A Novel Mutation in the Human Plasma Cholesteryl Ester Transfer Protein (CETP) Gene Leading to CETP-Deficiency in a Nova Scotian Patient: A Review of CETP Deficiency

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Human Cholesteryl Ester Transfer Protein (CETP) is a 476-residue hydrophobic plasma glycoprotein which catalyzes the hetero-exchange and net mass transfer of cholesteryl esters and triacylglycerols between plasma High Density Lipoprotein (HDL) and Very Low Density (VLDL) and Low Density Lipoproteins (LDL). CETP, together with the plasma enzyme Lecithin:Cholesterol Acyltransferase (LCAT), form integral parts of the Reverse Cholesterol Transport pathway by which cholesterol is removed from peripheral tissues and transported back to the liver for excretion or reutilization. We have recently identified a novel mutation (CT) at nucleotide 836 in Exon 9 of the Cholesteryl Ester Transfer Protein gene from a Caucasian subject resident in Nova Scotia. The patient is homozygous for the mutation which results in the conversion of 268 Arg into a STOP codon and a truncated, dysfunctional protein. This patient is the first Caucasian North American subject reported to have CETP deficiency. The majority of other subjects are of Japanese ancestry. Biochemically, homozygous CETP deficiency is characterized by a moderate hypercholesterolemia (7±0.8 mmol/L) entirely attributable to an elevated HDL cholesterol (4.2±1.0 mmol/L). LDL cholesterol is usually low (2±0.8 mmol/L). Lipoprotein composition is markedly altered with the HDL much larger, cholesteryl ester enriched and triglyceride poor; the LDL is polydisperse, triglyceride enriched, with a lower proportion of cholesteryl ester per particle. The VLDL is similarly cholesteryl ester poor. Here we review the structure and function of CETP, the consequences of CETP deficiency and hence its diagnosis and discuss the current opinions concerning the atherosclerotic risk associated with CETP deficiency and thus the advisability of treating this disorder with cholesterol lowering drugs.

INTRODUCTION

Atherosclerotic cardiovascular disease stemming from adverse plasma lipoprotein profiles remains a major cause of premature death and morbidity in North America. Within Canada, Nova Scotia has one of the highest prevalences of adverse risk factors for this disease. Although diet and lifestyle remain major determinants of plasma lipid levels and hence atherosclerotic risk, the significance of genetic components and their contribution to the overall risk profile of individuals and families is increasing as more gene mutations are identified and the consequences of gene-gene interactions better understood. Recently we have identified a novel mutation in the plasma cholesteryl ester transfer protein (CETP) gene from a Nova Scotian patient which results in a complete lack of CETP activity in her plasma and a resultant hypercholesterolemia (1). This patient is the first North American of Caucasian descent that has, to date, been identified with this rare genetic disorder. The purposes of this review article are to briefly describe the structure and function of CETP, to delineate the clinical and biochemical characteristics of CETP-deficiency, to describe the basis for accurate diagnosis and lastly to discuss potential atherosclerotic risk and possible therapeutic intervention. More comprehensive reviews are available for further reading (2-6).

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CETP STRUCTURE AND FUNCTION

Human CETP is a 476-residue, hydrophobic plasma glycoprotein of Molecular Weight 66-74 kDa which is synthesized by the liver, adipose tissue, spleen, and small intestine (7,8) and normally present in plasma at 1-3 mg/ml (9-11) where it preferentially associates with small High Density Lipoprotein (HDL) particles (9,12,13). The predicted α-helical content is about 40% with residues 300-315 possibly forming an amphipathic α-helix. Studies with monoclonal antibodies (10,14,15) and C-terminal deletion mutants (16) have identified the C-terminal 26 amino acid residues as critical in mediating the lipid transfer function of CETP. Other putative functional regions include a "hinge" domain, lipoprotein and HDL binding sites (**Figure 1**). The cDNAs for CETP in humans (7), rabbits (17) and cynomolgous monkeys (18) and a partial sequence (aa 188-476) for the hamster (19) have been cloned and sequenced and have shown a high degree of sequence homology at the amino acid level (>81%). The human CETP Gene has 16 exons encompassing 25 kbp and is located on chromosome 16q21 (20) close to the locus for lecithin:cholesterol acyltransferase (LCAT), (16q22). The genomic organization of the CETP gene shows marked similarity to that of the human Lipopolysaccharide Binding Protein, the Bacterial Permeability Increasing Protein, and the Phospholipid Transfer Protein suggesting that they are members of the same gene family, evolved from a common primordial gene and may share similar functional properties (21). The 5' proximal promoter of the human CETP gene contains the expected TATA box, proximal SP1 binding site and a putative site for the transcription activator C/EBP (-242 to -229bp) (22, 23). Tissue-specific expression elements for liver and spleen (-3,400 to -570bp), small intestine (-570 to -370bp) and adrenals (-370 to -138bp) have also been identified (24). In addition a positive sterol response element consisting of the tandem repeat sequence known to mediate the sterol down-regulation of the HMG-CoA reductase and LDL receptor genes has been identified between -206 and -189bp within the 5' sequence (24). In the case of the CETP gene, however, dietary cholesterol increases plasma CETP activity and CETP mRNA levels in a number of species including man (19,25-27). In the liver this is due entirely to increased CETP gene transcription (28).

Plasma CETP catalyzes the reciprocal transfer of cholesteryl esters from HDL to VLDL and LDL and triglycerides from VLDL and LDL to HDL (**Figure 2**). The requirement for a system by which cholesterol either acquired by or synthesized within peripheral tissues could be removed and transported back to the liver for bile acid synthesis or re-utilization is necessitated by the lack of ability of non-steroidogenic peripheral tissues to degrade or otherwise metabolize and export this essential lipid. This requirement was first recognized by Glomset (29). The elucidation of the "Reverse Cholesterol Transport" or RCT pathway has been the subject of intense research since the late '60s and has done much to rationalize the observed negative correlation between plasma HDL levels and atherosclerotic risk (30). Cholesterol efflux from cells is mediated by HDL, in particular by the nascent pre-β discoidal particles (31). Once acquired by HDL, the tissue-derived cholesterol is esterified by the plasma enzyme lecithin:cholesterol acyltransferase (LCAT) which maintains the cholesterol concentration gradient between tissues and

