Collomb, J., Sallas, F., Marsura, A., Finance, C. (1997) Beta cyclodextrin derivatives as carriers to enhance the antiviral activity of an antisense oligonucleotide directed toward a coronavirus intergenic consensus sequence. Arch. Virol. 142: 1585–1602

Adams, C. W. M., Abdulla, Y. H., Bayliss, O. B., Morgan, R. S. (1967) Modification of aortic atheroma and fatty liver in cholesterol-fed rabbits by intravenous injection of saturated and polyunsaturated lecithins. J. Pathol. Bacteriol. 94: 77–87

Ameli, S., Hultgardh-Nilsson, A., Cercek, B., Shah, P. K., Forrester, J. S., Ageland, H., Nilsson, J. (1994) Recombinant apolipoprotein A-I Milano reduces intimal thickening after balloon injury in hypercholesterolemic rabbits. Circulation 90: 1935–1941

Andersen, G. H., Robbins, F. M., Domingues, F. J. (1983) The utilisation of Schardinger dextrins by the rat. Toxicol. Appl. Pharmacol. 5: 257–266

Andersson, L.-O. (1996) Pharmacology of apolipoprotein A-I. Curr. Opin. Lipidol. 8: 225–228

Antenucci, R. N., Palmer, J. K. (1984) Enzymatic degradation of alpha and beta-cyclodextrins by bacteroides of human colon. J. Agric. Food Chem. 32: 1316–1321

Antlsperger, G. (1992) Uses for cyclodextrins.In: Hedges, A. R. (ed.) Mins. 6th Int. Symp. Cyclodextrins. Editions de Sante, Paris, pp 277–283

Asztalos, B., Roheim, P. S. (1995) Presence and formation of 'free apolipoprotein A-I-like' particles in human plasma. Arterioscler. Thromb. Vasc. Biol. 15: 1419–1423

Asztalos, B., Zhang, W., Roheim, P. S., Wong, L. (1997) Role of free apolipoprotein A-I in cholesterol efflux. Formation of pre-alpha-migrating high-density lipoprotein particles. Arterioscler. Thromb. Vasc. Biol. 17: 1630–1636

Atger, V. M., de la Llera Moya, M., Stoudt, G. W., Rodrigueza, W. V., Phillips, M. C., Rothblat, G. H. (1997) Cyclodextrins as catalysts for the removal of cholesterol from macrophage foam cells. J. Clin. Invest. 99: 773–780

Awad, A. B., Chen, Y. C., Fink, C. S., Hennessey, T. (1996) B-Sitosterol inhibits HT-29 human colon cancer cell growth and alters membrane lipids. Anticancer Res. 16: 2797–2804

Badimon, J. J., Badimon, L., Galvez, A., Dische, R., Fuster, V. (1989) High density lipoprotein plasma fractions inhibit aortic fatty streaks in cholesterol-fed rabbits. Lab. Invest. 60: 455–461

Badimon, J. J., Badimon, L., Fuster, V. (1990) Regression of atherosclerotic lesions by high density lipoprotein fraction in the cholesterol-fed rabbit. J. Clin. Invest. 85: 1234–1241

Banka, C. L., Black, A. S., Curtiss, L. K. (1994) Localization of an apolipoprotein A-I epitope critical for lipoprotein-mediated cholesterol efflux from monocytic cells. J. Biol. Chem. 269: 10288–10297

Barbaras, R., Puchois, P., Fruchart, J.-C., Ailhaud, G. (1987) Cholesterol efflux from cultured adipose cells is mediated by LpA-I particles but not LpA-I : LpA-II particles. Biochem. Biophys. Res. Commun. 142: 63–69

Bartholow, L. C., Geyer, R. P. (1982) Sterol efflux from mammalian cells induced by human serum albumin-phospholipid complexes: dependence on phospholipid acyl chain length, degree of saturation, and net charge. J. Biol. Chem. 257: 3126–3130

Bergeron, J., Frank, P. G., Scales, D., Meng, Q.-H., Castro, G., Marcel, Y. L. (1995) Apolipoprotein A-I conformation in reconstituted discoidal lipoproteins varying in phospholipid and cholesterol content. J. Biol. Chem. 270: 27429–27438

Bergeron, J., Frank, P. G., Emmanuel, F., Latta, M., Zhao, Y., Sparks, D. L., Rassart, E., Denefle, P., Marcel, Y. L. (1997) Characterization of human apolipoprotein A-I expressed in *Escherichia coli*. Biochim. Biophys. Acta 1344: 139–152

Bielicki, J. K., Johnson, W. J., Weinberg, R. B., Glick, J. M., Rothblat, G. H. (1992) Efflux of lipid from fibroblasts to apolipoproteins: dependence on elevated levels of cellular unesterified cholesterol. J. Lipid Res. 33: 1699–1709

Bielicki, J. K., McCall, M. R., Forte, T. M. (1999) Apolipoprotein A-I promotes cholesterol release and apolipoprotein E recruitment from THP-1 macrophage-like foam cells. J. Lipid Res. 40: 85–92

Bisgaier, C. L., Siebenkas, M. V., Williams, K. J. (1989) Effects of apolipoproteins A-IV and A-I on the uptake of phospholipid liposomes by hepatocytes. J. Biol. Chem. 264: 862–869

Bittman, R. (1988)In: Yeagle, P. L (ed.) Biology of Cholesterol. CRC, Boca Raton, Florida, pp 173–195

Boggs, J. M. (1987) Lipid intermolecular hydrogen bonding: influence on structural organization and membrane function. Biochim. Biophy. Acta 906: 353–404

Bolyan, D., Ganzler, K. (1998) Chiral separation of antisense oligonucleotides: a feasibility study. Biomed. Chromatogr. 12: 179–180

Braschi, S., Neville, T. A.-M., Vohl, M.-C., Sparks, D. L. (1999) Apolipoprotein A-I charge and conformation regulate the clearance of reconstituted high density lipoprotein *in vivo*. J. Lipid Res. 40: 522–532

Brasseur, R., de Meutter, J., Vanloo, B., Goormaghtigh, E., Ruysschaert, J. M., Rosseneu, M. (1990) Mode of assembly of amphipathic helical segments in model high-density lipoproteins. Biochim. Biophys. Acta 1043: 245–252

Bressolle, F., Audran, M., Pham, T. N., Vallon, J. J. (1996) Cyclodextrins and enantiomeric separations of drugs by liquid chromatography and capillary electrophoresis: basic principles and new developments. J. Chromatogr. B. Biomed. Appl. 687: 303–336

Brewster, M. E., Estes, K. S., Loftsson, T., Perchalski, R., Derendorf, H., Mullersman, G., Bodor, N. (1988) Inproved delivery through biological membranes. XXXL. Solubilization and stabilization of an estradiol chemical delivery system by modified β-cyclodextrins. J. Pharm. Sci. 77: 981–985

Brewster, M. E., Estes, K. S., Bodor, N. (1989) Development of non-surfactant formulation for alfaxalone through the use of chemically-modified cyclodextrine. J. Parenter Sci. Technol. 43: 262–265

Brewster, M. E., Anderson, W. R., Estes, K. S., Bodor, N. (1991) Development of aqueous parenteral formulations for carbamazepine through the use of modified cyclodextrins. J. Pharm. Sci. 80: 380–383

Brewster, M. E., Anderson, W. R., Meinsma, D., Moreno, D., Webb, A. I., Pablo, L., Estes, K. S., Derendorf, H., Bodor, N., Sawchuk, R., Cheung, B., Pop, E. (1997) Intravenous and oral pharmacokinetic evaluation of a z-hydroxypropyl-beta-cyclodextrin-based formulation of carbamazepine in the dog: comparison with commerically available tablets and suspensions. J. Pharm. Sci. 86: 335–339

