Clinical Significance of Plasma Apolipoprotein F in Japanese Healthy and Hypertriglyceridemic Subjects

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Aim: Apolipoprotein F (apo F), also known as lipid transfer inhibitory protein (LTIP), is a protein component of plasma lipoprotein classes including HDL and functions to inhibit lipid transfer between lipoproteins in vitro. To study the role of plasma apo F, a reliable and sensitive tool for the quantification would be needed.

Methods: We have developed a sandwich ELISA using two monoclonal antibodies for human plasma apo F, and analyzed apo F concentration in 397 Japanese healthy and 221 hypertriglyceridemic subjects.

Results: Our ELISA enables apo F to be assayed in the range of 0.6-25 μg/mL with intra- and inter-assay coefficients of variation less than 3.8% and 7.8%, respectively. In healthy subjects, plasma apo F concentration was 12.5 ± 2.9 μg/mL (mean ± SD), and was significantly higher in females than in males (p < 0.05). By linear regression analysis in healthy subjects, plasma apo F concentration correlated positively with HDL cholesterol and apo A-I levels, and in males but not in females, negatively with apo B and triglyceride levels. It also correlated negatively with intrinsic CETP activity measured using intrinsic apo B-containing lipoprotein as an acceptor, and positively with PLTP mass and apo J levels. Apo F concentration in hypertriglyceridemic patients (10.3 ± 3.1 μg/mL) was lower than in healthy controls (p < 0.0001) and correlated positively with PLTP mass.

Conclusions: Our ELISA is reliable and sensitive for the quantification of plasma apo F concentration. This system can be applicable for clinical significance in lipoprotein metabolism and reverse cholesterol transport.


Key words: Apolipoprotein F, Lipid transfer inhibitory protein, Reverse cholesterol transport

Abbreviations: apo F, apolipoprotein F; CBB, Coomassie brilliant blue; LCAT, lecithin: cholesterol acyltransferase; MAb, monoclonal antibody; rhapo F, recombinant human apolipoprotein F

Introduction

Apolipoprotein F (apo F) is a 29-kDa acidic glycoprotein associated with LDL¹, ² and HDL³-⁵ in human and mouse plasma⁶-⁸. It is also known as a lipid transfer inhibitor protein (LTIP) for its ability to inhibit lipid transfer among lipoproteins in vitro¹. Apo F is an endogenous inhibitor of cholesteryl ester transfer protein (CETP)-mediated cholesteryl ester (CE), triglyceride (TG) and phosphatidylcholine (PC) transfers and phospholipid transfer protein (PLTP)-mediated PC transfer¹, ⁹, ¹⁰. Apo F inhibits CETP...
activity by preventing CETP from binding to the lipoprotein surface\(^{11}\). By selective inhibition of lipid transfer to/from LDL, apo F enhances the net flux of CE from HDL to VLDL, facilitating the clearance of HDL-derived CE in the circulation and promoting reverse cholesterol transport (RCT).

Apo F mRNA is abundantly expressed in the liver and encodes a 22 amino acid signal peptide and 308 amino acids\(^{4}\). Plasma apo F is a 162 amino acid protein derived from the C-terminal portion of apo F proprotein by proteolytic cleavage at residues 146-147 (Arg/Ser). It contains one N-linked glycosylation site and numerous O-linked glycosylation sites, resulting in being acidic with an isoelectric point of 4.5 and 40\% greater molecular mass than predicted\(^{4}\).

Recent studies have shown that plasma apo F exists in two forms, one active and the other inactive\(^{7}\). Apo F bound to LDL has LTIP activity, whereas the HDL-associated apo F exists as part of the 470 kDa inactive complex. Although human apo F preferred to associate with the larger HDL particles in the gel filtration profile, apo F-containing particles show density in the HDL\(3\) range\(^{7,8}\). By immunoblotting analysis, plasma apo F associated with LDL was markedly higher in hypercholesterolemic subjects than in normolipidemic subjects, which was due to the distribution of apo F and its lipoprotein composition\(^{11,12}\). The clinical significance in normolipidemic and dyslipidemic subjects has also been studied in detail by a competitive enzyme immunoassay (EIA), and showed a decrease in the apo F level in hypertriglyceridemic males\(^{3}\).

