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Manipulation of Reverse Cholesterol Transport System-An Exploration for Rapid Regression of Athersclerosis

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Abstract: High plasma levels of Low Density Lipoprotein Cholesterol (LDL-C) triglycerides and low levels of High Density Lipoprotein Cholesterol (HDL-C) are strong and independent risk factors for Coronary Heart Disease (CHD). The first 2 abnormalities are managed by diet and a variety of drugs including statins cholesterol absorption inhibitors fibrates and niacin. Some of these drugs also elevate HDL albeit poorly. Appropriate treatments for HDL elevation are still needed. Low HDL-C is a common lipoprotein abnormality in patients with CHD. HDL mediates a broad variety of antiatherogenic effects within the cardiovascular system. An important antiatherogenic function of HDL is its ability to stimulate reverse cholesterol transport a process by which HDL is able to translocate excess cholesterol mass from cells within the subendothelial space and atheromatous plaque and deliver it back to the liver for elimination via the gastrointestinal tract. Among other functions HDL stimulates endothelial nitric oxide production and vasodilatation it is antithrombotic by reducing platelet aggregability and prostacyclin production. HDL also inhibits endothelial cell apoptosis reduces the oxidation of fatty acids in LDL-C particles and inhibits the expression of endothelial Cells Adhesion Molecules (ICAM-1 VCAM-1). Prospective epidemiologic studies indicate that high serum levels of HDL-C are atheroprotective while low levels of HDL-C are associated with increased risks for atherosclerotic disease and its clinical sequelae. Research in the past decade has greatly enhanced our understanding of HDL metabolism and has consequently offered potential therapeutic targets to address low HDL syndrome. Many new drugs and systems for treating patients with low HDL-C are currently being developed. HDL mimetics edible HDL Cholesterol Ester Transfer Protein (CETP) inhibitors endocannabinoid type-1 receptor antagonists novel Proliferators-Activated Receptor (PPAR) agonists and a systemic treatment designed to change the composition of the patient's own HDL to a super HDL particle to prevent the rupture of vulnerable plaque by causing rapid regression of atherosclerosis are being studied. The purpose of this review is to examine key players in HDL metabolism and therapeutics that modulate these targets.

Key words: Atherosclerosis, reverse cholesterol transport, cholesterol, high density lipoproteins

INTRODUCTION

Atherosclerosis is a slowly progressive disease of arteries that begins early in life but rarely produces symptoms until middle age. Atherosclerosis is characterized by focal fatty thickening in the inner aspects of vessels supplying blood to the heart brain and other vital organs. These lesions obstruct the lumen of the vessel and result in ischaemia of the tissue supplied by the vessel. Prolonged or sudden ischaemia may result in a clinical heart attack or stroke from which the patient may or may not recover. Atherosclerosis and its primary clinical manifestation CHD is the major cause of death in developed countries. Statistics indicate that in the USA cardiovascular disease claims more lives each year than the next 5 eading causes of death combined.

The association between cholesterol and atherosclerosis has been almost universally accepted and cholesterol is a major component of atherosclerotic plaques. Cholesterol accumulation within atherosclerotic plaque occurs when cellular cholesterol synthesis and/or influx into the arterial wall (from apo B-containing lipoproteins) exceeds cholesterol efflux (Spady, 1999). Parenchymal cells maintain cholesterol balance by downregulation of *De novo* cholesterol synthesis and LDL receptor expression whereas macrophages continue to acquire cholesterol from apo B-containing lipoproteins via pathways that are not subject to sterol-mediated feedback control. This results in a continuing cholesterol uptake by macrophages and their eventual conversion into lipid-laden atherosclerotic foam cells.

To reduce the cholesterol influx and thus to prevent or retard coronary artery diseases the current therapeutic modalities are aimed at reducing the concentrations of cholesterol-rich apo B-containing lipoproteins by dietary means hypolipidaemic drugs or extracorporeal removal of plasma lipoproteins.

The first step in the treatment of patients with elevated serum cholesterol is diet therapy. The use of diet as a primary mode of therapy requires a major effort of physicians nutritionists dietitians and other health professionals. Unfortunately diet therapy on its own is not overly successful. If this approach fails pharmaceutical therapy is engaged.