Brouillette, C. G., Anantharamaiah, G. M. (1995) Structural models of human apolipoprotein A-I. Biochim. Biophys. Acta 1256: 103–129

Brown, M. S., Goldstein, J. L. (1986) A receptor-mediated pathway for cholesterol homeostasis. Science 232: 34–47

Brown, A. J., Jessup, W. (1999) Oxysterols and atherosclerosis. Atherosclerosis 142: 1–28

Brown, D. A., London, E. (1998) Structure and origin of ordered lipid domains in biological membranes. J. Membr. Biol. 164: 103–114

Brown, M. S., Ho, Y. K., Goldstein, J. L. (1980) The cholesteryl ester cycle in macrophage foam cells. Continual hydrolysis and re-esterification of cytoplasmic cholesteryl esters. J. Biol. Chem. 255: 9344–9352

Brown, A. J., Leong, S. L., Dean, R. T., Jessup, W. (1997) 7-Hydroperoxycholesterol and its products in oxidized low density lipoprotein and human atherosclerotic plaque. J. Lipid Res. 38: 1730–1745

Bruckdorfer, K. R., Sherry, M. K. (1984) The solubility of cholesterol and its exchange between membranes. Biochim. Biophys. Acta 769: 187–196

Burns, C. H., Rothblat, G. H. (1969) Cholesterol excretion by tissue culture cells: effect of serum lipids. Biochim. Biophys. Acta 176: 616–625

Carpenter, T. O., Gerloczy, A., Pitha, J. (1995) Safety of parenteral hydroxylpropyl β-cyclodextrin. J. Pharm. Sci. 84: 222–225

Castle, C. K., Pape, M. E., Marotti, K. R., Melchior, G. W. (1991) Secretion of pre-beta-migrating apoA-I by cynomolgus monkey hepatocytes in culture. J. Lipid Res. 32: 439–447

Castro, G. R., Fielding, C. J. (1988) Early incorporation of cell-derived cholesterol into pre-β-migrating high-density lipoprotein. Biochemistry 27: 25–29

Chen, C., Applegate, K., King, W. C., Glomset, J. A., Norum, K. R., Gjone, E. (1984) A study of the small spherical high density lipoproteins of patients afflicted with familial lecithin : cholesterol acyltransferase deficiency. J. Lipid Res. 25: 269–282

Clay, M. A., Newnham, H. H., Forte, T. M., Barter, P. J. (1992) Cholesteryl ester transfer protein and hepatic lipase activity promote shedding of apoA-I from HDL and subsequent formation of discoidal HDL. Biochim. Biophys. Acta 1124: 52–58

Clejan, S., Bittman, R. (1984) Kinetics of cholesterol and phospholipid exchange between *Mycoplasma gallisepticum* cells and lipid vesicles. J. Biol. Chem. 259: 441–448

Croyle, M. A., Roessler, B. J., Amidon, G. L. (1997) Beta cyclodextrins enhance gene delivery to the intestine. Proc. 24th Intl Symp. Contr. Release Bioact. Mater., Stockholm, 675–676

Croyle, M. A., Roessler, B. J., Hsu, C.-P., Sun, R., Amidon, G. L. (1998) Beta cyclodextrins enhance adenoviral-mediated gene delivery to the intestine. Pharm. Res. 15: 1348–1355

Cserhati, T., Forgacs, E., Szejtli, J. (1996) Inclusion complex formation of antisense nucleotides with hydroxypropyl-β-cyclodextrin. Int. J. Pharm. 141: 1–7

Cyclolab (1995) Product Catalogue on Cyclodextrins. Budapest, Hungary

Cyclolab (1999) Cyclodextrin News, 13. http://hungary. com/cyclolab/cd_news (accessed)

Czarnecka, H., Yokoyama, S. (1995) Lecithin : cholesterol acyltransferase reaction on cellular lipid released by free apolipoprotein-mediated efflux. Biochemistry 34: 4385–4392

Daerr, W. H., Greten, H. (1982) In vitro modulation of the distribution of normal human plasma high-density lipoprotein subfractions through the lecithin : cholesterol acyltransferase reaction. Biochim. Biophys. Acta 710: 128–133

Dass, C. R. (1998) Targeted Delivery of DNA for Cancer Therapy. PhD thesis, Charles Sturt University

Dass, C. R., Burton, M. A. (1999) Lipoplexes and tumours. J. Pharm. Pharmacol. 51: 755–770

Dass, C. R., Walker, T. L., DeCruz, E. E., Burton, M. A. (1996) *In vitro* evaluation of ion-exchange microspheres as carriers of plasmid DNA. Pharm. Sci. 2: 401–405

Dass, C. R., DeCruz, E. E., Walker, T. L., Burton, M. A. (1997a) Tumor gene targeting using microspheres: cell culture and *in vivo* studies. Drug Delivery 4: 263–267

Dass, C. R., Walker, T. L., DeCruz, E. E., Burton, M. A. (1997b) Enhanced anticancer therapy mediated by specialized liposomes. J. Pharm. Pharmacol. 49: 972–975

Dass, C. R., Walker, T. L., DeCruz, E. E., Burton, M. A. (1997c) Cationic liposomes and gene therapy for solid tumours. Drug Delivery 4: 151–165

Dass, C.R., DeCruz, E. E., Walker, T. L., Burton, M. A. (1997d) Barriers to liposomal gene transfer into solid tumours. Australas. Biotech. 7: 155–159

Dass, C. R., Gelissen, I. C., Kritharides, L., Brown, A. J., Dean, R. T., Jessup, W. (1999a) 7-Ketocholesterol inhibits apoA-I-mediated cholesterol and phospholipid efflux from human foam cells. BCCH (Australasian Cardiovascular Research) 2nd Symposium Proceedings, August, Sydney

Dass, C. R., Walker, T. L., Kalle, W. H. J., Burton, M. A. (1999b) A microsphere-lipoplex (microplex) vector for targeted gene therapy of cancer. I. Construction and *in vitro* evaluation. Drug Delivery 6: 259–270

Dass. C. R., Walker, T. L., Kalle, W. H. J., Burton, M. A. (2000) A microsphere-lipoplex (microplex) vector for targeted gene therapy of cancer. II. *In vivo* biodistribution study in a solid tumour model. Drug Delivery 7: 15–20

Davidson, W. S., Lund-Katz, S., Johnson, W. J., Anantharamaiah, G. M., Palgunachari, M. N., Segrest, J. P., Rothblat, G. H., Phillips, M. C. (1994) The influence of apolipoprotein structure on the efflux of cellular free cholesterol to high density lipoprotein. J. Biol. Chem. 269: 22975–22982

Davidson, W. S., Gillotte, K. L., Lund-Katz, S., Johnson, W. J., Rothblat, G. H., Phillips, M. C. (1995a) The effect of high density lipoprotein phospholipid acyl chain composition on the efflux of cellular free cholesterol. J. Biol. Chem. 270: 5882–5890

Davidson, W. S., Rodrigueza, W. V., Lund-Katz, S., Johnson, W. J., Rothblat, G. H., Phillips, M. C. (1995b) Effects of particle size on the efflux of cellular free cholesterol. J. Biol. Chem. 270: 17106–17113

Dewailly, P., Decoopman, E., Desreumaux, C., Fruchart, J. C. (1976) Plasma removal of intravenous essential phospholipids in man. In: Peters, H. (ed.) Phosphatidylcholine. Springer Verlag, Berlin, pp 80–86

Duchene, D. (ed.) (1987) Cyclodextrins and their Industrial Use. Editions de Sante, Paris, France