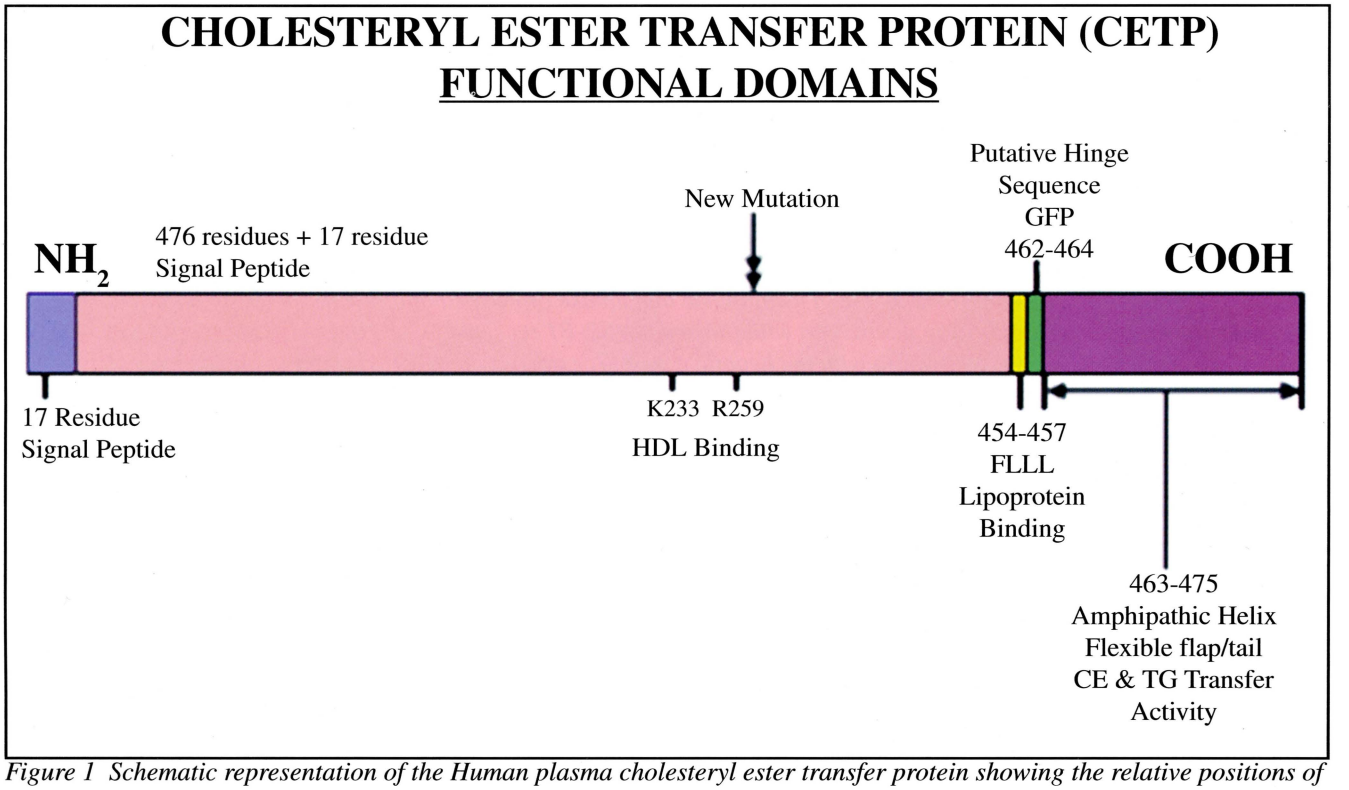

Figure 1 Schematic representation of the Human plasma cholesteryl ester transfer protein showing the relative positions of the functional domains and the location of the Nova Scotia mutation, 268 Arg→STOP.

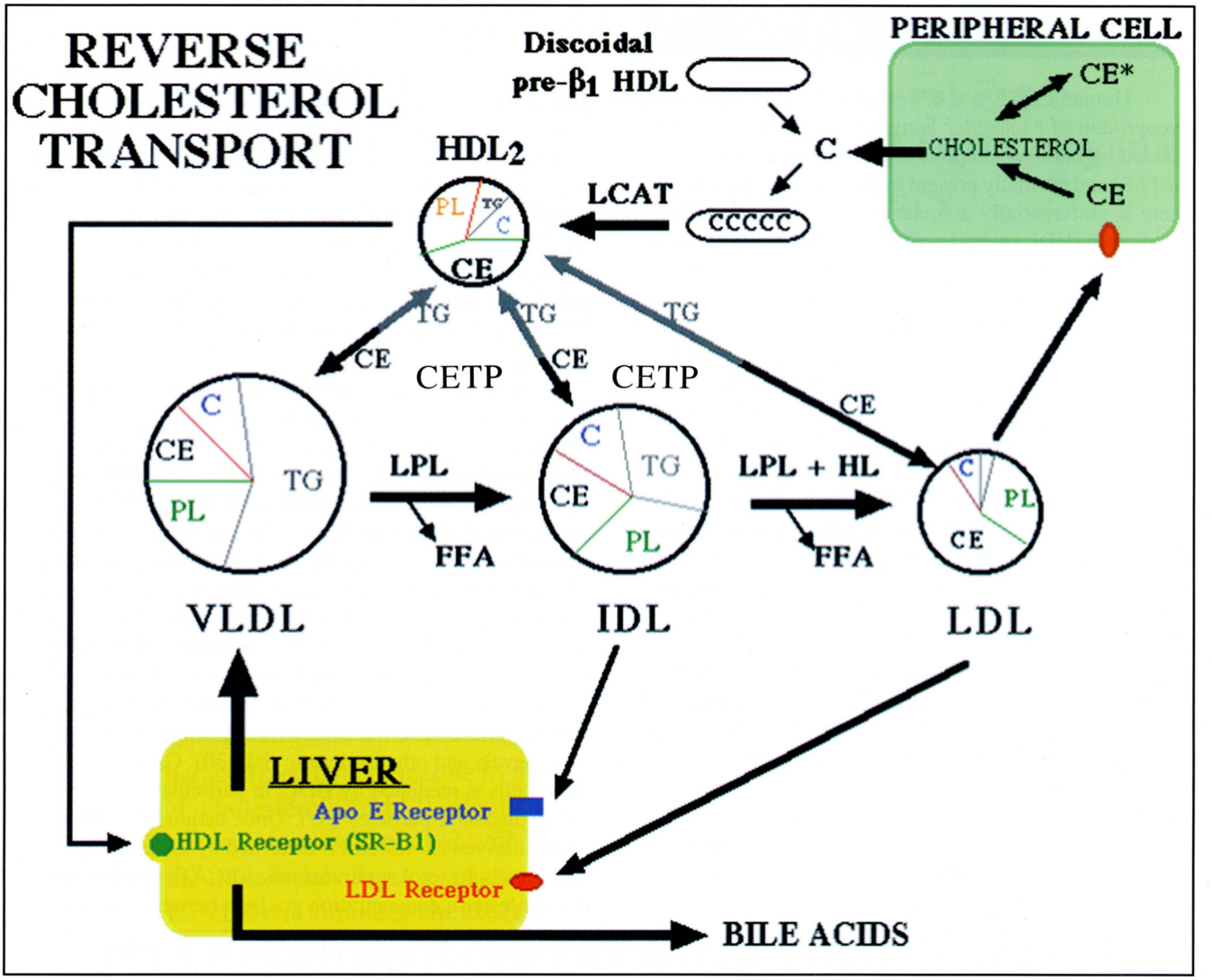

Figure 2 Lipoprotein metabolism and the reverse cholesterol transport pathway. Cholesterol, (C) either synthesized within or acquired by peripheral cells exits the cell and is acquired by small discoidal HDL particles (pre-β_1 HDL). The cell-derived cholesterol is then esterified by the plasma enzyme lecithin:cholesterol acyltransferase (LCAT) and the resultant cholesteryl ester (CE) moves to the core of the HDL particle rendering it both spherical and much larger (HDL_2). The HDL cholesteryl ester is then transferred to VLDL, IDL and LDL in exchange for triglycerides (TG) by plasma cholesteryl ester transfer protein (CETP). Triglyceride transferred to HDL is hydrolyzed by hepatic lipase (HL) in a similar manner to the hydrolysis of VLDL triglyceride by lipoprotein lipase (LPL). Tissue-derived CE transferred to VLDL, IDL and LDL is returned to the liver during the normal course of metabolism and uptake of these lipoprotein particles. In addition, HDL_2 CE may be directly removed following interaction with the hepatic SR-B1 receptor or, as HDL_2 also contains Apo E, by uptake of the particle following interaction with the hepatic Apo E receptor. Cholesterol returned to the liver may be utilized for bile acid synthesis or reutilized and re-exported as a small component of VLDL. PL = phospholipid; FFA = free fatty acid taken up by tissues at the site of LPL action.