Several drugs used singly or in combination are available. Cholestyramine and colestipol are bile acid sequestrants. These compounds are anion-exchange resins that can bind bile acids in the intestinal lumen. They reduce serum cholesterol both because of the reduced enterohepatic recirculation of bile acids and because the diminished intestinal content of bile acids lessens cholesterol absorption. Cholestyramine and colestipol administration lead to marked increase in fecal excretion of bile acids and a slight increase in the excretion of neutral steroids. Thus cholesterol the precursor of bile acids undergoes increased catabolism.

Nicotinic acid decreases hepatic formation of Very Low-Density Lipoproteins (VLDL) with a corresponding decrease in its metabolite LDL and consequently of its component cholesterol. The fibric acid derivatives clofibrate bezafibrate gemfibrozil and fenofibrate inhibit the synthesis of cholesterol in the liver and the intestinal mucosa. These drugs seem to exert a slight inhibition between the acetate and mevalonate stages in the cholesterol biosynthetic pathway.

The statins are powerful inhibitors of cholesterol biosynthesis. Their actions are based on the inhibition of the enzymatic rate-limiting step 3-Hydroxy-3 Methylglutaryl Coenzyme A (HMG-CoA) reductase in the pathway of cholesterol biosynthesis. These drugs are effective in reducing the lipid abnormalities to some extent and they also decrease both coronary and total mortality as shown in primary and secondary prevention trials. There have been more than 20 repeat coronary angiographic trials which have demonstrated that lowering plasma cholesterol slow the progression and some regression can occur in individual lesions. However when all the arteries are assessed current therapy does not lead to nett regression of coronary atherosclerosis.

Revascularisation and angioplasty are mechanical treatments that do not affect the underlying coronary atherosclerosis. In fact bypass accelerates the disease in the native arteries that are treated. Revascularisation techniques are mainly for symptom relief and in certain sub-sets of patients this may improve prognosis in the intermediate term.

LDL apheresis is a term used for procedures that mainly remove the LDL particle from plasma. LDL apheresis can be achieved by various means. The first reports of such a procedure involved the adsorption of LDL onto heparin-agarose beads (affinity chromatography) or the use of immobilized LDL-antibodies. Other methods have followed which include cascade filtration adsorption to immobilized dextran sulphate and LDL precipitation at low pH in the presence of Heparin (HELP). Each method specifically removes LDL but little or no HDL.

Notwithstanding the important progresses in the above described achievements CHD remains the major cause of deaths in the developed countries. This is probably due to the fact that there are other risk factors that are important in patients in addition to increased LDL-C concentrations. Therefore clearly there is a need for additional preventive and therapeutic interventions to complement the results of LDL lowering. Coronary atherosclerotic plaques which are unstable and prone to rupture have a thin fibrous cap and are usually rich in lipids and macrophages. In contrast plaques that are stable with less chance of rupture are lipid poor. Removal of these lipids with modified lipoprotein therapy is presently being investigated in humans. It appears that HDL has opposing properties to the apo B containing lipoproteins. Whereas on the one hand LDL is a causative factor of atherosclerosis ultimately resulting in the deposition of cholesterol within plaques it is becoming more acceptable that on the other hand HDL is responsible for removing cholesterol from the plaques. Hence the higher the ratio of plasma HDL to LDL the more beneficial for the patient. The liver plays a key role in the homeostasis of plasma levels of LDL (which is a metabolite of VLDL) and HDL. Endogenous cholesterol is synthesised in the liver and secreted in the form of VLDL. Within the vascular system VLDL is metabolised ultimately resulting in LDL. The cholesterol in LDL may depending on various circumstances end up in plaques. Evidence is mounting indicating that HDL is capable of removing cholesterol from the plaques back into the vascular circulation to the liver. The liver metabolises a large proportion of this HDL-C into bile salts which are then secreted through the biliary system into the intestines. This is known as Reverse Cholesterol Transport (RCT). Hence the layman terminology bad cholesterol (LDL) and good cholesterol (HDL).

There is now interest in increasing the levels of the good HDL-C which may ultimately result in clinical application of RCT for the treatment of atherosclerosis.