Favier, M.-L., Remesy, C., Moundras, C., Demigne, C. (1995) Effect of cyclodextrin on plasma lipids and cholesterol metabolism in the rat. Metabolism 44: 200–206

Ferezou, J., Riottot, M., Serougne, C., Cohen-Solal, C., Catala, I., Alquier, C., Parquet, M., Juste, C., Lafont, H., Mathe, D., Corring, T., Lutton, C. (1997) Hypocholesterolemic action of β-cyclodextrin and its effects on cholesterol metabolism in pigs fed a cholesterol-enriched diet. J. Lipid Res. 38: 86–100

Fielding, P. E., Fielding, C. J. (1980) Evidence for a cholesteryl ester transfer complex in human plasma. Proc. Natl Acad. Sci. USA 77: 3327–3330

Fielding, C. J., Fielding, P. E. (1981) Regulation of human plasma lecithin:cholesterol acyltransferase activity by lipoprotein acceptor cholesteryl ester content. J. Biol. Chem. 256: 2102–2104

Fielding, C. J., Fielding, P. E. (1995a) Molecular physiology of reverse cholesterol transport. J. Lipid Res. 36: 211–228

Fielding, P. E., Fielding, C. J. (1995b) Plasma membrane caveolae mediate the efflux of cellular free cholesterol. Biochemistry 34: 14288–14292

Flourie, B., Molis, C., Achour, L. (1993) Fate of β-cyclodextrin in the human intestine. J. Nutr. 123: 676–680

Forte, T. M., Goth-Goldstein, R., Nordhausen, R. W., McCall, M. R. (1993) Apolipoprotein A-I cell membrane interaction: extracellular assembly of heterogeneous nascent HDL particles. J. Lipid Res. 34: 317–324

Forte, T. M., Bielicki, J. K., Goth-Goldstein, R., Selmek, J., McCall, M. R. (1995) Recruitment of cell phospholipids and cholesterol by apolipoproteins A-II and A-I: formation of nascent apolipoprotein-specific HDL that differ in size, phospholipid composition, and reactivity with LCAT. J. Lipid Res. 36: 148–157

Forte, T. M., Bielicki, J. K., Knoff, L., McCall, M. R. (1996) Structural relationships between nascent apoA-I-containing particles that are extracellularly assembled in cell culture. J. Lipid Res. 37: 1706–1085

Fournier, N., de la Llera Moya, M., Burkey, B. F., Swaney, J. B., Paterniti, J., Moatti, N., Atger, V., Rothblat, G. H. (1996) Role of HDL phospholipid in efflux of cell cholesterol to whole serum: Studies with human apoA-1 transgenic rats. J. Lipid Res. 37: 1704–1711

Francone, O. L., Gurakar, A., Fielding, C. (1989) Distribution and functions of lecithin : cholesterol acyltransferase and cholesterol transfer protein in plasma lipoproteins. J. Biol. Chem. 264: 7066–7072

Frank, P. G., Bergeron, J., Emmanuel, F., Lavigne, J.-P., Sparks, D. L., Denefle, P., Rassart, E., Marcel, Y. L. (1997) Deletion of central α-helices in human apolipoprotein A-I: effect on phospholipid association. Biochemistry 36: 1798–1806

Friedman, M., Byers, S. O., Rosenman, R. H. (1957) Resolution of aortic atherosclerotic infiltration in the rabbit by phosphatide infusion. Proc. Soc. Exp. Biol. Med. 95: 586–588

Frijlink, H. W., Eissens, A. C., Hefting, N. R., Poelstra, K., Lerk, C. F., Meijer, D. K. F. (1991) The effect of parenterally administered cyclodextrins on cholesterol levels in the rat. Pharm. Res. 8: 9–16

Fugler, L., Clejan, S., Bittman, R. (1985) Movement of cholesterol between vesicles prepared with different phospholipids or sizes. J. Biol. Chem. 260: 4098–4102

Gelissen, I. C., Brown, A. J., Mander, E. L., Kritharides, L., Dean, R. T., Jessup, W. (1996) Sterol efflux is impaired from macrophage foam cells selectively enriched with 7-ketocholesterol. J. Biol. Chem. 271: 17852–17860

Gelissen, I. C., Kritharides, L., Dean, R. T., Jessup, W., Brown, A. J. (1999) Efflux of cholesterol and 7-ketocholesterol from macrophages: essential role of phospholipids. J. Lipid Res. 40: 1636–1646

Gerbhardt, D. O. E., Schicht, I. M., Paul, L. C. (1984) The immunochemical determination of apolipoprotein A-I and "free" apolipoprotein A-I in serum of patients on chronic haemodialysis. Ann. Clin. Biochem. 21: 301–305

Ghiselli, G., Rhode, M. F., Tanebaum, S., Krishnan, S., Gotto, A. M. (1985) Origin of apolipoprotein A-I polymorphism in plasma. J. Biol. Chem. 260: 15662–15668

Gillotte, K. L., Davidson, W. S., Lund-Katz, S., Rothblat, G. H., Phillips, M. C. (1996) Apolipoprotein A-I structural modification and the functionality of reconstituted high density lipoprotein particles in cellular cholesterol efflux. J. Biol. Chem. 271: 23792–23798

Gillotte, K. L., Davidson, W. S., Lund-Katz, S., Rothblat, G. H., Phillips, M. C. (1998) Removal of cellular cholesterol by pre-β-HDL involves plasma membrane microsolubilization. J. Lipid Res. 39: 1918–1928

Girao, H., Mota, M. C., Ramalho, J., Pereira, P. (1998) Cholesterol oxides accumulate in human cataracts. Exp. Eye Res. 66: 645–652

Glass, C. K., Pittman, R. C., Keller, G. A., Steinberg, D. (1983) Tissue sites of degradation of apoprotein A-I in the rat. J. Biol. Chem. 258: 7161–7167

Glomset, J. A. (1968) The plasma lecithin:cholesterol acyltransferase reaction. J. Lipid Res. 9: 155–167

Goldberg, D. I., Beltz, W. F., Pittman, R. C. (1991) Evaluation of pathways for the cellular uptake of high density lipoprotein cholesterol esters in rabbits. J. Clin. Invest. 87: 331–346

Goldstein, J. L., Brown, M. S. (1974) Binding and degradation of low density lipoproteins by cultured human fibroblasts. Comparison of cells from a normal subject and from a patient with homozygous familial hypercholesterolemia. J. Biol. Chem. 249: 5153–5162

Gordon, J. I., Sims, H. F., Lentz, S. R., Edelstein, C., Scanu, A. M., Strauss, A. W. (1983) Proteolytic cleavage of human preproapolipoprotein A-I. J. Biol. Chem. 258: 4037–4044

Guo, L. S. S., Hamilton, R. L., Goerke, J., Weinstein, J. N., Havel, R. J. (1980) Interaction of unilamellar liposomes with serum lipoproteins and apolipoproteins. J. Lipid Res. 21: 993–1000

Guyton, J. R., Black, B. L., Seidel, C. L. (1990) Focal toxicity of oxysterols in vascular smooth muscle cell culture: a model of the atherosclerotic core region. Am. J. Pathol. 137: 425–434

Habus, I., Zhao, Q., Agarwal, S. (1995) Hybridization properties, nuclease stability, and cellular uptake of the oligonucleotide-amino-β-cyclodextrins and adamantane conjugates. Bioconjug. Chem. 6: 327–331

Hara, H., Yokoyama, S. (1991) Interaction of free apolipoproteins with macrophages. J. Biol. Chem. 266: 3080–3086