HDL. The fate of the resultant spherical HDL particles containing the tissue-derived cholesterol ester is threefold, the relative contribution of each being species dependent (32). Firstly, as HDL particles acquire more core CE they enlarge and acquire multiple copies of Apo E at the expense of Apo A-1 permitting interaction with and uptake by the hepatic LDL (Apo B,E) or chylomicron remnant (Apo E) receptor. Secondly, in man and rabbits HDL-CE is transferred to the triglyceride-rich lipoproteins in exchange for triglycerides (Tg) by the action of the Cholesteryl Ester Transfer Protein (CETP) (33,34). Triglycerides acquired by HDL are lipolyzed by hepatic lipase and the HDL-CE transferred to VLDL, IDL and LDL is removed from the circulation during the normal metabolism of these particles. Thirdly, HDL may interact with the hepatic SR-B1 scavenger receptor (35) with the subsequent selective uptake of HDL-CE without HDL internalization or degradation (35,36). This pathway and the first are probably major in rats and mice which have very low plasma CETP activity (37).

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* In dose response studies in mildly to moderately hyperlipidemic patients (Fredrickson Type IIa and IIb) with LIPITOR 10-80 mg.
‡ At starting doses. Results in mildly to moderately hyperlipidemic patients (Fredrickson Type IIa and IIb). One year, double-blind, randomized multicentre study.

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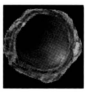

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THERAPEUTIC CLASSIFICATION: Lipid Metabolism Regulator

ACTIONS AND CLINICAL PHARMACOLOGY

LIPITOR (atorvastatin calcium) is a synthetic lipid-lowering agent. It is a selective, competitive inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase. This enzyme catalyzes the conversion of HMG-CoA to mevalonate, which is an early and rate-limiting step in the biosynthesis of cholesterol.
LIPITOR lowers plasma cholesterol and lipoprotein levels by inhibiting HMG-CoA reductase and cholesterol synthesis in the liver and by increasing the number of hepatic Low Density Lipoprotein (LDL) receptors on the cell-surface for enhanced uptake and catabolism of Low Density Lipoprotein (LDL).
LIPITOR reduces LDL-Cholesterol (LDL-C) and the number of LDL particles, and lowers Very Low Density Lipoprotein-Cholesterol (VLDL-C) and serum triglycerides (TG), as well as the number of apolipoprotein B (apo B) containing particles.
Atorvastatin is rapidly absorbed after oral administration; maximum plasma concentrations occur within 1 to 2 hours. Atorvastatin tablets are 95% to 99% bioavailable compared to solutions.
Mean distribution of atorvastatin is approximately 565 liters. Atorvastatin is ≥98% bound to plasma proteins.
Atorvastatin is extensively metabolized by cytochrome P-450 3A4 to ortho- and parahydroxylated derivatives and various beta-oxidation products. Approximately 70% of circulating inhibitory activity for HMG-CoA reductase is attributed to active metabolites.
Atorvastatin and its metabolites are eliminated by biliary excretion. Less than 2% of a dose of atorvastatin is recovered in urine following oral administration. Mean plasma elimination half-life of atorvastatin in humans is approximately 14 hours, but the half-life of inhibitory activity for HMG-CoA reductase is 20 to 30 hours due to the contribution of longer-lived active metabolites.

INDICATIONS AND CLINICAL USE

LIPITOR (atorvastatin calcium) is indicated as an adjunct to diet, at least equivalent to the American Heart Association (AHA) Step 1 diet, for the reduction of elevated total cholesterol, LDL-C, triglycerides (TG) and apolipoprotein B (apo B) in hyperlipidemic and dyslipidemic conditions, when response to diet and other nonpharmacological measures alone has been inadequate, including:

- Primary hypercholesterolemia (Type IIa),
- Combined (mixed) hyperlipidemia (Type IIb), including familial combined hyperlipidemia, regardless of whether cholesterol or triglycerides are the lipid abnormality of concern, and
- Heterozygous familial hypercholesterolemia.

In clinical trials, LIPITOR (10-80 mg/day) significantly improved lipid profiles in patients with a wide variety of hyperlipidemic and dyslipidemic conditions. In 2 dose-response studies in mildly to moderately hyperlipidemic patients (Fredrickson Types IIa and IIb), LIPITOR reduced the levels of total cholesterol (29-45%), LDL-C (39-60%), apo B (32-50%), TG (19-37%), and increased HDL-C levels (5-9%). Comparable responses were achieved in patients with heterozygous familial hypercholesterolemia, non-familial forms of hypercholesterolemia, combined hyperlipidemia, including familial combined hyperlipidemia and patients with non-insulin dependent diabetes mellitus. In patients with hypertriglyceridemia (TG>350 mg/dL), LIPITOR lowered TG levels by 27-42%. Limited data is available in homozygous familial hypercholesterolemia (FH). An open-label study with atorvastatin 80 mg/day in homozygote FH patients showed a LDL-C lowering of 30% for patients not on plasmapheresis and of 31% for patients who continued plasmapheresis. A LDL-C lowering of 35% was observed in receptor defective patients and of 19% in receptor negative patients (see PHARMACOLOGY, Clinical Studies).
For more details on efficacy results by pre-defined classification and pooled data by Fredrickson types, see PHARMACOLOGY, Clinical Studies.
Prior to initiating therapy with LIPITOR, secondary causes should be excluded for elevations in plasma lipid levels (e.g. poorly controlled diabetes mellitus, hypothyroidism, nephrotic syndrome, dysproteinemias, obstructive liver disease, and alcoholism), and a lipid profile performed to measure total cholesterol, LDL-C, HDL-C, and TG. For patients with TG <4.52 mmol/L (<400 mg/dL), LDL-C can be estimated using the following equation:

LDL-C (mmol/L) = total cholesterol - [(0.37 x (TG) + HDL-C)]
LDL-C (mg/dL) = total cholesterol - [(0.2 x (TG) + HDL-C)][1]

For patients with TG levels >4.52 mmol/L (>400 mg/dL), this equation is less accurate and LDL-C concentrations should be determined by ultracentrifugation.

CONTRAINDICATIONS

Hypersensitivity to any component of this medication.
Active liver disease or unexplained persistent elevations of serum transaminases exceeding 3 times the upper limit of normal (see WARNINGS).
Pregnancy and lactation (see PRECAUTIONS).

WARNINGS

Pharmacokinetic Interactions

The use of HMG-CoA reductase inhibitors has been associated with severe myopathy, including rhabdomyolysis, which may be more frequent when they are co-administered with drugs that inhibit the cytochrome P-450 enzyme system. Atorvastatin is metabolized by cytochrome P-450 isoform 3A4 and as such may interact with agents that inhibit this enzyme (see WARNINGS, Muscle Effects and PRECAUTIONS, Drug Interactions and Cytochrome P-450-mediated Interactions).

Hepatic Effects

In clinical trials, persistent increases in serum transaminases greater than three times the upper limit of normal occurred in <1% of patients who received LIPITOR. When the dosage of LIPITOR was reduced, or when drug treatment was interrupted or discontinued, serum transaminase levels returned to pretreatment levels. The increases were generally not associated with jaundice or other clinical signs or symptoms. Most patients continued treatment with a reduced dose of LIPITOR without clinical sequelae.
Liver function tests should be performed before the initiation of treatment, and periodically thereafter. Special attention should be paid to patients who develop elevated serum transaminase levels, and in these patients measurements should be repeated promptly and then performed more frequently.
If increases in alanine aminotransferase (ALT) or aspartate aminotransferase (AST) show evidence of progression, particularly if they rise to greater than 3 times the upper limit of normal and are persistent, the dosage should be reduced or the drug discontinued.
LIPITOR should be used with caution in patients who consume substantial quantities of alcohol and/or have a past history of liver disease. Active liver disease or unexplained transaminase elevations are contraindications to the use of LIPITOR; if such a condition should develop during therapy, the drug should be discontinued.