More recently, Lagor et al. have shown that mice overexpressing human CETP and apo F have a lipoprotein profile similar to human plasma, and decreased plasma HDL cholesterol due to the increased clearance of plasma HDL-CE\(^{8}\). They also found that the larger HDL isolated from mice overexpressing human apo F would be a potent acceptor for the efflux of cholesterol from macrophages. These results suggest that apo F plays an important role in lipoprotein metabolism and RCT.

In view of the possible importance of apo F in reverse cholesterol transport, we have developed a new sandwich ELISA and applied it to determine plasma apo F concentration in Japanese healthy and hypertriglyceridemic subjects, and its relation to plasma factors of lipoprotein metabolism.

**Materials and Methods**

**Subjects**

Blood from 397 apparently healthy volunteers (211 males, 186 females) without any medication, at BML Clinical Reference Laboratory (Saitama, Japan), and from 221 hypertriglyceridemic subjects (166 males and 55 females) at Dokkyo Medical University Nikko Medical Center, was collected after overnight fasting. EDTA-plasma was isolated immediately by centrifugation at 4°C and stored at −80°C until use. Lipid profiles are shown in Table 1 and 2. Healthy volunteers were not taking any medication. None of the hypertriglyceridemic subjects, who had hypertension (53\%) and diabetes mellitus (26\%), was taking lipid-lowering medication. In healthy subjects, concentrations of total and LDL cholesterol and TG were greater, and those of apo A-I and apo E were lower in males than in females. In hypertriglyceridemic subjects, there was no significant difference between the genders in the lipid parameters except for the HDL cholesterol level. This study was approved by the ethics committees of Dokkyo Medical University and BML. Informed consent was obtained from all subjects.

**Cloning of Human Apo F and Expression of Recombinant Human Apo F**

Human apo F cDNA was obtained by RT-PCR from mRNA of HepG2 cells as described elsewhere\(^{13}\). Briefly, PCR was carried out using 5'-gactttcgaggtggtgatgtatctcaagactcccagccca-3' as the sense primer and 5'-gacggatcctccctcccgacagaggactgtgaga-3' as the antisense primer for apo F cDNA1 and 5'-gacggatcctccctcccgacagaggactgtgaga-3' as the sense primer and 5'-gacggatcctccctcccgacagaggactgtgaga-3' as the antisense primer for apo F cDNA2\(\). The apo F cDNAs encoded amino acids 165-326 and 1-326 for cDNA1 and cDNA2, respectively. The apo F cDNAs were constructed with a 6 x His tag at the C-terminus. The apo F cDNA1 was separately subcloned into the pQE-30 plasmid (Qiagen) to yield the pQE-30/apo F vector. Escherichia coli JM109 (Toyobo, Tokyo, Japan) bearing the pQE-30/apo F plasmid was cultured in TB medium containing 50 mg/L ampicillin at 37°C. Expression was induced with 1 mM isopropyl thiogalactopyranoside and, after 5 h, the cells were harvested by centrifugation. The cells suspended in phosphate buffer (50 mM sodium phosphate and 0.5 M NaCl, pH 8.0) were disrupted by sonication. The insoluble fraction was pelleted by centrifugation at 30,000 g for 30 min at 4°C, and the supernatant was loaded onto a nickel-nitrilotriacetic...
by Coomassie brilliant blue (CBB) staining, was deter-

1. Males vs. females for healthy subjects:

   - Healthy subjects vs. hypertriglyceridemic subjects for each gender:

   Values shown are the mean ± SD.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Males</th>
<th>Females</th>
<th>All</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apo J (μg/mL)</td>
<td>46.2 ± 6.4</td>
<td>46.0 ± 6.3</td>
<td>46.1 ± 6.3</td>
</tr>
<tr>
<td>Intrinsic CETP activity (% of transfer)</td>
<td>15.5 ± 3.7</td>
<td>13.6 ± 3.2</td>
<td>14.6 ± 3.6</td>
</tr>
<tr>
<td>CETP mass (μg/mL)</td>
<td>2.2 ± 0.5</td>
<td>2.6 ± 0.6</td>
<td>2.4 ± 0.6</td>
</tr>
<tr>
<td>PLTP activity (μmol/mL/h)</td>
<td>7.4 ± 0.9</td>
<td>6.8 ± 0.8</td>
<td>7.1 ± 0.9</td>
</tr>
<tr>
<td>PLTP mass (μg/mL)</td>
<td>14.4 ± 4.6</td>
<td>16.6 ± 2.9</td>
<td>15.4 ± 4.1</td>
</tr>
</tbody>
</table>