Hara, H., Yokoyama, S. (1992) Role of apolipoproteins in cholesterol efflux from macrophages to lipid microemulsion: Proposal of a putative model for the pre-β high density lipoprotein pathway. Biochemistry 31: 2040–2046

Heideman, C. L., Hoff, H. F. (1982) Lipoproteins containing apolipoprotein A-I extracted from human aortas. Biochim. Biophys. Acta 711: 431–444

Ho, Y. K., Brown, M. S., Goldstein, J. L. (1980) Hydrolysis and excretion of cytoplasmic cholesteryl esters by macrophages: stimulation by high density lipoproteins and other agents. J. Lipid Res. 21: 391–398

Horowitz, B. S., Goldberg, I. J., Merab, J., Vannit, T., Ramakrishnan, R., Ginsberg, H. N. (1992) Role of the kidney in increased clearance of apoliprotein A-I in subjects with reduced high density lipoprotein cholesterol concentrations. In: Miller, N. E., Tall, A. R. (eds) High Density Lipoproteins and Atherosclerosis III. Excerpta Medica, Amsterdam, pp 215–222

Huang, Y., von Eckardstein, A., Assmann, G. (1993) Cell-derived unesterified cholesterol cycles between different HDLs and LDL for its effective esterification in plasma. Arterioscler. Thromb. 13: 445–458

Huang, Y., von Eckardstein, A., Wu, S., Maeda, N., Assmann, G. (1994) A plasma lipoprotein containing only apolipoprotein E and with gamma mobility on electrophoresis releases cholesterol from cells. Proc. Natl Acad. Sci. USA 91: 1834–1838

Irie, T., Uekama, K. (1997) Pharmaceutical applications of cyclodextrins. III. Toxicological issues and safety evaluation. J. Pharm. Sci. 86: 147–162

Irie, T., Uekama, K. (1999) Cyclodextrins in peptide and protein delivery. Adv. Drug Del. Rev. 36: 101–123

Irie, T., Fukunaga, K., Garwood, M. K., Carpenter, T. O., Pitha, J., Pitha, J. (1992) Hydroxypropylcyclodextrins in parenteral use. II. Effects on transport and disposition of lipids in rabbit and humans. J. Pharm. Sci. 81: 524–528

Ishida, B. Y., Frolich, J., Fielding, C. J. (1987) Prebeta-migrating high density lipoprotein: quantitation in normal and hyperlipidaemic plasma by solid phase radioimmunoassay following electrophoretic transfer. J. Lipid Res. 28: 778–786

Ishida, B. Y., Albee, D., Paigen, B. (1990) Interconversion of prebeta-migrating lipoproteins containing apolipoprotein A-I and HDL. J. Lipid Res. 31: 227–236

Janini, G. M., Muschik, G. M., Issaq, H. J. (1996) Electrokinetic chromatography in suppressed electroosmotic flow environment: Use of a charged cyclodextrin for the separation of enantiomers and geometric isomers. Electrophoresis 17: 1575–1583

Jauhiainen, M., Metso, J., Pahlman, R., Blomqvist, S., van Tol, A., Ehnholm, C. (1993) Human plasma phospholipid transfer protein causes high density lipoprotein conversion. J. Biol. Chem. 268: 4032–4036

Jessup, W., Brown, A. J., Dass, C. R., Gelissen, I. C., Dean, R. T., Kritharides, L. (1999) Sterol export from macrophage foam cells. Aust. Vasc. Biol. Soc. Confer., Adelaide, (abstract)

Jian, B., de la Llera-Moya, M., Royer, L., Rothblat, G., Francone, O., Swaney, J. B. (1997) Modification of the cholesterol efflux properties of human serum by enrichment with phospholipid. J. Lipid Res. 38: 734–744

Johnson, W. J., Mahlberg, F. H., Chacko, G. K., Phillips, M. C., Rothblat, G. H. (1988) The influence of cellular and lipoprotein cholesterol contents on the flux of cholesterol between fibroblasts and high density lipoproteins. J. Biol. Chem. 263: 14099–14106

Johnson, W. J., Mahlberg, F. H., Rothblat, G. H., Phillips, M. C. (1991) Cholesterol transport between cells and high density lipoproteins. Biochim. Biophys. Acta 1085: 273–298

Jonas, A., Kezdy, K., Williams, M. I., Rye, K.-A. (1988) Lipid transfers between reconstituted high density lipoprotein complexes and low density lipoproteins: effects of plasma protein factors. J. Lipid Res. 29: 1349–1357

Jonas, A., Kezdy, K. E., Wald, J. H. (1989) Defined apolipoprotein A-I conformations in reconstituted high density lipoprotein discs. J. Biol. Chem. 264: 4818–4824

Jonas, A., Steinmetz, A., Churgay, L. (1993) The number of amphipathic α-helical segments of apolipoproteins A-I, E, and A-IV determines the size and functional properties of their reconstituted lipoprotein particles. J. Biol. Chem. 268: 1596–1602

Karlin, J. B., Johnson, W. J., Benedict, C. R., Chacko, G. J., Phillips, M. C., Rothblat, G. H. (1987) Cholesterol flux between cells and high density lipoprotein: lack of relationship to specific binding of the lipoprotein to the cell surface. J. Biol. Chem. 262: 12557–12564

Kilsdank, E. P., Morel, D. W., Johnson, W. J., Rothblat, G. H. (1995) Inhibition of cellular cholesterol efflux by 25-hydroxycholesterol. J. Lipid Res. 36: 505–516

Klein, U., Gimpl, G., Farenholz, F. (1995) Alteration of the myometrial plasma membrane cholesterol content with β-cyclodextrin modulates the binding affinity of the oxytocin receptor. Biochemistry 34: 13784–13793

Knoche, B., Gehrcke, B., Konig, W. A., Wainer, I. W. (1996) Determination of the enantiomeric composition of mexiletine and its four hydroxylated metabolites in urine by enantioselective capillary gas chromatography. Chirality 8: 30–34

Koizumi, J., Kano, M., Okabayashi, K., Jadhav, A., Thompson, G. R. (1988) Behavior of human apolipoprotein A-1: phospholipid and apoHDL: phospholipid complexes *in vitro* and after injection into rabbits. J. Lipid Res. 29: 1405–1415

Koppaka, V., Silvestro, L., Engler, J. A., Brouillette, C. G., Axelsen, P. H. (1999) The srtucture of human lipoprotein A-I. J. Biol. Chem. 274: 14541–14544

Kreuzer, J., Lloyd, M. B., Bok, D., Fless, G. M., Scanu, A. M., Lusis, A. J., Haberland, M. E. (1994) Lipoprotein(a) displays increased accumulation compared with low-density lipoprotein in the murine arterial wall. Chem. Phys. Lipids 67/68: 175–190

Kritharides, L., Jessup, W., Mander, E., Dean, R. T. (1995) Apolipoprotein A-I-mediated efflux of sterols from oxidised LDL-loaded macrophages. Arterioscler. Thromb. Vasc. Biol. 15: 276–289

Kritharides, L., Kus, M., Brown, A. J., Jessup, W., Dean, R. T. (1996) Hydroxypropyl-β-cyclodextrin-mediated efflux of 7-ketocholesterol from macrophage foam cells. J. Biol. Chem. 271: 27450–27455

Kruth, H. S. (1997) The fate of lipoprotein cholesterol entering the arterial wall. Curr. Opin. Lipidol. 8: 246–252

Kruth, H. S., Skarlatos, S. L., Gaynor, P. M., Gamble, W. (1994) Production of cholesterol-enriched high density lipoproteins by human monocyte-derived macropahges is a mechanism that contributes to macrophage cholesterol efflux. J. Biol. Chem. 269: 24511–24518