Muscle Effects

Myopathy, defined as muscle aching or muscle weakness in conjunction with increases in creatinine phosphokinase (CPK) values to greater than ten times the upper limit of normal, should be considered in any patient with diffuse myalgia, muscle tenderness or weakness, and/or marked elevation of CPK. Patients should be advised to report promptly unexplained muscle pain, tenderness or weakness, particularly if accompanied by malaise or fever. LIPITOR therapy should be discontinued if markedly elevated CPK levels occur or myopathy is diagnosed or suspected.

[1] Friedewald, WT et al. *Clin Chem* 1972; 18(6):489-502.

The risk of myopathy and rhabdomyolysis during treatment with HMG-CoA reductase inhibitors is increased with concurrent administration of cyclosporine, fibric acid derivatives, erythromycin, niacin (nicotinic acid), azole antifungals or nefazodone. As there is no experience to date with the use of LIPITOR given concurrently with these drugs, with the exception of a pharmacokinetic study with erythromycin, the benefits and risks of such combined therapy should be carefully considered (see PRECAUTIONS, Drug Interactions).
Rhabdomyolysis has been reported in very rare cases with LIPITOR (see PRECAUTIONS, Drug Interactions). Rhabdomyolysis with renal dysfunction secondary to myoglobinuria has also been reported with HMG-CoA reductase inhibitors. LIPITOR therapy should be temporarily withheld or discontinued in any patient with an acute serious condition suggestive of a myopathy or having a risk factor predisposing to the development of renal failure secondary to rhabdomyolysis (such as severe acute infection, hypotension, major surgery, trauma, severe metabolic, endocrine and electrolyte disorders, and uncontrolled seizures).

PRECAUTIONS

General

The effects of atorvastatin-induced changes in lipoprotein levels, including reduction of serum cholesterol on cardiovascular morbidity or mortality or total mortality have not been established.
Before instituting therapy with LIPITOR (atorvastatin calcium), an attempt should be made to control elevated serum lipoprotein levels with appropriate diet, exercise, and weight reduction in overweight patients, and to treat other underlying medical problems (see INDICATIONS AND CLINICAL USE). Patients should be advised to inform subsequent physicians of the prior use of LIPITOR or any other lipid-lowering agents.

Effect on the Lens

Current long-term data from clinical trials do not indicate an adverse effect of atorvastatin on the human lens.

Effect on Ubiquinone (CoQ_{10}) Levels

Significant decreases in circulating ubiquinone levels in patients treated with atorvastatin and other statins have been observed. The clinical significance of a potential long-term statin-induced deficiency of ubiquinone has not been established. It has been reported that a decrease in myocardial ubiquinone levels could lead to impaired cardiac function in patients with borderline congestive heart failure (see SELECTED BIBLIOGRAPHY).

Effect on Lipoprotein(a)

In some patients, the beneficial effect of lowered total cholesterol and LDL-C levels may be partly blunted by a concomitant increase in Lp(a) levels. Until further experience is obtained, it is suggested, where feasible, that measurements of serum Lp(a) be followed up in patients placed on atorvastatin therapy (see SELECTED BIBLIOGRAPHY).

Hypersensitivity

An apparent hypersensitivity syndrome has been reported with other HMG-CoA reductase inhibitors which has included 1 or more of the following features: anaphylaxis, angioedema, lupus erythematous-like syndrome, polymyalgia rheumatica, vasculitis, purpura, thrombocytopenia, leukopenia, hemolytic anemia, positive ANA, ESR increase, eosinophilia, arthritis, arthralgia, urticaria, asthenia, photosensitivity, fever, chills, flushing, malaise, dyspnea, toxic epidermal necrolysis, erythema multiforme, including Stevens-Johnson syndrome. Although to date hypersensitivity syndrome has not been described as such, LIPITOR should be discontinued if hypersensitivity is suspected.

Use in Pregnancy

LIPITOR is contraindicated during pregnancy (see CONTRAINDICATIONS).
Atherosclerosis is a chronic process and discontinuation of lipid-lowering drugs during pregnancy should have little impact on the outcome of long-term therapy of primary hypercholesterolemia. Cholesterol and other products of cholesterol biosynthesis are essential components for fetal development (including synthesis of steroids and cell membranes). Since HMG-CoA reductase inhibitors decrease cholesterol synthesis and possibly the synthesis of other biologically active substances derived from cholesterol, they may cause harm to the fetus when administered to pregnant women.
There are no data on the use of LIPITOR during pregnancy. LIPITOR should be administered to women of child-bearing age only when such patients are highly unlikely to conceive and have been informed of the potential hazards. If the patient becomes pregnant while taking LIPITOR, the drug should be discontinued and the patient apprised of the potential risk to the fetus.

Nursing Mothers

In rats, milk concentrations of atorvastatin are similar to those in plasma. It is not known whether this drug is excreted in human milk. Because of the potential for adverse reactions in nursing infants, women taking LIPITOR should not breast-feed (see CONTRAINDICATIONS).

Pediatric Use

Treatment experience in a pediatric population is limited to doses of LIPITOR up to 80 mg/day for 1 year in 8 patients with homozygous familial hypercholesterolemia. No clinical or biochemical abnormalities were reported in these patients.

Geriatric Use

Treatment experience in adults 70 years or older (N=221) with doses of LIPITOR up to 80 mg/day has demonstrated that the safety and effectiveness of atorvastatin in this population was similar to that of patients <70 years of age. Pharmacokinetic evaluation of atorvastatin in subjects over the age of 65 years indicates an increased AUC. As a precautionary measure, the lowest dose should be administered initially (see PHARMACOLOGY, Human Pharmacokinetics; and SELECTED BIBLIOGRAPHY).

Renal Insufficiency

Plasma concentrations and LDL-C lowering efficacy of LIPITOR are similar in patients with moderate renal insufficiency compared with patients with normal renal function. However, since several cases of rhabdomyolysis have been reported in patients with a history of renal insufficiency of unknown severity, as a precautionary measure and pending further experience in renal disease, the lowest dose (10 mg/day) of LIPITOR should be used in these patients. Similar precautions apply in patients with severe renal insufficiency (creatinine clearance <30 mL/min); the lowest dosage should be used and implemented cautiously (see WARNINGS, Muscle Effects; PRECAUTIONS, Drug Interactions). Refer also to DOSAGE AND ADMINISTRATION.

Endocrine Function

HMG-CoA reductase inhibitors interfere with cholesterol synthesis and as such might theoretically blunt adrenal and/or gonadal steroid production. Clinical studies with atorvastatin and other HMG-CoA reductase inhibitors have suggested that these agents do not reduce plasma cortisol concentration or impair adrenal reserve and do not reduce basal plasma testosterone concentration. However, the effects of HMG-CoA reductase inhibitors on male fertility have not been studied in adequate numbers of patients. The effects, if any, on the pituitary-gonadal axis in premenopausal women are unknown.
Patients treated with atorvastatin who develop clinical evidence of endocrine dysfunction should be evaluated appropriately. Caution should be exercised if an HMG-CoA reductase inhibitor or other agent used to lower cholesterol levels is administered to patients receiving other drugs (e.g. ketoconazole, spironolactone or cimetidine) that may decrease the levels of endogenous steroid hormones.