CLINICAL EVIDENCE FOR THE BENEFITS OF RAISING HDL-C LEVELS

The relationship between low levels of HDL-C and the development of CHD can be inferred from epidemiological studies where even small differences in

the level of HDL-C are associated with substantial variations in the risk of major coronary events. Data from the Framingham population study indicated that at any given level of total cholesterol the relative risk of CAD increases with decreasing levels of HDL-C (Castelli *et al*., 1986).

In addition prospective clinical studies have demonstrated a link between low HDL-C and an increased risk of atherosclerosis. In the PROCAM study low levels of HDL-C were associated with a high incidence of atherosclerotic CAD. The relationship between HDL-C and the incidence of CHD in the Framingham Study Lipid Research Clinics Prevalence Mortality Follow-up Study Coronary Primary Prevention Trial control group and the Multiple Risk factor Intervention Trial control group was further examined. Analysis of these studies demonstrated that for every 1 mg^{-1}dL rise in plasma HDL-C the risk of CHD decreased by 2% in men and 3% in women and this was independent of LDL-C (Gordon *et al*., 1989).

The Veterans Affairs cooperative studies program High-density Intervention Trial (VA-HIT) assessed the effect of raising HDL-C levels on CHD risk in patients with low levels of both LDL-C and HDL-C. After 1 year gemfibrozil treatment in comparison with placebo significant effects on HDL-C and total cholesterol but not LDL-C were observed and this was associated with a reduction of 22% in nonfatal myocardial infarction (MI) or death due to CHD. For every 5% increase in plasma HDL-C CHD death or MI decreased by 11% (Rubins *et al*., 1999). However further studies are required to confirm these findings even though owing to the complexity of lipid metabolism it is difficult to isolate the effect of HDL-C on CHD.

ENHANCING HDL-C LEVELS WITH LIFESTYLE CHANGES

HDL is generally minimally affected by changes in the type of dietary fatty acids and substitution of carbohydrates for fat. Lifestyle changes to increase HDL are weight loss with obesity increased physical activity smoking cessation and alcohol consumption in moderate amounts. Smoking cessation results in plasma HDL-C increases on average 6-8 mg^{-1}dL.

ENHANCING HDL-C LEVELS WITH DRUGS

In addition to the dose-dependent reduction of LDL-C levels statins exert beneficial effects in the lipid profile; however these statins differ in their ability to raise HDL-C. A direct comparison of the lipid modifying effects of atorvastatin pravastatin lovastatin fluvastatin and simvastatin was performed in the CURVES study (Jones *et al*., 1998). Simvastatin and rosuvastatin seem to have the most beneficial although minimal effects on HDL-C.

Niacin and fibrates are more effective at increasing HDL-C levels but they produce only modest LDL-C lowering. Niacin raises HDL-C levels by up to 30% and increases of 10-15% have been reported with fibrates. Nicotinic acid and fibrates activate the Peroxisome Proliferator Activated Receptor α (PPARα) system resulting in raising HDL (Sirtori, 2006).

Ezetimibe is a selective cholesterol absorption inhibitor that blocks the uptake of dietary and biliary cholesterol by preventing its transport through the intestinal wall without affecting the passage of other fat-soluble nutrients. Ezetimibe can reduce LDL-C levels by up to 19% and moderately increases HDL-C by 3%. It is well tolerated when administered with a statin or fibrate with additive effects (Catapano and Ezetimibe, 2001).

Statin-fibrate combinations have only been studied in small trials. Such combination therapies do improve the entire lipid profile clotting factors insulin resistance and blood pressure (Wierzbicki *et al*., 2001). However, high incidences of myalgia and rhabdomyolysis have consistently been reported with combination therapies involving gemfibrozil and an excess of these adverse events resulted in the withdrawal of cerivastatin in 2001.

A combination of lovastatin plus extended release niacin produced greater effects on LDL-C HDL-C and Triglycerides (TG) levels than either of the two drugs alone; HDL-C levels were increased by 30% LDL-C decreased by 47%. However, adverse events such as cutaneous flushing resulted in the withdrawal of 7% of patients from the study (Kashvap *et al*., 2000).