Kuivenhoven, J. A., Stalenhoef, A. F., Hill, J. S., Dernacker, P. N., Errami, A., Kastelein, J. J., Pritchard, P. H.. (1996) Two novel molecular defects in the LCAT gene are associated with fish eye disease. Arterioscler. Thromb. Vasc. Biol. 16: 294–303

Kunitake, S. T., Mendel, C. M., Hennessy, L. K. (1992) Interconversion between apolipoprotein A-I-containing lipoproteins of pre-beta and alpha electrophoretic properties. J. Lipid Res. 33: 1807–1816

Laccotripe, M., Makrides, S. C., Jonas, A., Zannis, V. I. (1997) The carboxyl-terminal hydrophobic residues of apolipoprotein A-I affect its rate of phospholipid binding and its association with high density lipoprotein. J. Biol. Chem. 272: 17511–17522

Lefevre, M., Sloop, C. H., Roheim, P. S. (1988) Characterization of dog prenodal peripheral lymph lipoproteins. Evidence for the peripheral formation of lipoprotein-unassociated apoA-I with slow pre-β electrophoretic mobility. J. Lipid Res. 29: 1139–1148

Levrat, M.-A., Favier, M.-L., Moundras, C. (1994) Role of propionic acid and bile acids excretion in the hypocholesterolemic effects of oligosaccharides. J. Nutr. 124: 531–538

Li, Q., Czarnecka, H., Yokoyama, S. (1995) Involvement of a cellular surface factor(s) in lipid-free apolipoprotein-mediated cellular cholesterol efflux. Biochim. Biophys. Acta 1259: 227–234

Li, Q., Tsujita, M., Yokoyama, S. (1997) Selective down-regulation by protein kinase C inhibitors of apolipoprotein-mediated cellular cholesterol efflux in macrophages. Biochemistry 36: 12045–12052

Li, D., Weng, S., Yang, B., Zander, D. S., Saldeen, T., Nichols, W. W., Khan, S., Mehta, J. L. (1999) Inhibition of arterial thrombus formation by apoAI Milano. Arterioscler. Thromb. Vasc. Biol. 19: 378–383

Liang, H.-Q., Rye, K.-A., Barter, P. J. (1995) Cycling of apolipoprotein A-I between lipid-associated and lipid-free pools. Biochim. Biophys. Acta 1257: 31–37

Libby, P., Clinton, S. K. (1993) The role of macrophages in atherogenesis. Curr. Opin. Lipidol. 4: 355–363

Mahlberg, F. H., Rothblat, G. H. (1992) Cellular cholesterol efflux: Role of cell membrane kinetic pools and interaction with apolipoproteins A-I, A-II and Cs. J. Biol. Chem. 267: 4541–4550

Mahlberg, F. H., Glick, J. M., Lund-Katz, S., Rothblat, G. H. (1991) Influence of apolipoproteins A-I, A-II and C on the metabolism of membrane and lysosomal cholesterol in macrophages. J. Biol. Chem. 266: 19930–19937

Malmendier, C. L., Delcroix, C., Ameryckx, J. P. (1983) *In vivo* metabolism of human apoprotein A-1-phospholipid complexes. Comparison with human high density lipoprotein-apoprotein A-1 metabolism. Clin. Chim. Acta 131: 201–210

Marcel, Y. L., Jewer, D., Leblond, L., Weech, P. K., Milne, R. W. (1989) Lipid peroxidation changes the expression of specific epitopes of apolipoprotein A-I. J. Biol. Chem. 264: 19942–19950

Marttin, E., Verhoef, J. C., Merkus, F. W. (1998) Efficacy, safety and mechanism of action of cyclodextrins as absorption enhancers in nasal delivery of peptide and protein drugs. J. Drug Target. 6: 17–36

Mattsson-Hulten, L., Lindmark, H., Diczfalusy, U., Bjorkhem, I., Ottosson, M., Liu, Y., Bondjers, G., Wiklund, O. (1996) Oxysterols present in atherosclerotic tissue decrease the expression of lipoprotein lipase messenger RNA in human monocyte-derived macrophages. J. Clin. Invest. 97: 461–468

McCall, M. R., Forte, T. M., Shore, V. G. (1988) Heterogeneity of nascent high density lipoproteins secreted by the hepatoma-derived cell line, HepG2. J. Lipid Res. 29: 1127–1137

McCall, M. R., Tang, J. Y., Bielicki, J. K., Forte, T. M. (1995) Inhibition of lecithin:cholesterol acyltransferase and modification of HDL-apolipoproteins by aldehydes. Arterioscler. Thromb. Vasc. Biol. 15: 1599–1606

McLean, L. R., Phillips, M. C. (1984) Cholesterol transfer from small and large unilamellar vesicles. Biochim. Biophys. Acta 776: 21–26

Mendez, A. J. (1997) Cholesterol efflux mediated by apolipoproteins is an active cellular process distinct from efflux mediated by passive diffusion. J. Lipid Res. 38: 1807–1821

Mendez, A. J., Uint, L. (1996) Apolipoprotein-mediated cellular cholesterol and phospholipid efflux depend on a functional Golgi apparatus. J. Lipid Res. 37: 2510–2524

Mendez, A. J., Anantharamaiah, G. M., Segrest, J. P., Oram, J. F. (1994) Synthetic amphipathic helical peptides that mimic apolipoprotein A-I in clearing cellular cholesterol. J. Clin. Invest. 94: 1698–1705

Miyazawa, I., Ueda, H., Nagase, H., Endo, T., Kobayashi, S., Nagai, T. (1995) Eur. J. Pharm. Sci. 3: 153–162

Moundras, C., Behr, S. R., Demigne, C., Mazur, A., Remesy, C. (1994) Fermentable polysaccharides that enhance fecal bile acid excretion lower plasma cholesterol and apolipoprotein E-rich HDL in rats. J. Nutr. 124: 2179–2188

Muller, B. W., Albers, E. (1991) Effect of hydrotropic substances on the complexation of sparingly soluble drugs with cyclodextrin derivatives and the influence of cyclodextrin complexation on the pharmacokinetics of the drugs. J. Pharm. Sci. 80: 599–604

Musliner, T. A., Long, M. D., Forte, T. M., Nichols, A. V., Gong, E. L., Blanche, P. J., Krauss, R. M. (1991) Dissociation of high density lipoprotein precursors from apolipoprotein B-containing lipoproteins in the presence of unesterified fatty acids and a source of apolipoprotein A-I. J. Lipid Res. 32: 917–933

Nanjee, M. N., Crouse, J. R., King, J. M., Hovorka, R., Rees, S. E., Carson, E. R., Morgenthaler, J.-J., Lerch, P., Miller, N. E. (1996a) Effects of intravenous infusion of lipid-free apo A-I in humans. Arterioscler. Thromb. Vasc. Biol. 16: 1203–1214

Nanjee, M. N., Miller, N. E. (1996b) Effects of intravenous apolipoprotein A-I/phosphatidylcholine (apo AI/PC) discs in humans. Circulation 94: 2707

Neary, R. H., Gowland, E. (1987) Stability of free apolipoprotein A-I concentration in serum, and its measurement in normal and hyperlipidemic subjects. Clin. Chem. 33: 1163–1169

Neary, R., Bhatnagar, D., Durrington, P., Ishola, M., Arrol, S., Mackness, M. (1991) An investigation of the role of lecithin:cholesterol acyltransferase and triglyceride-rich lipoproteins in the metabolism of pre-beta high density lipoproteins. Atherosclerosis 89: 35–48

Neufeld, E. B., Cooney, A. M., Pitha, J., Dawidowicz, E. A., Dwyer, N. K., Pentchev, P. G., Blanchette, E. J. (1996) Intracellular trafficking of cholesterol monitored with a cyclodextrin. J. Biol. Chem. 271: 21604–21613

