Effect of postprandial lipaemia and Taq 1B polymorphism of the cholesteryl ester transfer protein (CETP) gene on CETP mass, activity, associated lipoproteins and plasma lipids

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A large number of studies in recent years have investigated the effects of hyperlipidaemias and diabetes on cholesteryl ester transfer protein (CETP) on neutral lipid transfer activity and plasma lipids. There has been an ongoing debate as to whether CETP is pro- or anti-atherogenic as it provides a mechanism for the transfer of cholesterol from the cardioprotective HDL subfraction to the potentially atherogenic LDL subfraction. This study was designed to investigate whether there was significant variability of CETP mass and activity in a large normolipidaemic population and whether there is an association between CETP and plasma lipoprotein composition. The presence of a known polymorphism of CETP gene (Taq 1B) was investigated to see if there was any association between this polymorphism and CETP mass and activity, and plasma lipids. There was significant ($P < 0{\cdot}0001$) increase in CETP mass and activity in plasma postprandially at 6 h. Using multiple stepwise regression analysis there was significant association with fasting CETP mass and activity ($\beta = 0{\cdot}055$; $P = 0{\cdot}002$) and triacylglycerol-rich lipoprotein ($\beta = 0{\cdot}013$; $P = 0{\cdot}005$) and postprandial CETP mass ($\beta = 0{\cdot}254$; $P = 0{\cdot}007$). Repeated-measures analysis showed a strong association between the absence of Taq 1B polymorphism and low CETP mass and elevated HDL- and HDL_2-cholesterol and HDL-phospholipid concentrations than did those who were homozygous or heterozygous for the presence of the restriction site.

Cholesteryl ester transfer protein: Taq 1B polymorphism: Postprandial lipaemia

Increases in cholesteryl ester transfer protein (CETP) activity during postprandial lipaemia have been reported previously (Tall *et al*. 1986) where a 2–3-fold increase in cholesteryl ester transfer was reported. As a consequence of CETP activity, there is a transfer of cholesteryl ester from HDL particles to VLDL and LDL particles with a reciprocal transfer of triacylglycerol (TAG). Alterations in HDL size and composition occur as a result of CETP activity. Precursors to HDL are pre beta HDL, which contain apolipoprotein (Apo) A1 proteins with a small amount of phospholipid. These pre beta HDL can bind to cells promoting efflux of cellular cholesterol (Fielding & Fielding, 1995). They may combine with chylomicron remnants produced during postprandial lipaemia and accumulate cholesteryl ester and phospholipids. This results in the formation of mature HDL, which is facilitated through the actions of lecithin–cholesterol acyl transferase (Skinner, 1994). Mature HDL can be divided into HDL_2 and HDL_3 based on their lipid and lipoprotein composition. HDL subfractions are dynamic and constantly change from more dense HDL_3 to less dense HDL_2 particularly during postprandial lipaemia through the activities of CETP. TAG-rich HDL_2 is catabolised by hepatic lipase resulting in the formation of HDL_3. VLDL and intermediate-density lipoprotein accept cholesteryl ester from HDL with a reciprocal exchange of TAG into HDL as a result of CETP activity. VLDL and intermediate-density lipoprotein are catabolised to form LDL. A number of publications suggest that CETP activity may be pro-atherogenic because it results in the reduction of HDL-cholesterol, increased production of dense atherogenic LDL and increased catabolism of TAG-rich HDL by hepatic lipase (Tall, 1993; Lagrost *et al*. 1994). The increase in CETP mass, activity and the redistribution of cholesteryl ester from HDL to VLDL and LDL lipoprotein fractions during postprandial lipaemia suggests the pro-atherogenic effect of CETP may be elevated during lipaemia. Cholesterol intake (Quinet, 1990; Fielding & Fielding 1995), dietary *trans* fatty acid consumption (Abbey & Nestel, 1994), alcohol consumption (Savolainen *et al*. 1990), and genetic variations all account for differences

Abbreviations: Apo, apolipoprotein; CETP, cholesteryl ester transfer protein; TAG, triacylglycerol.