Values shown are the mean ± SD.
Males vs. females for healthy subjects: *p < 0.005, **p < 0.0005, ***p < 0.0001; males vs. females for hypertriglyceridemic subjects: healthy subjects vs. hypertriglyceridemic subjects for each gender: *p < 0.0001.

acetic acid agarose column (Qiagen). The recombinant pro-

Preparation of MAbs Against Apo F

Monoclonal antibodies (MAbs) against apo F were obtained as previously described13-16. Balb/c mice were immunized with 25 μg purified rhapo F, and spleen cells from mice were fused with Sp2/0 cells17. The supernatants of hybridoma cells were screened by ELISA using plates coated with purified rhapo F (100 ng/well) and by immunoblotting. Positive hybridoma cells were cloned at least four times by limiting dilu-
thion and injected intraperitoneally into pristane-primed Balb/c mice. The IgG fraction was isolated from ascitic fluid using protein A-Sepharose FF (GE Healthcare) according to the manufacturer’s instructions, dialyzed at 4°C against PBS, and stored at ~80°C. The specifici-

gated anti-mouse IgG (Zymed Laboratories) as the second antibody. Bound antibodies were detected with an enhanced chemiluminescence kit (Perkin-Elmer).
the plate had been washed, 100 μL substrate solution (50 mM citrate-phosphate buffer, pH 5.0) containing 0.4 g/L o-phenylenediamine dihydrochloride and 0.15 mL/L H₂O₂ was added to each well. After 0.5 h, the reaction was stopped by the addition of 100 μL of 2 mol/L H₂SO₄. The absorbance was measured at 492 nm with a microplate reader. Purified bacterial rhapo F and pooled culture medium from CHO-K1 cells served as primary and secondary calibrators, respectively.

When purified rhapo F was added to samples of plasma (n=3) in sufficient amounts to increase the total apo F concentration by 25-100 ng/mL, the final concentrations given by the ELISA averaged 100% (90-116%) of those predicted. The intra- and interassay coefficients of variation of the ELISA were 1.9-3.8% (n=10) and 2.9-7.8% (n=10), respectively. No interference with the ELISA was observed with hemoglobin (5.0 g/L), bilirubin (0.3 g/L), or triacylglycerol (4.25 g/L). Storage of plasma and serum samples at 4℃ for 14 days did not affect the apo F concentration, as determined by ELISA (data not shown).

Assays for CETP, PLTP and Apo J

The intrinsic cholesteryl ester transfer protein (CETP) activity and mass concentration were carried out as previously described¹⁸). Intrinsic CETP activity was determined as the rate of 3H-labeled CE transfer from donor HDL to acceptor VLDL/LDL¹⁹). A plasma sample (95 μL) was incubated with 3H-labeled HDL (5 μL) for 4 h at 4℃ or 37℃. After VLDL/LDL was precipitated with 10 μL of 4% phosphotungstate and 2M MgCl₂ reagent, the radioactivity of the supernatant was measured. Intrinsic CETP activity was calculated as the difference in radioactivity between samples incubated at 37℃ and 4℃ as follows; CETP activity (%)=[(radioactivity at 4℃)−(radioactivity at 37℃)]/(radioactivity at 4℃)×100). Each assay was carried out in triplicate. Intrinsic CETP activity correlated significantly with CETP mass (r=0.180, p=0.0099), LDL cholesterol (r=0.431, p<0.0001) and HDL cholesterol (r=-0.356, p<0.0001).

Phospholipid transfer protein (PLTP) activity, and the mass concentrations of PLTP and apo J were assessed as described elsewhere¹⁶, ²⁰). PLTP activity related positively with its mass concentration (r=0.196, p=0.0047).