New, R. R. C. (1994) Preparation of liposomes. In: Liposomes – A Practical Approach. IRL, Oxford, pp 33–104

Nichols, V., Gong, E. L., Blanche, P. J., Forte, T. M. (1983) Characterization of discoidal complexes of phosphatidylcholine, apolipoprotein A-I and cholesterol by gradient gel electrophoresis. Biochim. Biophys. Acta 750: 353–364

Nielsen, J. H., Olsen, C. E., Jensen, C., Skibsted, L. H. (1996) Cholesterol oxidation in butter and dairy spread during storage. J. Dairy Res. 63: 159–167

Nievelstein, P. F. E. M., Fogelman, A. M., Mottino, G., Frank, J. S. (1991) Lipid accumulation in rabbit aortic intima 2 hours after bolus infusion of low density lipoprotein. Arteriscler. Thromb. 11: 1795–1805

Nunez, J. F., Swaney, J. B. (1984) Interaction between hepatic microsomal membrane lipids and apolipoprotein A-I. J. Biol. Chem. 259: 9141–9148

Ohta, T., Nakamura, R., Ikeda, Y., Shinohara, M., Miyazaki, A., Horiuchi, S., Matsuda, I. (1992) Differential effect of subspecies of lipoprotein containing apolipoprotein A-I on cholesterol efflux from cholesterol-loaded macrophages: functional correlation with lecithin : cholesterol acyltransferase. Biochim. Biophys. Acta 1165: 119–128

Ohvo, H., Slotte, J. P. (1996) Cyclodextrin-mediated removal of sterols from monolayers: effects of sterol structure and phospholipids on desorption rate. Biochemistry 35: 8018–8024

Olivier, P., Verwaerde, F., Hedges, A. R. (1991) Subchronic toxicity of orally administered beta-cyclodextrin in rats. J. Am. Coll. Toxicol. 10: 407–419

Oram, J. F., Yokoyama, S. (1996) Apolipoprotein-mediated removal of cellular cholesterol and phospholipids. J. Lipid Res. 37: 2473–2491

Oram, J. F., Brinton, E. A., Bierman, E. L. (1983) Regulation of HDL receptor activity in cultured human skin fibroblasts and human arterial smooth muscle cells. J. Clin. Invest. 72: 1611–1621

Oram, J. F., Johnson, C. J., Brown, T. A. (1987) Interaction of high density lipoprotein with its receptor on cultured fibroblasts and macrophages: evidence for reversible binding at the cell surface without internalisation. J. Biol. Chem. 262: 2405–2410

Palgunachari, M. N., Mishra, V. K., Lund-Katz, S., Phillips, M. C., Adeyeye, S. O., Alluri, S., Anantharamaiah, G. M., Segrest, J. P. (1996) Only the two end helices of eight tandem amphipathic helical domains of human apo A-I have significant lipid affinity. Implications for HDL assembly. Arterioscler. Thromb. Vasc. Biol. 16: 328–338

Parente, R. A., Lentz, B. E. (1984) Phase behavior of large unilamellar vesicles composed of synthetic phospholipids. Biochemistry 23: 2353–2359

Paszty, C., Maeda, N., Verstuyft, J., Rubin, E. M. (1994) Apolipoprotein A-I transgene corrects apolipoprotein E deficiency-induced atherosclerosis in mice. J. Clin. Invest. 94: 899–903

Patel, R. P., Diczfalusy, U., Dzeletovic, S., Wilson, M. T., Darley-Usmar, V. M. (1996) Formation of oxysterols during oxidation of low density lipoprotein by peroxynitrite, myoglobin, and copper. J. Lipid Res. 37: 2361–2371

Peluso, M. R., Dixon, J. L. (1997) Use of cyclodextrin to deliver and to modulate apolipoprotein B-100 production in HepG2 cells. Lipids 2: 811–823

Phillips, M. C., McLean, L. R., Stoudt, G. W., Rothblat, G. H. (1980) Mechanism of cholesterol efflux from cells. Atherosclerosis 36: 409–422

Phillips, M. C., Johnson, W. J., Rothblat, G. H. (1987) Mechanisms and consequences of cellular cholesterol exchange and transfer. Biochim. Biophys. Acta 906: 223–276

Phillips, J. C., Wriggers, W., Li, Z., Jonas, A., Schulten, K. (1997) Predicting the structure of apolipoprotein A-I in reconstituted high density lipoprotein disks. Biophys. J. 73: 2337–2346

Phillips, J. C., Wriggers, W., Li, Z., Jonas, A. (1998) HDL and apoA-I. http://www.ks.uluc.edu/Research/apoa1/ (accessed December 1998).

Ponsin, G., Sparrow, J. T., Gotto, A. M., Pownall, H. J. (1986) *In vivo* interaction of synthetic acylated apopeptides with high density lipoproteins in rat. J. Clin. Invest. 77: 559–567

Pownall, H. J., Massey, J. B., Kusserow, S. K., Gotto, A. M. (1978) Kinetics of lipid-protein interactions: interaction of apolipoprotein A-1 from human plasma high density lipoproteins with phosphatidylcholine. Biochemistry 17: 1183–1188

Pownall, H. J., Massey, J. B., Sparrow, J. T., Gotto, A. M. (1987) In: Gotto, A. M. (ed.) Plasma Lipoproteins. Elsevier, Amsterdam, pp 95–127

Rapp, J. H., Lespine, A., Hamilton, R. L., Colyvas, N., Chaumeton, A. H., Tweedie-Hardman, J., Kotite, L., Kunitake, S. T., Havel, R. J., Kane, J. P. (1994) Triglyceride-rich lipoproteins isolated by selective-affinity anti-apolipoprotein B immunosorption from human atherosclerotic plaque. Arterioscler. Thromb. 14: 1767–1774

Riottot, M., Olivier, P., Huet, A., Caboche, J.-J., Parquet, M., Khallou, J., Lutton, C. (1993) Hypolipidemic effects of β-cyclodextrin in the hamster and in the genetically hypercholesterolemic Rico rat. Lipids 28: 181–188

Rodrigueza, W. V., Pritchard, P. H., Hope, M. J. (1993) The influence of size and composition on the cholesterol mobilizing properties of liposomes *in vivo*. Biochim. Biophys. Acta 1153: 9–19

Rodrigueza, W. V., Williams, K. J., Rothblat, G. H., Phillips, M. C. (1997a) Remodeling and shuttling. Mechanisms for the synergistic effects between different acceptor particles in the mobilization of cellular cholesterol. Arterioscler. Thromb. Vasc. Biol. 17: 383–393

Rodrigueza, W. V., Mazany, K. D., Essenburg, A. D., Bisgaier, C. L., Williams, K. J. (1997b) Large versus small unilamellar vesicles mediate reverse cholesterol transport *in vivo* into two distinct hepatic metabolic pools. Implications for the treatment of atherosclerosis. Arterioscler. Thromb. Vasc. Biol. 17: 2132–2139

Rodrigueza, W. V., Klimuk, S. K., Pritchard, P. H., Hope, M. J. (1998) Cholesterol mobilization and regression of atheroma in cholesterol-fed rabbits induced by large unilamellar vesicles. Biochim. Biophys. Acta 1368: 306–320

Roerdink, F., Dijkstra, J., Hartman, G., Bolscher, B., Scherphof, G. (1981) The involvement of parenchymal, Kupffer and endothelial liver cells in the hepatic uptake of intravenously injected liposomes. Biochim. Biophys. Acta 677: 79–89