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in CETP mass and activities in normolipidaemics. Where there are genetic variants of CETP, for example the absence of Taq 1B polymorphism (Bernard *et al*. 1998), or splicing defects which are common in the Japanese population (Inazo *et al*. 1990; Koizumi *et al*. 1991), there are increased HDL-cholesterol concentrations.

A number of previous studies have investigated the effect and frequency of the Taq 1B polymorphism of the CETP gene (Freeman *et al*. 1993; Hannuksula *et al*. 1994; Kuivenhoven *et al*. 1997; Kuivenhoven *et al*. 1998). Kuivenhoven *et al*. (1998) noted that the frequency of Taq 1B polymorphism in a group of patients with coronary atherosclerosis was B+ B+, 35 %, B+ B–, 49 % and B– B–, 16 %. Data from many of these previous studies indicate those who show the absence of the Taq 1B cutting site (B– B–) have lower CETP mass and higher HDL-cholesterol concentrations (Kondo *et al*. 1989; Freeman *et al*. 1990). It is hypothesised that an altered HDL-cholesterol concentration in these individuals is a direct result of the presence or absence of the Taq 1B polymorphism on the CETP gene. However, a mechanism as to how this occurs is not forthcoming.

The present investigation was designed to address whether there is an increase in CETP mass and activity in response to a test meal containing a typical amount of fat (40 g) in a large normolipidaemic study group. Although the effects of acute fat ingestion on CETP mass and activity have been previously studied, the majority have measured either CETP mass or activity but not both. Many of the previous studies on CETP have concentrated on CETP mass and activity in hyperlipidaemic subjects in order to ascertain if their condition results in altered CETP. In this present study, a large number of lipid variables were analysed to try and ascertain if CETP activities could be attributed to greater coronary risk factors in a normolipidaemic population. Finally an investigation into the effects of the Taq 1B polymorphism on CETP mass or activity and associated lipids in fasting and postprandial states was carried out.

Methods

Study subjects

The study was conducted in the nutrition laboratory at the Trinity Centre, St James's Hospital, Dublin, Republic of Ireland. Sixty-three non-smoking healthy subjects were recruited from the personnel of Trinity College Dublin and St James's Hospital, Dublin. Biochemical exclusion criteria included fasting plasma cholesterol $< 7{\cdot}0$ mmol/l, plasma TAG $< 2{\cdot}0$ mmol/l, plasma glucose < 110 mg/dl, plasma γ-glutamyltransferase < 60 IU/l, haemoglobin > 12 g/dl and a BMI < 30. Each study day began between 07.30 and 08.00 hours, following a 12 h overnight fast. Subjects were asked to abstain from alcohol and exercise 24 h before a postprandial day. During postprandial investigation each subject was asked to abstain from food and drink with the exception of water, decaffeinated coffee and diet drinks. The test meal was prepared each day. Each subject received 40 g fat which was high in monounsaturated fatty acids (Flora Sunflower Oil, Van Den Bergs Ltd, Crawley, Sussex, UK), 150 g skimmed milk and 5 g orange-flavoured Modjul system (Scientific Hospital Supplies International, Liverpool, UK) which was mixed with water.

A 21 gauge 32 mm venous catheter (Abbott Ireland Ltd, Dublin, Republic of Ireland) was inserted into the antecubital vein of the non-dominant forearm. Blood samples for CETP analysis were collected in 5 ml citrated tubes (0·106 M-sodium citrate) (Starstead monovette). Blood samples for lipid analysis were collected into 10 ml heparinised vacutainers. All blood samples were drawn before the test meal was administered. The test meal was consumed within 15 min. A further blood sample was drawn 6 h after the test meal. All samples were centrifuged (3000 r.p.m. for 15 min) at room temperature. Plasma was removed, mixed, and aliquots of 0·5 ml fractions were made. Samples for CETP analysis were snap-frozen under liquid N_2 and stored (–70°C) until CETP mass and activity was assayed. Plasma for lipid analysis was stored at –20°C.