Other Laboratory Methods

Plasma total and HDL cholesterol, triacylglycerol, apo A-I and apo B concentrations were measured with a Hitachi 7450 automated analyzer using commercial kits (Sekisui Medical, Tokyo, Japan). HDL
Characterization of Anti-Apo F MAbs

Two MAbs specific for apo F were established: MAb C2A and MAb 10A5. Their specificities were examined by SDS-PAGE and immunoblotting under reducing condition. When human plasma was subjected to SDS-PAGE, MAbs reacted with a single protein (Fig. 1B), the molecular mass of which (33 kDa under reducing conditions) was similar to that previously reported for human apo F. Both antibodies reacted with the purified recombinant human apo F protein, and neither inhibited the interaction of the other to apo F coated on a microtiter plate (data not shown), suggesting that they react with different epitopes of apo F protein.

The distribution of apo F in human plasma was analyzed by two methods, ultracentrifugation and size-exclusion chromatography. After ultracentrifugation of pooled plasma, apo F distributed mainly in the subfractions of LDL and HDL3, partly in HDL2 subfraction by SDS-PAGE and Western blotting (Fig. 2). When human plasma was fractioned by size-exclusion chromatography, and the eluted fractions were analyzed, apo F eluted at a position corresponding to smaller HDL (data not shown).

Statistical Analysis

Results are expressed as the mean ± SD. ANOVA was used for group comparisons. Correlations were analyzed by Spearman’s rank order correlation coefficient. \( P < 0.05 \) was considered significant.

Results

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Standardization of ELISA for Plasma Apo F Concentration

Sandwich ELISA for plasma apo F was established using MAb C2A for capture and biotinylated MAb 10A5 for detection, in which the combination of two MAbs, C2A and 10A5, showed higher absor-
The system showed a dose-dependent response to purified bacterial apo F, to CHO culture medium expressing apo F, and to plasma, and the reactivity was equal to both bacterial and mammalian apo F (Fig. 1). For calibration of the ELISA, purified bacterial apo F was used as the primary calibrator. When subjected to SDS-PAGE and visualized by CBB staining, the purified bacterial apo F showed a single major 33 kDa band (Fig. 1A), in which the purity of protein was >95% of total protein as determined by gel scanning using the Intelligent Quantifier system. The protein concentration of this primary bacterial apo F calibrator, assayed using a bicinchoninic acid protein kit with BSA as calibrator, was typically 54 μg/mL.

To obtain a calibration curve for the ELISA, dilutions of the primary calibrator were made in PBS containing 1 g/L Tween 20 to provide 1.25-25 ng of apo F protein per well (1.25-25 μg/mL). When the apo F culture medium, as a secondary calibrator, was diluted in PBS containing 1 g/L Tween 20 to cover the apo F concentration range 12.5-250 ng/mL, the curve was identical to that obtained with the primary calibrator (Fig. 3). The ELISA had linearity up to 250 ng/mL and was suitable for quantifying apo F concentration as low as 6.25 ng/mL. Linearity was also confirmed with serially diluted plasma or serum samples of several concentrations (18.0 to 248.1 ng/mL) (data not shown). To avoid potential non-linearity caused by very low or high absorbance, the apo F concentrations in plasma samples were measured using several dilutions (1:10 to 1:2,560). At the lowest dilution of 1:10 to 1:20, the results obtained with plasma were not identical to those obtained with the recombinant proteins (Fig. 4). A 100-fold dilution of plasma sample, in which the diluted aliquot gave an absorbance between 0.5 to 1.2 was chosen for routine use.

To avoid any effects of differences between samples in their lipid or apolipoprotein compositions, the detergent Tween 20 was included in the diluent. We examined several detergents for sample dilution using the Detergent Starter Kit II (Wako Pure Chemical Industries) as previously described. Plasma sample diluted (100-fold) and apo F culture medium diluted (10-fold) with PBS containing 1 g/L Tween 20 gave the highest absorbance, but most other detergents gave lower or less absorbance than that in Tween 20; therefore, we chose PBS containing 1 g/L Tween 20 as the sample diluent (data not shown). The intra-
and interassay variation in the ELISA was 1.9-3.8% and 2.9-7.8%, respectively.