NOVEL TREATMENTS INCREASING REVERSE CHOLESTEROL TRANSPORT

Recombinant apolipoprotein AI/HDL: There is substantial support for the feasibility of apo A-I infusions in human subjects. In the first of these studies apoA-I/phosphatidyl choline discs were infused over 4 h into 7 healthy men with following results (Nanjee *et al*., 1999). The rise of plasma apoA-I was greatest in small pre-beta-migrating lipoproteins not present in the infusate; there was a simultaneous increase in the levels of HDL unesterified cholesterol. After stopping the infusion the concentrations of HDL unesterified cholesterol apoA-I and small pre-beta-HDL particles decreased and those of HDL cholesterol esters and large alpha migrating HDL increased. Apo B-containing lipoproteins became enriched in cholesterol esters. It was concluded that the

infusion of apoA-I/phosphatidyl complexes resulted in an increased intravascular production of small pre-beta-HDL *in vivo* and that this was associated with an increase in the efflux and esterification of unesterified cholesterol from fixed tissues. However it was not possible to determine which fixed tissues (liver spleen aorta) were the sources of the new cholesterol in plasma HDL.

In another study the effect of proapo A-I/phospholipid complexes on the fecal sterol excretion was explored (Eriksson *et al*., 1999). After intravenous infusion of recombinant proapoA-I/phospholipid complexes into four subjects with heterozygous familial hypercholesterolemia there was a 30% increase in fecal bile salt excretion and a 39% increase in neutral sterol excretion corresponding to the removal of approximately 500 mg dL^{-1} excess of cholesterol after infusion. Control studies with infusion of only liposomes in two patients had no effect on the cholesterol excretion. Equally important was the observation that serum lathosterol a marker for the rate of cholesterol synthesis *in vivo* was unchanged suggesting that the nett increase in cholesterol excretion reflected an enhanced RCT. Although it was not possible to identify the precise source of the excess excreted cholesterol it was speculated that repeated treatments with proapo A-I/phospholipid complexes may reduce cholesterol in the arterial wall to some extent. Clinical trials will be necessary to evaluate the antiatherogenic potential of such therapy. Finding a way to increase the efflux of cholesterol from foam cells within the arterial wall and delivering this cholesterol to the liver for excretion may be the key to achieving timely regression of atherosclerotic lesions.

Both of these studies have shown that the infusion of apo A-I/phospholipid complexes into human subjects at least in the short term is a clinically safe procedure that may enhance the efflux of cholesterol from the arterial wall and possibly lead to the regression of atherosclerotic lesions.

In a human clinical trial it was reported that 5 weekly infusions of an Apo A-I Milano/phospholipid complex produced significant regression of coronary atheroma as shown by Intravascular Ultra Sound (IVUS). These results are promising nevertheless some important unanswered questions remain. For example it was interesting to note that although rapid regression was obtained the efficacy of the Apo A-I Milano/phospholipid therapy was not concentration dependent over a three-fold range (Nissen *et al*., 2003).

Other agents that mimic HDL are being developed with the aim of reversing atherosclerosis. These HDL mimetics include Apo A-1 mutants and peptide mimetics of Apo A-1. Although cell culture work shows promise for Apo A-1 peptide mimetics one should not overlook the fact that these peptides when associated with lipid in the vascular pool will aggregate to a large new molecule with conformational novelties that the body may consider as a foreign lipid-protein complex which could result in consequential rejection by the body and creation of antibodies which would be disastrous.

It was recently shown that in subjects with low HDL-C levels CETP inhibition markedly increased HDL-C levels and also decreased LDL-C levels both when administered as single therapy and when administered in combination with a statin (Brousseau *et al*., 2004). CETP is a plasma glycoprotein that facilitates the transfer of cholesteryl esters from HDL-C to apolipoprotein B-containing lipoproteins. The relation of CETP activity to the risk of CHD remains controversial (Barter *et al*., 2004). It is not clear whether CETP-deficient persons are protected from CHD; they may even be at increased risk (Zhong *et al*., 1996). This phenomenon has recently been expressed when clinical trials with torcetrapib a CETP inhibitor were withdrawn world wide in 2006 because of serious adverse events. It is imperative to understand that HDL-C is only 1 factor involved in RCT. RCT is not a static process but a continuous dynamic metabolic process. HDL-C is transported and delivered to the liver through interaction with the Scavenger Receptor class B-type-1 (SR-B1). However HDL is present in the vascular circulation in various conformational forms. SR-B1 may be specific for one particular conformational form of HDL which may not be induced by CETP inhibition. This may result in increases of HDL-C due to CETP inhibition but not clearance by the liver of the increased HDL-C. Relatively high HDL-C plasma levels do not necessarily reflect the efficacy and antiatherogenicity of RCT (Eckardstein *et al*., 2001). The efficiency of cholesterol flux through the RCT pathways is clinically more relevant than the HDL-C concentrations. The concentrations of HDL subclasses the mobilization of cellular lipids for efflux and the kinetics of HDL metabolism are important determinants of RCT and the risk of atherosclerosis. Furthermore it remains to be determined whether increased HDL-C concentrations will ultimately result in formation of cholesterol gallstones in the biliary system.