Laboratory methods

Analysis of plasma TAG (TAG PAP Biomerieux, Etoile, France), cholesterol (Biomerieux PAP), non-esterified fatty acids (Acyl Co A synthetase-acyl Co A oxidase, Wako Chemicals Gmbh, Neuss, Germany), phospholipid (Biomerieux PAP), ApoA1 (Biomerieux) were carried out. HDL-cholesterol, TAG, phospholipid and ApoA1 were measured using the methods mentioned previously following precipitation of HDL with Immuno Quantolip HDL (Immuno AG, Vienna, Austria) precipitating reagent. All determinations were performed on a Technicon RA-XT Chemistry Analyser (Technicon Inc., Tarrytown, NY, USA)

CETP activity in plasma was determined using a fluorescent transfer method (WAK-Chemi Medical, Bad Soden, Germany). A fluorescent-labelled cholesterol linoleate is sequestered within a donor particle in a quenched state. CETP facilitates the transfer of cholesteryl ester to an acceptor particle where the fluorescence is unquenched. The increase in fluorescence is proportional to the rate of transfer activity. Total CETP mass in plasma was determined using an alkaline phosphatase-based sandwich ELISA assay as described previously by Clarke *et al*. (1995).

DNA analysis

Genomic DNA was isolated from whole blood by haemolysing the red blood cells. White blood cells were pelleted and washed with Tris EDTA (pH 7·5) until no trace of red blood cells was evident. The white blood cells were treated with proteinase K for 45 min, after which it was denatured. Amplification of a 213 base pair fragment of the CETP gene containing the Taq 1B was carried out using polymerase chain reaction. The forward (GGT CCT AGC TGC ATT GCA AAC) and reverse (GAT GGA GCC TCC GTC GTC ACC TGA) primers were synthesised on a 391 DNA synthesiser (Applied Biosystems, Perkin Elmer Corp., Foster City, CA, USA). The polymerase chain reaction consisted of a 7 min denaturation at 94°C, then 35 cycles of 94°C for 1 min, 55°C for 1 min and 72°C for 1 min 12 s and finally a 5 min extension at 72°C. This polymerase chain reaction programme was carried out on 25 μl reactions containing

1·25 mM-dATP, -dCTP, -dGTP, -dTTP, 6 % 20 mM-Mg^{2+} buffer, 0·6 units and 1 μl DNA in 15 mM-Mg^{2+} buffer, plus the forward and reverse primers. The polymerase chain reaction product (5 μl) was digested with 1 Unit Taq 1B (Boehringer Mannheim, Mannheim, Germany) for 4 h at 65°C. Digestion products were run on 3 % agarose gel. Subjects who did not have restriction site had a band at 213 base pairs (B− B−), heterozygotes had bands at 213, 142 and 71 base pairs (B+ B−), while subjects who cut had bands at 142 and 71 base pairs (B+ B+).

Data analysis

All statistical analysis was carried out on an Apple Macintosh statistical package, Data Desk 4.1 (Data Description Inc., New York, NY, USA. The distribution of the data for each variable was assessed. Some of the variables were transformed to normalise the distribution of some of the data sets ($\sqrt{}$HDL, log of CETP data). Paired Student's *t* test was used to analyse the difference in variables between fasting and 6 h postprandial samples. Pearson product moment correlation analysis was used to investigate the relationship between plasma lipid and lipoprotein concentrations and CETP metabolism. Stepwise multiple regression analysis was used to investigate the factors which determined CETP mass and activity in the fasted and 6 h postprandial state. One-way ANOVA was used to compare significant difference in CETP mass, activity and plasma lipids according to Taq 1B polymorphism grouping.