**Plasma Apo F Concentrations in Healthy Subjects**

Plasma apo F concentrations in healthy subjects are presented in Table 3. The average apo F concentration was 12.5 ± 3.1 μg/mL, ranging from 6.1 to 22.0 μg/mL in females and from 4.7 to 21.6 μg/mL in males, being higher in females than in males \( p < 0.005 \). In all healthy subjects combined (both sexes pooled), plasma apo F concentrations was positively correlated with HDL cholesterol \( (r = 0.362, p < 0.0001 \text{ for all}; r = 0.452, p < 0.0001 \text{ for males}; r = 0.202, p = 0.0059 \text{ for females}) \) and apo A-I \( (r = 0.383, p < 0.0001 \text{ for all}; r = 0.457, p < 0.0001 \text{ for males}; r = 0.246, p = 0.0159 \text{ for females}) \), and negatively correlated with triglyceride \( (r = -0.221, p < 0.0001 \text{ for all}; r = -0.302, p < 0.0001) \) and apo B \( (r = -0.239, p = 0.0122) \).

**The Relationship of Plasma Apo F Concentration to Lipid Transfer Proteins in Healthy Subjects**

The relationship of plasma apo F with lipid parameters in healthy subjects is shown in Table 4. Plasma apo F concentration was correlated negatively with CE transfer (intrinsic CETP) activity determined using intrinsic substrate \( (r = -0.183, p = 0.0087 \text{ for all}; r = -0.193, p = 0.0454 \text{ for males}) \) but not with CETP mass concentration (Fig. 5). In addition, it was also related positively with PLTP mass concentration \( (r = 0.404, p < 0.0001 \text{ for all}; r = 0.246, p = 0.0004 \text{ for females}) \) and apo J concentration \( (r = 0.246, p = 0.0004 \text{ for all}; r = 0.210, p = 0.0275 \text{ for females}) \) (Fig. 6).

By multiple regression analysis of healthy combined subjects, 32% of variance in intrinsic CETP activity was explained by the covariates of CETP mass, apo F and triglyceride concentration. Of these variables, CETP mass and triglyceride were most strongly associated with intrinsic CETP activity \( (p < 0.0001) \).
Apo F ELISA

Plasma Apo F Concentrations in Hypertriglyceridemic Subjects

We also measured apo F concentrations in hypertriglyceridemic subjects. Apo F concentrations in hypertriglyceridemic subjects were 10.2 ± 3.3 μg/mL for males and 10.7 ± 2.6 μg/mL for females, respectively, and were significantly lower than those of for both), while apo F (p = 0.0191) was also a significant independent contributor in the model; however, apo F was not a significant contributor when analyzed for each gender (p = 0.17 for males and p = 0.0747 for females).

Fig. 5. Relationship of plasma apo F concentration to intrinsic CETP activity (upper panel) and CETP mass concentration (lower panel) in healthy subjects. Correlation coefficients are given under Results. ○, males; ●, females.

Fig. 6. Relationship of plasma apo F concentration to PLTP activity (upper panel) and PLTP mass concentration (lower panel) in healthy subjects. Correlation coefficients are given under Results. ○, males; ●, females.

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healthy controls for each gender ($p=0.0072$ for males and $p=0.0016$ for females, respectively) (Table 3). In hypertriglyceridemic males, but not females, apo F related positively with total cholesterol ($r=0.229$, $p=0.0033$) and LDL and HDL cholesterol ($r=0.180$, $p=0.0209$ and $r=0.340$, $p<0.0001$, respectively), and negatively with triglyceride ($r=-0.193$, $p=0.0132$). Similar to healthy subjects, apo F was positively related with PLTP mass in hypertriglyceridemic male subjects ($r=0.391$, $p<0.0001$ for all; $r=0.430$, $p<0.0001$ for males; $r=0.139$, $p=0.31$ for females).

**Discussion**

There have been few studies of the relation of apo F to lipoprotein metabolism in humans. For a better understanding of the function of apo F, there is a need for reliable and sensitive methods for its quantification. To this end, we prepared a series of MAbs against recombinant apo F expressed by *E. coli* and CHO cells, and used them to develop a sandwich ELISA. By SDS-PAGE and immunoblotting, both MAbs recognized a single protein in recombinant apo F and human plasma of ~33 kDa under reducing conditions, indicating that they reacted with a linear epitope of apo F protein. This is the same as previous reports of plasma apo F.