THE PLASMA DELIPIDATION PROCESS (PDP)

The importance of apolipoproteins in lipid metabolism is well recognized (Cham, 1978). To investigate the transport of cholesterol from peripheral tissues to the liver for clearance by RCT (Ross and Golmest, 1976; Biesbroeck *et al*., 1983; Oram *et al*., 1983; Ho *et al*., 1980; Sirtori *et al*., 1999) researchers have

employed systems in which the unidirectional efflux of cholesterol from cells growing in culture can be monitored. The cell culture studies approaches have been translated in much interest aimed at enhancing cholesterol efflux from the arterial wall and promoting its transport to the liver for excretion in RCT. The initial step in RCT is efflux of cholesterol from cell membranes and/or extracellular cholesterol to acceptor particles. A physiological acceptor for cholesterol *in vivo* appears to be nascent HDL particles which are discoidal prebeta-migrating HDL complexes of phospholipid and apoA-I.

It is also possible that apo A-I on its own without phospholipid is a good substrate for the efflux of cellular phospholipid and cholesterol. Other HDL containing apolipoproteins such as apo E may also be involved in RCT. The ATP-Binding Cassette transporter (ABCA1) is a key mediator of cellular cholesterol and phospholipid efflux to apoA-I. Comparatively HDL has only minor effects on lipid efflux (Wang *et al*., 2000). The ABCA1 pathway is now regarded an important therapeutic target for mobilising excess cholesterol from tissue macrophages and protecting against atherosclerosis (Oram and Vaughan, 2000).

Another cellular lipoprotein receptor SR-B1 for HDL plays an important role in delivering HDL directly to the liver. HDL-C that is taken up by the liver may then excreted in the form of bile salts and cholesterol (Trigatti *et al*., 2000).

Nascent HDL particles which are thought to be the *in vivo* acceptors for cholesterol are secreted by liver and small intestine. They are also formed at low concentrations during the metabolism of triglyceride-rich lipoproteins from excess surface material (phospholipid and soluble apolipoproteins). These discoidal pre-β-migrating HDL complexes accept cellular cholesterol which is then esterified by Lecithin-Cholesterol Acyl Transferase (LCAT) to cholesteryl esters. In this way nascent HDL is converted to the α-migrating spherical HDL found in plasma. HDL cholesteryl esters are cleared from plasma through several pathways. In the presence of CETP a portion of the HDL cholesteryl esters is transferred to lower-density lipoproteins (in exchange for triglyceride) and ultimately may be returned to the liver via the LDL receptor. HDL-C is also delivered directly to the liver through interaction with the scavenger receptor SR-B1. The liver accounts for approximately 75% of total HDL cholesterol ester turnover (Jones *et al*., 1998). The final step in RCT pathway is excretion of cholesterol from the liver into bile either directly or after conversion to bile salts. Figure 1 illustrates a simplified view cholestrol related lipoprotiens metabolism.