Results

Sixty-three subjects, thirty-nine males and twenty-four females, participated in this study. The total study group had a mean age of 32·4 (SD 9·6) years, a mean weight of 73·48 (SD 13·65) kg and a mean BMI of 24·14 (SD 2·62) kg/m^2. The effect of postprandial lipaemia on CETP was investigated at two hourly intervals over 8 h. Maximal CETP mass and activity occurred at 6 h, 2 h after peak TAG production. Therefore, the 6 h time point was used to access the effect of lipaemia on CETP in this investigation. Fasting and 6 h postprandial CETP mass, activity and lipid concentrations are given in Table 1. There was a significant increase in CETP mass ($P = 0·0014$) and activity ($P \leq 0·0001$) from the fasted state to 6 h after ingestion of test meal. Plasma TAG and plasma non-esterified fatty acids were significantly increased ($P \leq 0·0001$) during postprandial lipaemia at 6 h, while plasma LDL concentrations were significantly ($P = 0·01$) reduced. The compositions of the HDL subfractions are given in Table 2. Total phospholipid, ApoA1 and TAG concentrations were significantly increased ($P \leq 0·0001$) at 6 h. Total HDL-cholesterol did not increase significantly. Plasma concentrations of HDL_3-ApoA1 and -TAG were significantly ($P < 0·05$) increased 6 h postprandially, whilst cholesterol and phospholipid concentrations were not altered significantly. All of the lipids of the HDL_2 subfraction increased significantly 6 h after ingestion of test meal, with the increase in HDL_2-TAG being the most significant ($P \leq 0·0001$). There was a strong positive relationship between CETP mass and activity in the fasted and postprandial states for the total study group (r 0·470, $P \leq 0·0001$).

Plasma lipids and cholesteryl ester transfer protein metabolism

Fasting CETP mass was significantly correlated with age (r 0·310, $P = 0·012$). Postprandial CETP mass (6 h) was significantly correlated with BMI (r 0·270, $P = 0·032$) and body weight (r 0·265, $P = 0·035$). Postprandial CETP activity was not significantly associated with age, weight and BMI. Stepwise multiple regression analysis of fasting CETP mass demonstrated that fasting plasma cholesterol was the most important factor ($\beta = 0·354$; $P = 0·0003$), followed by age ($\beta = 0·0233$; $P = 0·012$). Stepwise multiple regression analysis of CETP mass at 6 h showed that fasting CETP mass was the most important determinant ($\beta = 0·0192$; $P = 0·0001$), followed by fasting LDL-cholesterol concentration ($\beta = 0·047$; $P = 0·0031$), HDL_3-TAG concentration ($\beta = 0·898$; $P = 0·0101$) and HDL_3-ApoA1 concentration ($\beta = -0·240$; $P = 0·015$). Multiple stepwise regression analysis of fasting CETP activity showed that fasting CETP mass was the principle determinant ($\beta = 26·746$; $P = 0·0001$), while fasting plasma non-esterified fatty acids had a small effect ($\beta = 43·002$; $P = 0·06$). Stepwise multiple regression analysis

Table 1. Plasma cholesteryl ester transfer protein mass and activity and plasma lipids in the fasted state and at 6 h following an acute fat load in healthy normolipidaemic subjects*

(Mean values and standard deviations for sixty-three subjects)

	Time			
	0 hours		6 hours	
	Mean	SD	Mean	SD
CETP mass (μg/ml)	2·040	0·686	2·314†	0·985
CETP activity (pmol/NBD-CE transfer/3 h)	29·851	7·232	39·011††	12·45
Plasma triacylglycerol (mmol/l)	0·987	0·467	1·071††	0·477
Plasma NEFA (mmol/l)	0·482	0·185	0·616†	0·199
Plasma cholesterol (mmol/l)	4·982	1·048	4·926†	0·989
Plasma LDL-cholesterol (mmol/l)	3·621	0·785	3·582†	0·653

CETP, cholesteryl ester transfer protein; NBD-CE, nitrobenzoxadiol-fluorophore–cholesteryl ester; NEFA, non-esterified fatty acids.

* For details of procedures see pp. 204–205.

Mean values were significantly different from those at 0 h: †$P < 0·0014$, ††$P < 0·0001$.