Rooney, M., Tamura-Lis, W., Lis, L. J., Yachnin, S., Kucuk, O., Kauffman, J. W. (1986) The influence of oxygenated sterol compounds on dipalmitoylphosphatidylcholine bilayer structure and packing. Chem. Phys. Lipids 41: 81–92

Ross, R. (1995) Cell biology of atherosclerosis. Annu. Rev. Physiol. 57: 791–804

Rothblat, G. H., Phillips, M. C. (1982) Mechanism of cellular cholesterol efflux from cells. Effects of acceptor structure and composition. J. Biol. Chem. 257: 4775–4782

Rothblat, G. H., Mahlberg, F. H., Johnson, W. J., Phillips, M. C. (1992) Apolipoproteins, membrane cholesterol domains, and the regulation of cholesterol efflux. J. Lipid Res. 33: 1091–1097

Rothblat, G. H., de la Llera-Moya, M., Atger, V., Kellner-Weibel, G., Williams, D. L., Phillips, M. C. (1999) Cell cholesterol efflux: integration of old and new observations provides new insights. J. Lipid Res. 40: 781–796

Rubin, E. M., Krauss, R. M., Spangler, E. A., Verstuyft, J. G., Clift, S. M. (1991) Inhibition of early atherogenesis in transgenic mice by human apolipoprotein A-I. Nature 353: 265–267

Saito, H., Miyako, Y., Handa, T., Miyajima, K. (1997) Effect of cholesterol on apolipoprotein A-I binding to lipid bilayers and emulsions. J. Lipid Res. 38: 287–294

Salehian, B., Wang, C., Alexander, G., Davidson, T., McDonald, V., Berman, N., Dudley, R. E., Ziel, F., Swerdloff, R. S. (1995) Pharmacokinetics, bioefficacy, and safety of sublingual testosterone cyclodextrin in hypogonadal men: comparison to testosterone enanthate – a clinical research center study. J. Clin. Endocrinol. Metabol. 80: 3567–3575

Savion, N., Kotev-Emeth, S. (1993) Role of apolipoproteins A-I, A-II and C-I in cholesterol efflux from endothelial and smooth muscle cells. Eur. Heart J. 14: 930–935

Scherphof, G. L., Morselt, H. (1984) On the size-dependent disintegration of small unilamellar phosphatidylcholine vesicles in rat plasma: evidence of complete loss of vesicle structure. Biochem. J. 221: 423–429

Schmitz, G., Niemann, R., Brennhausen, B., Krauss, R. M., Assmann, G. (1985) Regulation of high density lipoprotein receptors in cultured macrophages: role of acyl CoA : cholesterol acyltransferase. EMBO J. 4: 2773–2779

Schwenke, D. C., Carew, T. E. (1989a) Initiation of atherosclerotic lesions in cholesterol-fed rabbits. I. Focal increases in arterial LDL concentration precede development of fatty streak lesions. Arteriosclerosis 9: 895–907

Schwenke, D. C., Carew, T. E. (1989b) Initiation of atherosclerotic lesions in cholesterol-fed rabbits. II. Selective retention of LDL vs. selective increases in LDL permeability in susceptible sites of arteries. Arteriosclerosis 9: 908–918

Segrest, J. P., Jones, M. K., de Loof, H., Brouillette, C. G., Venkatachalapathi, Y. V., Anantharamaiah, G. M. (1992) The amphipathic helix in the exchangeable apolipoproteins: a review of secondary structure and function. J. Lipid Res. 33: 141–166

Segrest, J. P., Garber, D. W., Brouillette, C. G., Harvey, S. C., Anantharamaiah, G. M. (1994) The amphipathic alpha helix: a multifunctional structural motif in plasma apolipoproteins. Adv. Prot. Chem. 45: 303–369

Semple, S. C., Chonn, A., Cullis, P. R. (1998) Interactions of liposomes and lipid-based carrier systems with blood proteins: Relation to clearance behaviour in vivo. Adv. Drug Del. Rev. 32: 3–17

Soma, M. R., Donetti, E., Parolini, C., Sirtori, C. R., Fumagalli, R., Franceschini, G. (1995) Recombinant apolipoprotein A-I_{Milano} dimer inhibits carotid intimal thickening induced by perivascular manipulation in rabbits. Circ. Res. 76: 405–411

Soutar, A. K., Knight, B. L., Myant, N. B. (1982) The characterization of lipoproteins in the high density fraction obtained from patients with familial lecithin : cholesterol acyltransferase deficiency and their interaction with cultured human fibroblasts. J. Lipid Res. 23: 380–390

Sparks, D. L., Phillips, M. C., Lund-Katz, S. (1992) Effect of cholesterol on the charge and structure of apolipoprotein A-I in recombinant high-density lipoproteins particles. J. Biol. Chem. 267: 25830–25838

Sparks, D. L., Davidson, W. S., Lund-Katz, S., Phillips, M. C. (1993) The conformation of apolipoprotein A-I in discoidal and spherical recombinant high-density lipoproteins particles. J. Biol. Chem. 268: 23250–23257

Sparks, D. L., Anantharamaiah, G. M., Segrest, J. P., Phillips, M. C. (1995) Effect of the cholesterol content of reconstituted LpA-I on lecithin : cholesterol acyltransferase activity. J. Biol. Chem. 270: 5151–5157

Stein, O., Stein, Y. (1973) The removal of cholesterol from aortic muscle cells in culture and Lundschutz ascites cells by fractions of human high-density lipoprotein. Biochim. Biophys. Acta 326: 232–244

Stein, Y., Stein, O. (1979)In: Scanu, A. M., Wissler, R. W., Getz, G. S. (eds) Biochemistry of Atherosclerosis. Marcel Dekker, New York, pp 313–324

Stein, Y., Glangeaud, M. C., Fainaru, M., Stein, O. (1975) The removal of cholesterol from aortic muscle cells in culture and Lundschutz ascites cells by fractions of human high-density apolipoprotein. Biochim. Biophys. Acta 380: 106–118

Sun, D., Gilboe, D. D. (1994) Effect of of the platelet-activating factor antogonist BN 50739 and its diluents on mitochondrial respiration and membrane lipids during and following cerebral ischemia. J. Neurochem. 62: 1929–1938

Suzuki, M., Sato, A. (1985) Nutritional significance of cyclodextrins: indigestability and hypolipidemic effects of cyclodextrin. J. Nutr. Sci. Vitaminol. 31: 209–223

Sviridov, D., Pyle, L. E., Fidge, N. (1996a) Identification of a sequence of apolipoprotein A-I associated with the efflux of intracellular cholesterol to human serum and apolipoprotein A-I containing particles. Biochemistry 35: 189–196

Sviridov, D., Pyle, L. E., Fidge, N. (1996b) Efflux of cellular cholesterol and phospholipid to apolipoprotein A-I mutants. J. Biol. Chem. 271: 33277–33283

Swaney, J. B. (1983) Reconstitution of apolipoprotein A-I from human high-density lipoprotein with bovine brain sphingomyelin. J. Biol. Chem. 258: 1254–1259

Szedlacsek, S. E., Wasowicz, E., Hulea, S. A., Nishida, H. I., Kummerow, F. A., Nishida, T. (1995) Esterification of oxysterols by human plasma lecithin-cholesterol acyltransferase. J. Biol. Chem. 270: 11812–11819

Szejtli, J. (1988) In: Cyclodextrin Technology. Davies, J. E. D. (ed) Kluwer, Dordrecht, The Netherlands, pp 84–86, 163–169

Tabas, I., Marathe, S., Keesler, G. A., Beatini, N., Shiratori, Y. (1996) Evidence that the initial up-regulation of phosphatidylcholine biosynthesis in free cholesterol-loaded macrophages is an adaptive response that prevents cholesterol-induced cellular necrosis. J. Biol. Chem. 271: 22773–22782