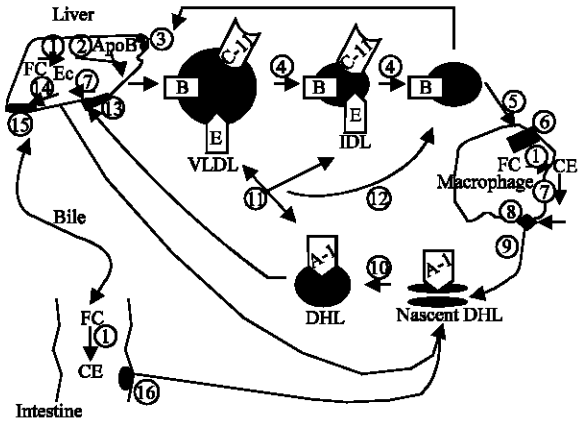

Fig. 1: Simplified view of cholesterol-related lipoproteins metabolism

Acyl Coenzyme A: Cholesterol Acyltransferase (ACAT)
Microsomal Transfer Protein (MTP)
Low-Density Lipoprotein (LDL) receptor
Lipoprotein Lipase (LPL)
Oxidation
CD36 Scavenger Receptor A (SR-A)
Cholesterol Ester Hydrolase (CEH)
Adenosine triphosphate Binding Cassette protein type AI (ABCA-I)
cholesterol efflux
Lecithin Cholesterol Acyl Transferase (LCAT)
Cholesterol Ester Transfer Protein (CETP)
Phospholipid Transfer Protein (PLTP)
Scavenger Receptor BI (SR-BI)
27-hydroxylase
Adenosine triphosphate Binding Cassette GI transport (ABCGI)
Illeal Bile Acid Transport (IBAT)

FC = Free (unesterified) Cholestero	CE = Cholesterol Ester
B or apo B = Apolipoprotein B	AI or apo AI = Apolipoprotein AI
C-II = Apolipoprotein C-II	E = Apolipoprotein E
HDL = High-Density Lipoprotein	VLDL = Very-low-density Lipoprotein
IDL = Intermediate low-Density Lipoprotein	

In order to generate substantial autologous apolipoproteins in serum or plasma a technique was developed to rapidly remove cholesterol triglyceride phospholipid and unesterified fatty acids from the serum or plasma without protein denaturation.

Serum may be delipidated with a biphasic solvent system comprising of Diisopropyl Ether (DIPE) or a

mixture of DIPE (or halogenated DIPE) and butanol (Cham and Knowles, 1976). The serum proteins including the apolipoproteins remain in solution in the aqueous phase while organic phase contains the dissolved lipids.

Diethyl Ether (DEE) or activated charcoal is used to wash out traces of butanol in the aqueous phase. It was reported that it is imperative that DEE and DIPE are rendered peroxide-free prior to use. Peroxides may alter proteins resulting in changes in their metabolism (Cham and Knowles, 1976a-c; Hayase *et al*., 1980; Tans *et al*., 1979; Cham, 1976, 1977a, b).

Lipid classes are selectively and accurately extracted from plasma without damage to lipid-related enzyme activities (Cham and Knowles, 1976). Electrophoretic and immunoelectrophoretic studies have shown that the proteins in plasma are unaffected but that lipid is removed by the delipidation procedure (Hayase *et al*., 1980; Tans *et al*., 1979; Cham and Knowles, 1976b, c: Cham, 1977a, b).

Delipidation of plasma does not affect the concentration of the ionic constituents proteins pH and enzyme activities. Delipidation of plasma appears to remove only lipids (Cham and Knowles, 1976a). Delipidation of tissue extracts does not affect the tissue enzymes.

After delipidation the apolipoproteins including apolipoprotein B remain in solution in the aqueous phase (Cham and Knowles, 1976 a-c; Cham, 1977 b; Slater *et al*., 1980). The DIPE-butanol delipidation procedure has been applied to plasma or lipoprotein fractions for the assay of apolipoprotein A-I (Curry *et al*., 1976; Avogaro *et al*., 1978; Kostner *et al*., 1979; Joukianen *et al*., 1982; Koren *et al*., 1985 a, b). A-II (Curry *et al*., 1976; Arogaro *et al*., 1978) B (Koren *et al*., 1985; Klein and Zilversmit, 1984) C (Jaukiainen *et al*., 1982; Staprams and Fells, 1977; Curry *et al*., 1981) D (Jaukiainen *et al*., 1982; Koren *et al*., 1985a, b; Klien and Zilversmit, 1984; Chamet *et al*., 1981; Staprans and Felts, 1977; Curry *et al*., 1977 1980, 1981) and E (Jaukiainen *et al*., 1982; Meunier *et al*., 1986).