Table 2. Concentrations of cholesterol, phospholipid and triacylglycerol (TAG) in the high-density lipoprotein (HDL) subfractions in the fasted and postprandial states following an acute fat load in healthy normolipidaemic subjects*

(Mean values and standard deviations for sixty-three subjects)

	Total HDL				HDL_3				HDL_2			
	0 h		6 h		0 h		6 h		0 h		6 h	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Cholesterol (mmol/l)	1·162	0·310	1·174	0·358	0·917	0·254	0·887	0·285	0·248	0·221	0·332††	0·254
Phospholipid (mmol/l)	1·047	0·230	1·168†††	0·296	0·856	0·205	0·875	0·278	0·213	0·174	0·335††	0·279
ApoA1 (mmol/l)	0·582	0·223	0·726†††	0·106	0·494	0·212	0·570†	0·176	0·126	0·119	0·187†	0·171
TAG (mmol/l)	0·053	0·019	0·070†††	0·03	0·045	0·017	0·055†	0·027	0·011	0·011	0·021†††	0·019

Apo, apolipoprotein.
* For details of procedures see pp. 204–205.
Mean values were significantly different from those at 0 h: †$P < 0·05$, ††$P < 0·005$, †††$P \leq 0·0001$.

Table 3. Fasting levels of plasma cholesteryl ester transfer protein (CETP) mass, activity, total high-density lipoprotein (HDL) and HDL_2-cholesterol and HDL-phospholipid concentrations according to CETP Taq 1 B genotype in healthy normolipidaemic subjects*

(Mean values and standard deviations for sixty-three subjects)

	Genotype						Statistical significance of difference between means (one-way ANOVA): *P*
	B– B– (*n* 6)		B– B+ (*n* 34)		B+ B+ (*n* 23)		
	Mean	SD	Mean	SD	Mean	SD	
CETP mass (μg/ml)	1·342	0·773	2·119	0·623	2·105	0·674	0·002
CETP activity (pmol/NBD-CE transfer/min)	26·116	7·229	29·72	10·130	31·042	9·075	NS
HDL-cholesterol (mmol/l)	1·506	0·598	1·114	0·237	1·140	0·292	0·017
HDL_2-cholesterol (mmol/l)	0·526	0·405	0·200	0·173	0·245	0·173	0·05
HDL-phospholipid (mmol/l)	1·321	0·290	1·011	0·212	1·027	0·197	0·002

NBD-CE, nitrobenzoxadiol-fluorophore–cholesteryl ester.
* For details of procedures see pp. 204–205.

of 6 h CETP activity showed that fasting cholesterol was the principle determinant ($\beta = 0·055$; $P = 0·0025$) followed by TAG-rich lipoprotein ($\beta = -0·013$; $P = 0·005$), and mass at 6 h ($\beta = 0·254$; $P = 0·0070$).

Taq 1B genotype

The distribution of CETP Taq 1B alleles within this study group was: B+ B+ 36·5 %, B+ B– 53·9 %, B– B– 9·52 %. ANOVA was used on the comparison of the lipid concentrations of the B+ B+, B+ B– and B– B– polymorphisms to see if Taq 1B restriction site exhibited any influence on the lipid profile of subjects grouped according to particular polymorphism; results are shown in Table 3. Those individuals which are homozygous for the absence of the Taq 1B polymorphism (B– B–) had a significantly ($P < 0·05$) lower fasting CETP mass, higher fasting HDL, HDL_2-cholesterol and HDL-phospholipid concentration than did those individuals who were heterozygous (B+ B–) or homozygous (B+ B+) for the presence of Taq 1B polymorphism (see Table 3). A similar trend was noted when 6 h CETP and 6 h plasma lipids were compared according to polymorphism grouping; however, significant statistical association was not achieved.