Drug Interactions

Concomitant Therapy with Other Lipid Metabolism Regulators: Combined drug therapy should be approached with caution as information from controlled studies is limited.

Bile Acid Sequestrants:

Patients with mild to moderate hypercholesterolemia: LDL-C reduction was greater when LIPITOR 10 mg and colestipol 20 g were coadministered (-45%) than when either drug was administered alone (-35% for LIPITOR and -22% for colestipol).
Patients with severe hypercholesterolemia: LDL-C reduction was similar (-53%) when LIPITOR 40 mg and colestipol 20 g were coadministered when compared to that of LIPITOR 80 mg alone. Plasma concentration of

atorvastatin was lower (approximately 26%) when LIPITOR 40 mg plus colestipol 20 g were coadministered compared to LIPITOR 40 mg alone.
However, the combination drug therapy was less effective in lowering the triglycerides than LIPITOR monotherapy in both types of hypercholesterolemic patients (see PHARMACOLOGY, Clinical Studies).
When LIPITOR is used concurrently with colestipol or any other resin, an interval of at least 2 hours should be maintained between the two drugs, since the absorption of LIPITOR may be impaired by the resin.
Fibric Acid Derivatives (Gemfibrozil, Fenofibrate, Bezafibrate) and Niacin (Nicotinic Acid): Although there is no experience with the use of LIPITOR given concurrently with fibric acid derivatives and niacin, the benefits and risks of such combined therapy should be carefully considered. The risk of myopathy during treatment with other drugs in this class is increased with concurrent administration (see WARNINGS, Muscle Effects).
Coumarin Anticoagulants: LIPITOR had no clinically significant effect on prothrombin time when administered to patients receiving chronic warfarin therapy (see SELECTED BIBLIOGRAPHY).
Digoxin: Coadministration of multiple doses of LIPITOR and digoxin increased steady-state plasma digoxin concentrations by approximately 20%. Patients taking digoxin should be monitored closely and appropriately.
Oral Contraceptives: Coadministration of LIPITOR with an oral contraceptive, containing 1 mg norethindrone and 35 µg ethinyl estradiol, increased plasma concentrations (AUC levels) of norethindrone and ethinyl estradiol by approximately 30% and 20%, respectively. These increases should be considered when selecting an oral contraceptive.
Antacids: Administration of aluminum and magnesium based antacids, such as Maalox® TC Suspension, with LIPITOR decreased plasma concentrations of LIPITOR by approximately 35%. LDL-C reduction was not altered but the triglyceride-lowering effect of LIPITOR may be affected.
Cimetidine: Administration of cimetidine with LIPITOR did not alter plasma concentrations or LDL-C lowering efficacy of LIPITOR, however, the triglyceride-lowering effect of LIPITOR was reduced from 34% to 26%.
Cytochrome P-450-mediated Interactions: Atorvastatin is metabolized by the cytochrome P-450 isoenzyme, CYP 3A4. Erythromycin, a CYP 3A4 inhibitor, increased atorvastatin plasma levels by 40%. Coadministration of CYP 3A4 inhibitors, such as grapefruit juice, macrolide antibiotics (including eryhromycin), immunosuppressants (cyclosporin), azole antifungal agents (i.e. itraconazole, ketoconazole), or some antidepressants (e.g. nefazodone), may have the potential to increase plasma concentrations of HMG CoA reductase inhibitors, including LIPITOR (see SELECTED BIBLIOGRAPHY). Caution should thus be exercised with concomitant use of these agents (see WARNINGS, Pharmacokinetic Interactions, Muscle Effects; DOSAGE AND ADMINISTRATION).
In a study with healthy subjects, coadministration of maximum doses of both atorvastatin (80 mg) and terfenadine(120 mg), a CYP 3A4 substrate, was shown to produce modest increases in AUC values. The QTc interval remained unchanged. However, since an interaction between these two drugs cannot be excluded in patients with predisposing factors for arrhythmia, (e.g. preexisting prolonged QT interval, severe coronary artery disease, hypokalemia), caution should be exercised when these agents are coadministered (see WARNINGS, Pharmacokinetic Interactions; DOSAGE AND ADMINISTRATION).
Antipyrine: Antipyrine was used as a non-specific model for drugs metabolized by the microsomal hepatic enzyme system (cytochrome P-450 system). LIPITOR had no effect on the pharmacokinetics of antipyrine, thus interactions with other drugs metabolized via the same cytochrome isozymes are not expected.
Erythromycin: In healthy individuals, plasma concentrations of atorvastatin increased approximately 40% with coadministration of LIPITOR and erythromycin, a known inhibitor of CYP 3A4 (see WARNINGS, Muscle Effects).
Other Concomitant Therapy: In clinical studies, LIPITOR was used concomitantly with antihypertensive agents and estrogen replacement therapy without evidence to date of clinically significant adverse interactions. Interaction studies with specific agents have not been conducted.
Patients with Severe Hypercholesterolemia: Higher drug dosages (80 mg/day) required for some patients with heterozygous familial hypercholesterolemia or severe hypercholesterolemia are associated with increased plasma levels of atorvastatin. **Caution should be exercised in such patients who are also severely renally impaired, elderly, or are concomitantly being administered digoxin or CYP 3A4 inhibitors (see WARNINGS, Pharmacokinetic Interactions, Muscle Effects; PRECAUTIONS, Drug Interactions and DOSAGE AND ADMINISTRATION).**

Drug/Laboratory Test Interactions

LIPITOR may elevate serum transaminase and creatinine phosphokinase levels (from skeletal muscle). In the differential diagnosis of chest pain in a patient on therapy with LIPITOR, cardiac and noncardiac fractions of these enzymes should be determined.

ADVERSE REACTIONS

LIPITOR is generally well-tolerated. Adverse reactions have usually been mild and transient. In controlled clinical studies (placebo-controlled and active-controlled comparative studies with other lipid lowering agents) involving 2502 patients, <2% of patients were discontinued due to adverse experiences attributable to LIPITOR. Of these 2502 patients, 1721 were treated for at least 6 months and 1253 for 1 year or more.
Adverse experiences occurring at an incidence ≥1% in patients participating in placebo-controlled clinical studies of LIPITOR and reported to be possibly, probably or definitely drug related are shown in Table 1 below:

TABLE 1. Associated Adverse Events Reported in ≥ 1% of Patients in Placebo Controlled Clinical Trials

	Placebo % (n=270)	LIPITOR % (n=1122)
GASTROINTESTINAL		
Constipation	1	1
Diarrhea	1	1
Dyspepsia	2	1
Flatulence	2	1
Nausea	0	1
NERVOUS SYSTEM		
Headache	2	1
MISCELLANEOUS		
Pain	<1	1
Myalgia	1	1
Asthenia	<1	1

The following additional adverse events were reported in clinical trials; not all events listed below have been associated with a causal relationship to LIPITOR therapy: Muscle cramps, myositis, myopathy, paresthesia, peripheral neuropathy, pancreatitis, hepatitis, cholestatic jaundice, anorexia, vomiting, alopecia, pruritus, rash, impotence, hyperglycemia, and hypoglycemia.
Post-marketing experience: Very rare reports of severe myopathy with or without rhabdomyolysis have been reported (see WARNINGS, Muscle Effects; PRECAUTIONS, Renal Insufficiency and Drug Interactions).
Ophthalmologic observations: see PRECAUTIONS.
Laboratory Tests: Increases in serum transaminase levels have been noted in clinical trials (see WARNINGS).