Other lipid-associated proteins in biological fluids are also unaffected when their lipid is removed by the delipidation procedure (Pattnaik *et al*., 1978; Fax and Hansson, 1978; Rustow *et al*., 1979; Ockner *et al*., 1982). Removal of lipid from such proteins does not affect their physiological role and in fact this delipidation procedure has also been used to estimate cholesteryl ester transfer/exchange activity in serum or plasma (Groener *et al*., 1984, 1986).

The versatility of this biphasic organic solvent system has tremendous possibilities for the delipidation of biological fluids. The composition of the specific ether-alcohol mixture and the energy applied for the delipidation process can be adjusted to obtain a particular outcome. For example different lipids can be extracted simultaneously but the rates of extraction differ for the particular lipids and indeed it is possible to extract all neutral lipids but no phospholipid from plasma (Cham and Knowles, 1976a, b). The amount of energy applied to the delipidation process determines whether delipidation occurs within minutes or seconds (Cham and Knowles, 1976 a, b). The ratio of organic solvent to plasma can be very variable. Ordered delipidation can be achieved in plasma. Alpha lipoproteins (HDL) are firstly delipidated by this solvent system followed by pre-beta (VLDL) and beta (LDL) lipoproteins in plasma (Cham and Knowles, 1976 a). Thus by choosing the appropriate organic solvent system and the amount of energy applied for delipidation it is possible to remove a particular class of lipid from plasma and/or to delipidate a particular lipoprotein fraction in the plasma (Cham and Knowles, 1976a, b). Using this delipidation procedure it is now possible to obtain plasma with little or no altered LDL but the partially delipidated plasma contains modified HDL particles with no cholesterol. The non-denaturing properties of delipidation of biological fluids with these specific organic solvent mixtures have paved the way for possible clinical applications.

In vitro studies have shown that delipidated plasma obtained after extraction with a mixture of DIPE-butanol is capable of recombining with lipid (Cham and Knowles, 1976 c). Cell culture studies have established that partially delipidated LDL exhibits a binding activity to cultured human fibroblasts identical to that obtained with the untreated LDL. This means that receptors on cells will recognize LDL even if the LDL contains virtually no cholesterol. It was also shown that delipidated LDL did not stimulate cellular cholesteryl ester (ACAT) synthesis indicating that no cholesterol was presented to those cells by delipidated LDL (Slater and Robertson, 1979; Shakespear and Postle, 1979; Slater *et al*., 1980).

Partial delipidation of HDL with DIPE-butanol mixtures converts the spherical lipoprotein particles to disc and amorphous forms but this does not alter receptor binding activity (Slater *et al*., 1980; Innerarity and Mahley, 1978). It is now accepted that disc and amorphous forms of HDL (cholesterol-depleted) are better substrates for removing intracellular cholesterol and are more effective in RCT than in native HDL.

Delipidated serum effluxes cholesterol from human mononuclear leukocytes. When delipidated serum substantially depletes the contents of cellular cholesterol stimulation of intracellular sterol synthesis occurs by a factor of eight to eventually meet the needs of cholesterol for cells survivals. The intracellular sterol synthesis can be inhibited by the addition of LDL. This indicates that cells preferentially utilize cholesterol given to them rather that synthesising their own cholesterol. The activity of HMG-Co. A reductase (the rate limiting enzyme for the

synthesis of cholesterol) in skin and lung fibroblasts is increased 5 to 30 fold in media containing delipidated serum after substantial removal of endogenous cellular cholesterol when compared with media supplemented with non-delipidated serum. Addition of non-delipidated LDL to the cells resulted in the inhibition of cholesterol synthesis within the cells. It was subsequently established that the component in the delipidated serum responsible for the removal of cholesterol from cells grown in culture was delipidated HDL and that delipidated HDL is a much better (more than 6 fold) cholesterol acceptor than is non-delipidated HDL (Slater *et al*., 1980; Innerarity and Mahley, 1978).

Intra-and extracellular events that occur when extra hepatic cells are exposed to delipidated lipoproteins (apolipoproteins) and delipidated plasma are summarised in the Table 1. The delipidation system used in all these studies was with DIPE-butanol. No other unrelated organic solvent procedures have been able to derive similar observations.

Positive attributes observed in the Table for delipidated plasma have resulted in a Plasma Delipidation Procedure (PDP) which has been applied *in vivo* to animals and it has been shown that the PDP results in acute reduction of circulating plasma lipids (Cham *et al*., 1995). In addition a readily extravascular cholesterol pool in hypercholesterolemic animals was rapidly mobilized by PDP. PDP did not affect any of the studied haematological and biochemical parameters.