Discussion

In a study by Tall *et al.* (1986) there was a 1·1–1·7-fold increase in cholesteryl ester transfer in response to a 135 g fat-rich test meal. In the present study there was an increase of 1·3-fold in CETP activity ($P = 0·0001$), and 1·13-fold increase in CETP mass ($P = 0·0001$) during postprandial lipaemia in response to a 40 g fat test meal. Investigation of the rate of production of CETP postprandially in eighteen subjects in the fasted state and at two hourly intervals for 8 h demonstrated that maximal CETP mass and activity were found at 6 h after ingestion of test meal, similar to the findings of Tall *et al.* (1986). The 6 h samples were used as the time point for CETP and lipid analysis to see the effect of maximal CETP production on plasma lipids after ingestion of the test meal. Castro & Fielding (1985) demonstrated that cholesteryl ester transfer doubled during postprandial lipaemia. Previous studies show that increases in CETP mass during postprandial lipaemia in type 2 diabetics and normal control subjects were significant (Lottenberg *et al.* 1996*b*). Discrepancies as to increases in CETP activity between studies may be explained by fat composition. A test meal rich in monounsaturated fatty acids (which was administered in this present study) tends to produce a lower postprandial response than does a saturated fat meal (Groener *et al.* 1991; de Bruin *et al.* 1993) as would the lower dose of fat administered in the test meal. Lottenberg *et al.* (1996*a*) demonstrated that increases in CETP activity were also accounted for by increases in substrate availability during lipaemia.

In the present study analysis of HDL subfractions showed the HDL_2 subfraction had the greatest increase in lipid

composition 6 h postprandially, with HDL_2-TAG showing the most significant increase (Patsch *et al*. 1984, 1987), followed by HDL_2-cholesterol, -phospholipid and -ApoA1 concentrations. Total HDL-cholesterol did not change significantly 6 h postprandially in this study as was demonstrated previously by Karpe *et al*. (1993). The HDL_3 subfraction showed the lowest change in composition during postprandial lipaemia, with significant ($P < 0{\cdot}05$) increases in HDL_3-TAG and -ApoA1.

The finding of the present study that fasting plasma cholesterol is the most important determinant of fasting CETP mass is supported by the study of Kahri *et al*. (1994) and Tato *et al*. (1995). In other studies, this association failed to attain statistical significance (Jones *et al*. 1996; Tato *et al*. 1997). Tato *et al*. (1995) also observed associations between postprandial CETP mass and both BMI and weight, but they failed to reach statistical significance. The present study did find these associations to be statistically significant, possibly because of a normolipidaemic study group used in this investigation compared with the study of Tato *et al*. (1995) where the study group was hypercholesterolaemic.

Multiple stepwise regression analysis demonstrated that fasting plasma cholesterol was the primary determinant of CETP mass followed closely by age which confirms a link between CETP, plasma cholesterol and age, suggesting that plasma cholesterol may determine CETP mass. These data suggest that high CETP levels may be a consequence of elevated plasma cholesterol levels (Bagdade *et al*. 1992; Inazu *et al*. 1992). Multiple stepwise regression analysis showed that the predictors of 6 h postprandial CETP mass were LDL-cholesterol ($\beta = 0{\cdot}047$), HDL_3-TAG ($\beta = 0{\cdot}898$; $P = 0{\cdot}01$) both of which are functional indicators of CETP activity and HDL_3-ApoA1 ($\beta = -0{\cdot}240$; $P = 0{\cdot}015$) which is important in regulation of CETP activity (Guyard-Dangremont *et al*. 1994) and binding of CETP to HDL (MacPhearson *et al*. 1996).

Regression analysis showed that fasting CETP activity was associated with CETP mass; this has been shown in other studies (Clarke *et al*. 1995; Tato *et al*. 1995; Jones *et al*. 1996). CETP activity was determined using a new quick fluorimetric assay. Rapid analysis of samples was possible and use of radioactive substrates was excluded. When fluorimetric CETP activity assay was compared with an established CETP mass assay (Clarke *et al*. 1995), satisfactory correlation was achieved considering that plasma used for the CETP activity assay contained many of endogenous plasma proteins which inhibit or activate CETP activity unlike many previous methods of measuring CETP activity (Groener *et al*. 1986). This present study is one of the first to carry out a comparison of this new method for the measurement of CETP activity with an established method for the measurement of CETP mass.