SYMPTOMS AND TREATMENT OF OVERDOSAGE

There is no specific treatment for atorvastatin overdosage. Should an overdose occur, the patient should be treated symptomatically and supportive measures instituted as required. Due to extensive drug binding to plasma proteins, hemodialysis is not expected to significantly enhance atorvastatin clearance.

DOSAGE AND ADMINISTRATION

Patients should be placed on a standard cholesterol-lowering diet [at least equivalent to the American Heart Association (AHA) Step 1 diet] before receiving LIPITOR, and should continue on this diet during treatment with LIPITOR. If appropriate, a program of weight control and physical exercise should be implemented.
Primary Hypercholesterolemia and Combined (Mixed) Hyperlipidemia, Including Familial Combined Hyperlipidemia
The recommended dose of LIPITOR is 10 mg once a day. The majority of patients achieve and maintain target cholesterol levels with LIPITOR 10 mg/day. A significant therapeutic response is evident within 2 weeks, and the maximum response is usually achieved within 2-4 weeks. The response is maintained during chronic therapy. Doses can be given at any time of the day, with or without food, and should preferably be given in the evening. Doses should be individualized according to baseline LDL-C levels, the desired LDL-C target (such as that recommended by the US National Cholesterol Education Program [NCEP] and/or the Canadian Consensus Conference Guidelines), the goal of therapy and the patient's response. Adjustments of dosage, if necessary, should be made at intervals of 4 weeks or more. The recommended dose range for most patients is 10 to 40 mg/day. The maximum dose is 80 mg/day, which may be required in a minority of patients (see section below).
Cholesterol levels should be monitored periodically and consideration should be given to reducing the dosage of LIPITOR if cholesterol falls below the targeted range such as that recommended by guidelines.
The following reductions in total cholesterol and LDL-C levels have been observed in 2 dose-response studies, and may serve as a guide to treatment of patients with mild to moderate hypercholesterolemia:

TABLE 2. Dose-Response in Patients With Mild to Moderate Hypercholesterolemia (Mean Percent Change from Baseline)[a]

Lipid Parameter	LIPITOR Dose (mg/day)			
	10 (N=22)	20 (N=20)	40 (N=21)	80 (N=23)
Total-C: 7.1 mmol/L[b] (273 mg/dL)[b]	-29	-33	-37	-45
LDL-C: 4.9 mmol/L[b] (190 mg/dL)[b]	-39	-43	-50	-60

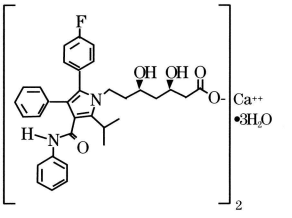
[a] Results are pooled from 2 dose-response studies
[b] Mean baseline values

Severe Hypercholesterolemia
In patients with severe hypercholesterolemia, including heterozygous familial hypercholesterolemia, higher dosages (up to 80 mg/day) may be required (see WARNINGS, Pharmacokinetic Interactions, Muscle Effects and PRECAUTIONS, Drug Interactions).

Concomitant Therapy
See PRECAUTIONS, Drug Interactions.
Dosage in Patients With Renal Insufficiency
See PRECAUTIONS.

PHARMACEUTICAL INFORMATION

Drug Substance
Proper Name: Atorvastatin calcium
Chemical Name: [R-(R*,R*)]-2-(4-fluorophenyl)-ß, δ-dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenylamino) carbonyl]-1H-pyrrole-1-heptanoic acid, calcium salt (2:1) trihydrate
Empirical Formula: $(C_{33}H_{34}FN_2O_5)_2Ca \bullet 3H_2O$
Molecular Weight: 1209.42
Structural Formula:



Description: Atorvastatin calcium is a white to off-white crystalline powder that is practically insoluble in aqueous solutions of pH 4 and below. Atorvastatin calcium is very slightly soluble in distilled water, pH 7.4 phosphate buffer and acetonitrile, slightly soluble in ethanol, and freely soluble in methanol.
Tablet Composition: Each tablet contains either 10 mg, 20 mg or 40 mg atorvastatin as the active ingredient. Each tablet also contains the following non-medicinal ingredients: calcium carbonate, candelilla wax, croscarmellose sodium, hydroxypropyl cellulose, lactose monohydrate, magnesium stearate, microcrystalline cellulose, hydroxypropyl methylcellulose, polyethylene glycol, talc, titanium dioxide, polysorbate 80 and simethicone emulsion.
Stability and Storage Recommendations: Store at controlled room temperature 15 to 25°C.

AVAILABILITY OF DOSAGE FORMS

LIPITOR (atorvastatin calcium) is available in dosage strengths of 10 mg, 20 mg and 40 mg atorvastatin per tablet.
10 mg: White, elliptical, film-coated tablet, coded "10" on one side and "PD 155" on the other. Available in bottles of 90 tablets.
20 mg: White, elliptical, film-coated tablet, coded "20" on one side and "PD 156" on the other. Available in bottles of 90 tablets.
40 mg: White, elliptical, film-coated tablet, coded "40" on one side and "PD 157" on the other. Available in bottles of 90 tablets.

References:
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CETP DEFICIENCY

To date only ten unique mutations in the CETP gene that lead to a functional CETP deficiency have been reported (1,38-43) as shown in **Figure 3**. Three of these mutations (41-43) involve base changes at the intron/exon splice donor or junction sites which result in a lack of intron removal or correct mRNA splicing. Four other mutations (1,38-40) generate stop codons or frameshift which generate proximal stop codons leading to the putative expression of truncated non-functional proteins. Only three of the known mutations (38,43) result in single amino acid changes. By far the majority of subjects expressing these mutated alleles are of Japanese ancestry with a particularly high prevalence in the region of Omagari city in northern Japan (44). Phenotypically, familial CETP deficiency is characterized by an HDL hypercholesterolemia where the HDL cholesteryl ester content is increased, leading to a larger particle with decreased triglyceride content (45). The VLDL is cholesteryl ester poor (1,45,46) however the LDL cholesteryl ester content, although lower than normal, is maintained at significant levels due to the action of plasma LCAT (1). Plasma Apo A-1, A-IV and E are elevated commensurate with the high levels of HDL (46). In Japanese subjects Apo B levels are reduced due to an apparent increase in the LDL catabolic rate (47). In our Caucasian subject (1) however her Apo B levels were near normal (94 mg/dl) suggesting that the hypercatabolism of LDL may be a co-inherited trait independent of CETP deficiency in the Japanese subjects.