PDP caused *in vivo* changes in the lipoprotein profiles. The observed changes were more pronounced in hypercholesterolemic animals. Pre-alpha-lipoproteins and pre-beta HDL which are complexes known to be anti-atherogenic lipid-protein conjugates are generated in substantially large quantities during *in vivo* PDP (Cham *et al*., 1996). These conjugates are involved with recruitment of cell-membrane cholesterol and play a major role in RCT which leads to prevention and regression of atherosclerosis.

Table 1: Effects of delipidated plasma and delipidated lipoproteins relative to non delipidated lipoproteins on extra hepatic cellular cholesterol metabolism

Metabolic Events	Delipidated LDL	Delipidated HDL	Delipidated plasma
LDL Binding	Not affected (but less cholesterol presented to cell)	----------	Not affected (but less cholesterol Presented to cell)
LDL receptors	Not affected	Not affected	Not affected
ACAT	Not stimulated	not stimulated	Not stimulated
CEH	Not stimulated	Stimulated	Stimulated
HMGCoA	Stimulated	Stimulated	Reductase
Stimulated			
HDL Receptor Binding	----------	Enhanced	Enhanced
Cholesterol Efflux		----------	Stimulated
Stimulated			

PDP resulted in immediate and sustainable reduction for 2.5 h of plasma unesterified cholesterol concentration after intravascular infusion of autologous delipidated plasma. The extent of the relative reduction of unesterified cholesterol concentration in normocholesterolemic animals was much higher when compared to hypercholesterolemic animals. These observations indicate that PDP was mobilising existing stores of cholesterol. PDP induced changes in the ratio of unesterified to total cholesterol in the plasma of the normocholesterolemic animals but not in the hypercholesterolemic animals. In the hypercholesterolemic animals the LCAT activity was not affected by PDP whereas in the normocholesterolemic animals the LCAT activity was acutely reduced and this reduction in activity but not concentration was sustained for up to 2.5 h. Saturated LCAT kinetics occurred in the hypercholesterolemic animals but because of the sustainable reduction in the LCAT substrate unesterified cholesterol in the normocholesterolemic animals no saturated LCAT kinetics were observed in the PDP-treated normocholesterolemic animals. LCAT obeyed the Michaelis-Menten relationship. These results indicate that after PDP there was a more available pool of unesterified cholesterol which served as substrate for LCAT in the hypercholesterolemic animals relative to normocholesterolemic animals. These events are of considerable importance in the overall regulation of cholesterol transport from peripheral tissues to the plasma. PDP triggers these events which may have major implications in RCT (Kostner *et al*., 1997). These *in vivo* observations are supported by the cell culture *in vitro* observations as shown in the Table 1. Clear quantitative (Cham and Smith, 1994) as well as qualitative (Cham *et al*., 2005) evidence of rapid regression of atherosclerosis using PDP in animals have been obtained. Multiple PDP *in vivo* treatments also result in impressive mobilisation of excessive adipose tissue (Cham *et al*., 2005). PDP has two main effects. The circulating vascular lipid pool is reduced when plasma is delipidated (Cham *et al*., 1995) and the tissue levels of lipid are reduced as a consequence of mobilisation of lipid from these tissues by the delipidated apolipoproteins (Cham *et al*., 1994, 1996; Kostner *et al*., 1997; Cham and Smith, 1994). These effects result in reversal of atherosclerosis. Clinical trials in humans using PDP are currently underway.

CONCLUSION

While reducing LDL-C levels is the priority for the treatment of dyslipoproteinemia not enough coronary events are prevented despite aggressive LDL-C lowering.

Of the lipid-modifying drugs available statins are generally accepted as the therapy of choice however new treatments with beneficial effects upon HDL-C are emerging and may provide additional benefit for the reduction of CHD risk. To date large studies have demonstrated that drugs in particular the statins are excellent for retarding the progression of atherosclerosis. However these drugs do not appear to cause regression of the disease. Data regarding RCT have been available for some time. It is appropriate that this metabolic pathway be fully explored as a possible means for finally achieving rapid regression of atherosclerosis in clinical settings.

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