Plasma non-esterified fatty acid concentration approached significance ($P = 0{\cdot}06$) with CETP activity in this study as was also demonstrated by Largrost *et al*. (1995). Postprandial CETP activity at 6 h was associated with plasma cholesterol ($P = 0{\cdot}002$), TAG-rich lipoprotein ($P = 0{\cdot}005$) and mass ($P = 0{\cdot}007$) at 6 h. This association demonstrates that CETP activity in postprandial lipaemia is perhaps influenced by the availability of cholesteryl ester and TAG. Lottenberg *et al*. (1996*a*), Lassel *et al*. (1998) and Mann (1991) have all demonstrated the importance of substrate availability in the regulation of CETP activity.

The frequency of the Taq 1B polymorphism of CETP was similar in our study group to that found in other studies (Bernard *et al*. 1998; Kuivenhoven *et al*. 1998). The relationship between the Taq 1B polymorphism and CETP mass, activity, and plasma lipids was investigated. The presence of the Taq 1B restriction site resulted in significantly higher ($P < 0{\cdot}002$) fasting CETP and lower HDL-cholesterol concentrations (as has been demonstrated by Hannuksela *et al*. (1994), Kahri *et al*. (1998), and Kuivenhoven *et al*. (1998)) over those individuals who were homozygous for the absence of the polymorphism. We also found a significant effect ($P < 0{\cdot}05$) of Taq 1B restriction fragment length polymorphism on HDL_2-cholesterol concentrations (Freeman *et al*. 1993). The absence of Taq 1B restriction (B− B−) cutting site resulted in increased total HDL-phospholipid concentrations, a finding which has not been reported in any previous studies. These data seem to indicate that different alleles of CETP gene in a normolipidaemic population have an effect on CETP mass, HDL-cholesterol and -phospholipid concentrations. There is a lower concentration of CETP in those with B− B− allele. It could be hypothesised that there is less transfer of cholesteryl ester from HDL resulting in a greater concentration of cholesteryl ester being retained in the HDL fraction due to a lower concentration of CETP. There was a non-significant difference in CETP activity between the genotypes. These results conflict with previous studies (Freeman *et al*. 1993) which have suggested that association of HDL-cholesterol concentrations with Taq 1B was only identified in extreme situations, i.e. smokers, alcoholics, or α-lipoproteinaemic but not in normolipidaemic subjects. From the data in this study it would seem that in the normal population depending on their Taq 1B genotype, there is either an increased risk or decreased risk of being susceptible to CHD as a result of the CETP genotype. There was no statically significant association between the presence and absence of Taq 1B polymorphism and 6 h CETP, HDL-cholesterol and -phospholipid concentrations. This lack of association may be as a result of increased lipid concentration during lipaemia masking the effect of Taq 1B polymorphism on plasma CETP and lipoprotein composition.

In summary, the findings of this present study were that there was a very significant increase in CETP mass, activity, plasma TAG, and HDL_2 lipids in response to a test meal. CETP concentrations were associated with plasma cholesterol concentrations. Elevated CETP resulted in high LDL : HDL ratios demonstrating the pro-atherogenic effect of CETP in normolipidaemics. Increased neutral lipid exchange between lipoproteins as a result of CETP activity occurred during lipaemia suggesting the pro-atherogenic effects of CETP may be increased during postprandial lipaemia. Polymorphisms of CETP were also important in determining basal CETP and HDL-cholesterol and -phospholipid concentrations indicating that there is a genetic predisposition to either anti- or pro-atherogenic effects of CETP (elevated CETP concentrations resulting in low HDL-cholesterol). The data in this study implies that CETP may be pro-atherogenic at certain concentrations in

normolipidaemic subjects and that this effect may be exacerbated during lipaemia.

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