DIAGNOSIS OF CETP DEFICIENCY

The plasma lipid, apolipoprotein, CETP mass and activity values reported for three studies of Japanese CETP deficient subjects (42,44,48) are shown in **Table 1**. Definitive diagnosis of familial CETP deficiency is not yet available at the level of the routine hospital clinical chemistry laboratory due to the current lack of commercially available immunoassays for plasma CETP mass and difficulties inherent in the measurement of plasma CETP activity (49). These tests are however available within the research environment (9,12) and referral of plasma samples to the appropriate laboratory is encouraged. Despite this the primary care physician can obtain data which is highly suggestive of this defect by the use of available laboratory data. Specifically, the patients

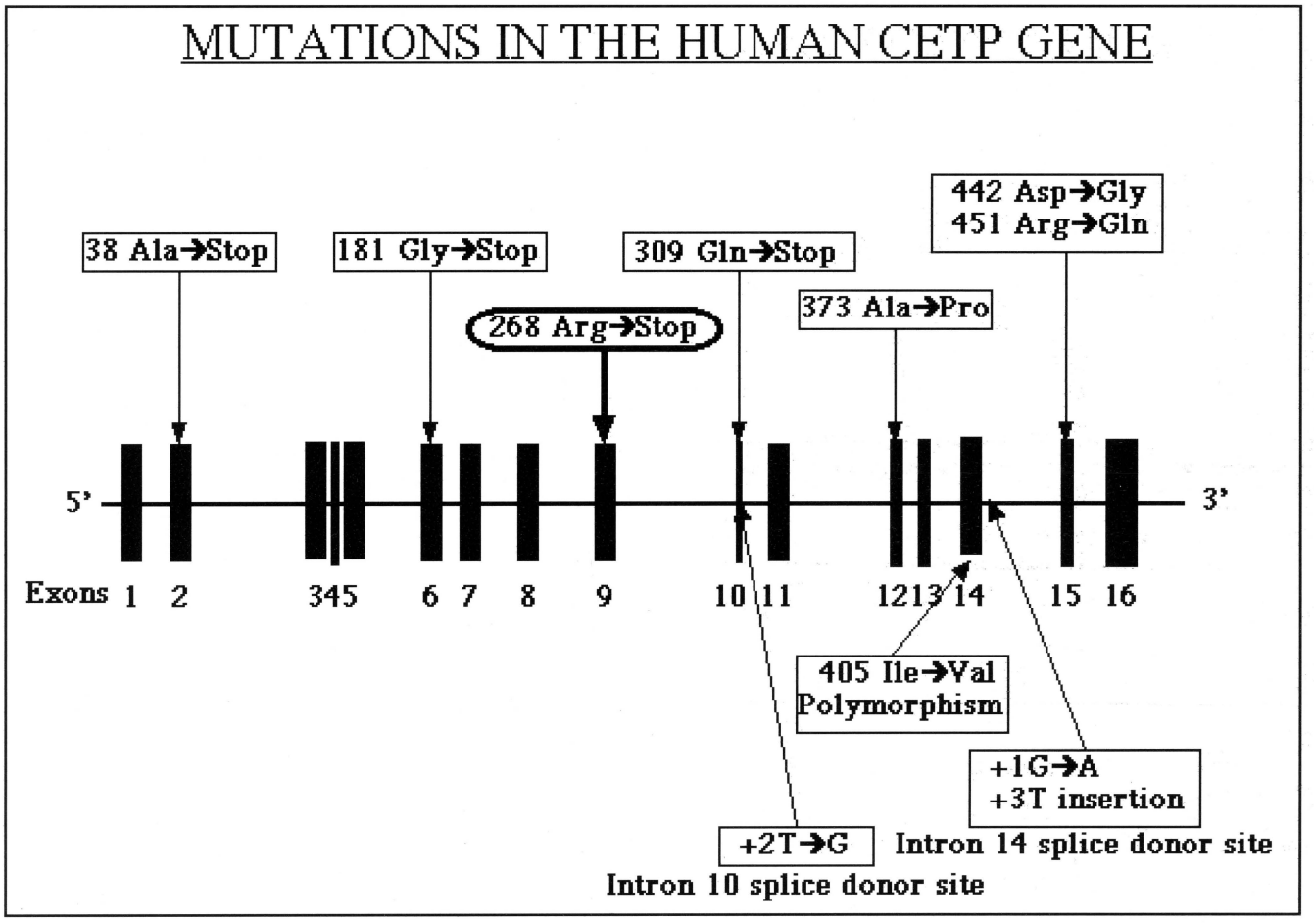

Figure 3 Schematic representation of the human cholesteryl ester transfer protein gene located on chromosome 16q21 and containing 16 exons with 15 introns which together with the 5' promoter region spans 25 kbp. The nature and positions of all mutations reported to date are shown. The Nova Scotia mutation in exon 9 is indicated by the rounded box.

	CETP Deficient			
	Homozygotes	Heterozygotes	Controls	Reference
Total Cholesterol (mmol/L)	7.01±0.83 (10)***	5.04±1.14 (20)	4.45±0.59 (10)	42
		5.59±1.08 (52)*	4.40±0.44 (20)	48
		5.87±0.86 (16)***	4.40±0.57 (20)	44
HDL Cholesterol (mmol/L)	4.24±1.01 (10)***	1.71±0.39 (20)**	1.16±0.26 (10)	42
		2.64±0.72 (52)**	1.24±0.28 (20)	48
		1.74±0.70 (16)*	1.24±0.21 (20)	44
LDL Cholesterol (mmol/L)	1.99±0.80 (10)*	2.87±1.11 (20)	2.77±0.42 (10)	42
		2.81±0.45 (16)	2.90±0.30 (20)	44
Triglycerides (mmol/L)	1.69±1.34 (10)	1.07±0.59 (20)	0.98±0.34 (10)	42
		1.10±0.52 (52)	0.87±0.29 (20)	48
		1.03±0.47 (16)	0.99±0.28 (20)	44
Apo A-I (g/L)	2.13±0.47 (10)***	1.49±0.43 (20)*	1.17±0.2 (10)	42
		2.12±0.33 (52)**	1.34±0.16 (20)	48
		1.50±0.31 (16)*	1.34±0.13 (20)	44
Apo B (g/L)	0.54±0.14 (10)*	0.66±0.20 (20)*	0.89±0.13 (10)	42
		0.81±0.20 (52)	0.83±0.16 (20)	48
		0.85±0.31 (16)	0.82±0.12 (20)	44
CETP mass (mg/L)	0±0 (10)***	1.4±0.3 (20)***	2.2±0.6 (10)	42
CETP activity (units)	0±0 (10)***	8.5±2.7 (52)**	20.4±2.1 (20)	48

*** = $p<0.001$ vs. Controls, ** = $p<0.01$ vs. Controls, * = $p<0.05$ vs. Controls

Table 1 Literature values for the plasma parameters observed in CETP deficiency relative to normal controls. All subjects are of Japanese ancestry. The number of subjects analyzed in each study is in parenthesis. Detailed compositional analyses of individual lipoprotein fractions are reported in references (1) and (45).

(homozygotes) will present with a moderate hypercholesterolemia which is entirely attributable to hyperalphalipoproteinemia or high HDL with concomitant elevations in the major HDL apoprotein, Apo A-1. Total cholesterol in heterozygotes is in the high-normal range and uninformative. However, their HDL cholesterol and Apo A-1 is significantly elevated when compared with controls. Thus the physician requires both total plasma cholesterol and HDL cholesterol values, the latter being obtained by difference following Heparin-Dextran precipitation of VLDL+LDL and measurement of the supernatant. LDL cholesterol is calculated using the Friedwald equation and in homozygous CETP deficient patients is usually low with normal values in herterozygotes of Japanese heritage. The LDL cholesterol values are reflected by the low Apo B values as this apoprotein is the major protein constituent of LDL. Our homozygous Nova Scotian patient, however, had normal Apo B levels (1). We speculate that this may be attributed to the imposition of CETP deficiency upon a different genetic background. Additional studies on LDL turnover must be conducted in order to test this possibility. Triglyceride levels in CETP deficiency are essentially normal and uninformative. However, if the clinical laboratory is capable of separating VLDL from whole plasma by ultracentrifugation the very low proportion of VLDL cholesteryl esters relative to VLDL triglycerides in homozygotes for CETP deficiency is a strong indicator of the lack of CETP activity. A relative decrease in the proportion of cholesteryl ester to triglyceride in the VLDL+LDL fraction in homozygote CETP-deficient plasma will be significantly less pronounced than in the VLDL alone. This is due to the fact that the LDL, although relatively triglyceride enriched, has near normal proportions of cholesteryl ester per particle and is usually present in much higher concentrations than VLDL. In the event that the above positive indicators of CETP deficiency are observed whole blood samples should be referred to a research laboratory for the determination of CETP mass (1,9,12), CETP activity (49) and molecular genetic analysis (1). Given the apparent rarity of CETP deficiency in the Caucasian population, Caucasian CETP deficient patients in Nova Scotia will most likely carry the mutation recently reported by us (1). This mutation can be rapidlydetected using a single base mismatched, 26 bp reverse PCR

primer which produces a single Mae III Restriction Fragment Length Polymorphic (RFLP) site upon amplification of the mutated DNA sequence (1). Other known mutations (**Figure 3**) can be screened for using similar techniques and the appropriate primers.