Tall, A. R. (1993) Plasma cholesteryl ester transfer protein. J. Lipid Res. 34: 1255–1274

Tall, A. R., Shipley, G. G., Small, D. M. (1976) Conformational and thermodynamic properties of apoA-I of human plasma high density lipoproteins. J. Biol. Chem. 251: 3749–3755

Tanaka, F., Suga, M., Nishikawa, H., Muranaka, H., Ando, M. (1997) Effects of pulmonary surfactant on macrophage migration: Suppression of chemokinesis by surfactant phospholipid and enhancement of chemotaxis by surfactant protein. Respirology 2: 119–126

Tansey, J. T., Thuren, T. Y., Jerome, W. G., Hantgan, R. R., Grant, K., Waite, M. (1997) Hydrolysis of phosphatidylcholine by hepatic lipase in discoidal and spheroidal recombinant high-density lipoprotein. Biochemistry 36: 12227–12234

Til, H. P., Bar, A. (1998) Subchronic (13-week) oral toxicity study of γ-cyclodextrin in dogs. Regul. Toxicol. Pharmacol. 27: 159–165

Toyoda, K., Shada, T., Uneyama, C., Takada, K., Takahashi, M. (1997) Carcinogenicity study of beta-cyclodextrin in F344 rats. Food Chem. Toxicol. 35: 331–336

van de Bovenkamp, P., Kosmeijer-Schuil, T. G., Katan, M. B. (1988) Quantification of oxysterols in Dutch foods: egg products and mixed diets. Lipids 23: 1079–1085

Vine, D. F., Croft, K. D., Beilin, L. J., Mamo, J. C. (1997) Absorption of dietary cholesterol oxidation products and incorporation into rat lymph chylomicrons. Lipids 32: 887–893

Voglino, L., McIntosh, T. J., Simon, S. A. (1998) Modulation of the binding of signal peptides to lipid bilayers by dipoles near the hydrocarbon-water interface. Biochemistry 37: 12241–12252

von Eckardstein, A. (1996) Cholesterol efflux from macrophages and other cells. Curr. Opin. Lipidol. 7: 308–319

von Eckardstein, Funke, H., Walter, M., Altland, K., Benningahaven, A., Assmann, G. (1990) Structural analysis of human apolipoprotein A-I variants. Amino acid substitutions are non-randomly distributed throughout the apolipoprotein A-I primary structure. J. Biol. Chem. 265: 8610–8617

Waalkens-Berendsen, D. H., Verhagen, F. J. J., Bar, A. (1998) Embryotoxicity and teratogenicity study with γ-cyclodextrin in rats. Regul. Toxicol. Pharmacol. 27: 166–171

Wald, J. H., Goormaghtigh, E., de Mauter, J., Ruysschaert, J.-M., Jonas, A. (1990) Investigation of the lipid domains and apolipoprotein A-I orientation in reconstituted high-density lipoproteins by fluorescence and IR methods. J. Biol. Chem. 265: 20044–20050

Walker, T. L., Dass, C. R., Burton, M. A., DeCruz, E. E. (1997) The status of antisense oligodeoxynucleotides as therapeutic agents. Int. J. Pharm. Med. 11: 263–268

Walker, T. L., DeCruz, E. E., Dass, C. R., Burton, M. A. (1998) A method for intratumoral continuous infusion of antisense oligodeoxynucleotides. J. Pharm. Sci. 87: 387–389

Westman, J. Roobol, C., Heymans, C., Carlson, L. A., Wulfert, E. (1993) Efflux of cholesterol from cholesterol loaded macrophages by incubation with synthetic HDL-particles. Scand. J. Lab. Invest. 53: 773–782

Westman, J., Roobol, C., Carlson, L. A., Wulfert, E. (1995) In vitro reverse cholesterol transport from THP-1-derived macrophage-like cells with synthetic HDL particles consisting of proapolipoprotein AI and phosphatidylcholine. Scand. J. Clin. Lab. Invest. 55: 23–33

Westman, J., Kallin, B., Bjorkhem, I., Nilsson, J., Diczfalusy, U. (1998) Sterol 27-hydroxylase and apoAI/phospholipid-mediated efflux of cholesterol from cholesterol-laden macrophages: evidence for an inverse relation between the two mechanisms. Arterioscler. Thromb. Vasc. Biol. 18: 554–561

Williams, K. J., Scanu, A. M. (1986) Uptake of endogenous cholesterol by a synthetic lipoprotein. Biochim. Biophys. Acta 875: 183–194

Williams, K. J., Tabas, I. (1995) The response-to-retention hypothesis of early atherogenesis. Arterioscler. Thromb. Vasc. Biol. 15: 551–561

Williams, K. J., Werth, V. P., Wolff, J. A. (1984) Intravenously administered lecithin liposomes: a synthetic antiatherogenic lipid particle. Perspect. Biol. Med. 27: 417–423

Winegar, D. A., Salisbury, J. A., Sundseth, S. S., Hawke, R. L. (1996) Effects of cyclosporin on cholesterol 27-hydroxylation and LDL receptor activity in HepG2 cells. J. Lipid Res. 37: 179–191

Yancey, P. G., Bielicki, J. K., Johnson, W. J., Lund-Katz, S., Palgunachari, M. N., Anantharamaiah, G. M., Segrest, J. P., Phillips, M. C., Rothblat, G. H. (1995) Efflux of cellular cholesterol and phospholipid to lipid-free apolipoproteins and class A amphipathic peptides. Biochemistry 34: 7955–7965

Yancey, P. G., Rodrigueza, W. V., Kilsdonk, E. P. C., Stoudt, G. W., Johnson, W. J., Phillips, M. C., Rothblat, G. H. (1996) Cellular cholesterol efflux mediated by cyclodextrins: demonstration of kinetic pools and mechanism of efflux. J. Biol. Chem. 271: 16026–16034

Zannis, V. I., Kurnit, D. M., Breslow, J. L. (1982) Hepatic apolipoprotein A-I and apolipoprotein E and intestinal apolipoprotein A-I are synthesized in precursor isoprotein forms by organ cultures of human fetal tissues. J. Biol. Chem. 257: 536–544

Zhao, Q., Temsamani, J., Agarwal, S. (1995) Use of cyclodextrin and its derivatives as carriers for oligonucleotide delivery. Antisense Res. Dev. 5: 185–192

Zhao, Q., Temsamani, J., Tadarola, P. L., Jiang, Z., Agarwal, S. (1996a) Effect of different chemically modified oligodeoxynucleotides on immune stimulation. Biochem. Pharmacol. 51: 173–182

Zhao, Q., Temsamani, J., Tadarola, P. L., Jiang, Z., Agarwal, S. (1996b) Modulation of oligonucleotide-induced immune stimulation by cyclodextrin analogs. Biochem. Pharmacol. 52: 1537–1544

Zhao, Y., Sparks, D. L., Marcel, Y. L. (1996c) Effect of apolipoprotein A-I and surface lipid composition of reconstituted discoidal HDL on cholesterol efflux from cultured fibroblasts. Biochemistry 35: 16510–16518

Zhao, Y., Sparks, D. L., Marcel, Y. L. (1996d) Specific phospholipid association with apolipoprotein A-I stimulates cholesterol efflux from human fibroblasts. Studies with reconstituted sonicated lipoproteins. J. Biol. Chem. 271: 25145–25151

Zorich, N. L., Kezdy, K. E., Jonas, A. (1987) Properties of discoidal complexes of human apolipoprotein A-I with phosphatidylcholines containing various fatty acid chains. Biochim. Biophys. Acta 919: 181–189