THE HYPERCHOLESTEROLEMIA OF CETP DEFICIENCY: TO TREAT OR NOT TO TREAT

The atherogenic susceptibility of patients with CETP deficiency and hence the need for pharmacological intervention is presently a matter of some debate. Based only upon total plasma cholesterol determinations, which are significantly elevated in homozygous CETP deficient subjects, prescription of cholesterol lowering drugs such as the statins would be indicated and this indeed is what initially occurred with the Nova Scotia patient recently characterized by us. Although the decision concerning the advisability of pharmacological intervention must reside with the attending physician and the patient, pure CETP deficiency when superimposed upon a normal genetic background and uncomplicated by the co-inheritance of other traits which may adversely affect the plasma lipoprotein profile, may be one form of hypercholesterolemia in which pharmacological intervention is contraindicated at this time. Inspection of the plasma lipoprotein profile in CETP deficient subjects shows that the majority of the plasma cholesterol is associated with large HDL particles (1). LDL cholesterol is either near normal (Nova Scotian patient) or low (Japanese subjects) and this is reflected respectively in the normal or low levels of Apo B, the major protein component of VLDL and LDL. Due to the lack of CETP-mediated cholesteryl ester transfer, the VLDL contains much reduced proportions of cholesteryl ester. As one VLDL molecule produces one molecule of IDL and thence one LDL molecule it would be expected that the LDL would be equally cholesteryl ester poor and thus less atherogenic. In fact this is not the case and the LDL particles in CETP deficient subjects, although containing less cholesteryl ester per particle than normal LDL, are still relatively enriched in this lipid due to cholesterol esterification by the enzyme LCAT (1,45). The LDL particles are also small and polydisperse when compared to normal (50,51) and have a lower affinity for the LDL receptors (52) but despite this appear to be cleared more rapidly from the plasma than normal LDL (48). Thus on the basis of current wisdom the plasma lipoprotein profile produced in CETP-deficiency would, on balance, be considered anti-atherogenic due to the high levels of protective HDL and low levels of atherogenic LDL. Indeed it was initially suggested that CETP deficiency represented a "Longevity Syndrome" (42,53). However, when more heterozygotes were available for study it became apparent that the defective allele did not segregate with a higher frequency than normal into the elderly population (44) as would be expected if the defect exerted a protective effect against atherosclerotic cardiovascular disease. Given these findings the current discussion now focuses upon the potential pro or anti-atherogenic effects of high plasma concentrations of large cholesteryl ester-rich HDL particles in these subjects and the residual potential of the small HDL particles to mediate cholesterol efflux from peripheral vascular tissues. Currently these questions are unresolved as is the activity of the human equivalent of the hepatic SR-B1 receptor thought to directly remove plasma HDL cholesteryl ester (Figure 2). Clinical data produced from Japanese CETP-deficient subjects has recently revealed a "U" shaped relationship between HDL cholesterol and the incidence of ischemic ECG changes (44). Patients with HDL cholesterol between 1.55 and 1.81 mmol/L had a significantly decreased incidence when compared with subjects having an HDL cholesterol of 1.03 mmol/L. However females with an HDL cholesterol of 2.23 mmol/L had the same incidence of ischemic ECG changes as normal females with an HDL cholesterol of 1.03 mmol/L. Males, in contrast, had a somewhat elevated incidence when their HDL cholesterol was at or above 2.32 mmol/L The data indicate that heterozygotes with moderately elevated HDL cholesterol levels are protected against ischemic ECG changes and should not be treated with cholesterol lowering drugs.

Despite the above considerations there have been reports of CETP-deficient kindreds in which premature atherosclerotic vascular disease has been noted (54,55). This may be due to the co-inheritance of additional genetic factors which result in a pro-atherogenic plasma lipoprotein profile and/or the presence of other classical risk factors such as diet, hypertension, diabetes or smoking, particularly when studying Japanese-American CETP-deficient subjects living in Hawaii and consuming a North American diet (54). In terms of genetic background effects, hyperalphalipoproteinemic Japanese subjects heterozygous for CETP deficiency who also had reduced levels of hepatic lipase had a significantly elevated incidence of atherosclerotic cardiovascular disease when compared with heterozygous CETP-deficient subjects with normal hepatic lipase activity or with controls (55). Genetic deficiency of hepatic lipase has been associated with coronary artery disease (56-58), however, subjects with low CETP and hepatic lipase showed neither the high plasma triglyceride levels nor the accumulation of remnant lipoproteins characteristic of hepatic lipase deficiency alone. Thus, the accumulation of remnant lipoproteins cannot be attributed to the increased atherogenicity in heterozygous CETP-deficient – hepatic lipase deficient patients. A reasonable hypothesis stems from the report of Newnham and Barter (59) who showed that CETP and hepatic lipase act synergistically in the formation of small HDL particles thought to be important in promoting cholesterol efflux from peripheral cells. Studies in double heterozygotes with Familial Hypercholesterolemia (FH) and Cholesteryl Ester Transfer Protein Deficiency (60) have shown that the elevations in plasma HDL resulting from the CETP deficiency were ineffective in preventing the coronary artery disease produced by the elevated LDL levels in FH. This strengthens the observation that HDL-mediated cholesterol efflux from vascular tissue is not enhanced in CETP deficient subjects.

of familial CETP deficiency in a Caucasian subject resident in Nova Scotia. Previously this deficiency has only been reported in Japanese patients and in a few Caucasians in Europe. In Homozygotes, CETP deficiency is characterized by a moderate hypercholesterolemia attributable to a marked hyperalphalipoproteinemia with low levels of LDL cholesterol. Recent evidence supports the view that CETP deficiency would be inaccurately defined as a "Longevity Syndrome" due to the anti-atherosclerotic effects of the elevated plasma HDL in these subjects. Equally, although homozygous CETP deficiency, may result in an increased incidence of ischemic ECG changes it does not appear to markedly enhance atherosclerotic risk unless combined with other genetic insufficiencies or traditional risk factors. Thus pharmacological intervention may only be indicated when these compounding risk factors are present and/or there is a family history of premature coronary artery disease.

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AUTHOR BIOGRAPHY

Peter Dolphin received his BSc (Hon) in Physiology and Biochemistry in 1968, his PhD in Biochemistry in 1971 and his DSc in Biochemistry in 1989, all from the University of Southampton (UK). He was a Post-Doctoral Fellow and Assistant Professor in Biochemistry at McGill prior to joining the Dalhousie Biochemistry department in 1978. He is presently a full professor in the same department, a past-president of the Canadian Society for Biochemistry, Molecular and Cellular Biology and current president of the Canadian Federation of Biological Societies. His research is funded by MRC and the Heart and Stroke Foundation of Nova Scotia.

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