This ELISA was suitable for assaying plasma apo F concentration in the range of 0.6-25 μg/mL. No differences in the dilution curves were observed between recombinant apo F purified from *E. coli* and CHO cells (primary and secondary calibrators) and human plasma. Intra- and inter-assay coefficients of variation were <5%. Our results indicated that our ELISA is specific, accurate, reproducible and sufficiently sensitive for a wide range of applications. Our assay is more reliable, sensitive and reproducible than previously reported assays, and has a wider range of linearity (0.6-25 μg/mL vs. 0.97-15.56 μg/mL) than the competitive ELISA of Morton *et al.*

Sandwich ELISAs are generally easier to perform with good inter-assay precision than electroimmunoassay and competitive ELISAs, as the latter requires purified apo F.

We used our ELISA to measure the plasma apo F concentration in 397 Japanese healthy and 221 hypertriglyceridemic subjects. No healthy subject was taking lipid-lowering medication. The plasma apo F concentrations we observed in healthy subjects tended to be lower than those observed by others, using an electroimmunoassay, estimated plasma apo F concentration of 27 μg/mL. Morton *et al.* reported that plasma apo F concentration was 83.5 ± 4.8 μg/mL (mean ± SD) in 76 normolipidemic subjects by competitive ELISA using polyclonal antibodies raised against recombinant LTIP-GST fusion protein. Similarly, we have previously observed an inconsistency in quantity. This is probably related at least in part to the relatively high affinity of our calibrator of purified recombinant apo F from CHO cells. A second consideration concerns the dilution of the plasma sample used in the assay studied. In our assay, a 100-fold dilution of plasma was used routinely, whereas in addition to the longer incubation time (overnight), they used a lower dilution (20-fold). Relatively low dilutions with a long incubation might lead to non-specific binding of apo F to the plate, resulting in the true concentration being over-estimated. A non-methodological factor that might have contributed to the differences between the concentrations observed in different studies is that of sample size. The previous study was smaller than ours. Another possibility may be the protein concentration of the calibrator. In our assay, a primary calibrator of rhp F from *E. coli* (the purity of >90%) reacted dose-dependently with rhp F from CHO cells and the plasma sample (Fig. 4); however, they determined the protein concentration by multiple quantitative immunoblotting using the Positope as a calibrator in combination with His-tag antibody. This may overestimate and/or underestimate due to the affinity of the antibody. Our study is also the first in Japanese subjects, raising the possibility of an ethnic effect and/or disease association on apo F concentration; however, the discrepancy should be clarified further.

In the present study, a higher apo F concentration in females than in males was observed in healthy subjects. The opposite observation has been reported in normolipidemic subjects. This may be due to the subjects studied. In the previous report, the studied subjects over 60 years old or with hypertriglyceridemia had lower apo F concentration (56% of normolipidemic subjects) in this study, triglyceride was 2-fold higher in males, and 27% of males had higher triglyceride (>150 mg/dL). Increased triglyceride may partly cause a decrease of apo F levels by increased plasma clearance of apo F. The other possibility was the age of subjects studied. The mean age of subjects was over 60 years old in the previous study. In our study, the higher apo F in females was still significant in the subjects aged over 45 years old (yrs) (11.8 ± 3.7 μg/mL vs. 14.0 ± 3.5 μg/mL, $p=0.0027$) but not in those under 45 yrs (12.1 ± 3.1 μg/mL vs. 12.7 ± 2.6 μg/mL, $p=0.08$). In addition, healthy females below 45 years old (91%) had lower apo F concentration than those over 45 yrs (12.7 ± 2.6 μg/mL vs. 14.0 ± 3.5 μg/mL, $p=0.0250$), but this was not the case in healthy males.
(12.1 ± 3.1 μg/mL vs. 11.8 ± 3.7 μg/mL, $p=0.26$). The effect of the menstrual cycle could also not be excluded. This suggests that age may also influence the plasma apo F level. Furthermore, the result by multivariate analysis that apo F tended to be an independent determinant of intrinsic CETP activity in healthy females explained in part that lower intrinsic CETP activity despite higher CETP mass in females was compatible with higher apo F in females.

The distribution of plasma apo F was confirmed by immunoblotting of the isolated lipoprotein fractions (Fig. 2) and size exclusion chromatography (data not shown). This is consistent with the evidence that plasma apo F exists predominantly in HDLs as a component of apo F-containing HDLs in very small amount and in LDLs in a very small amount. We also observed a correlation between apo F and plasma apo B levels, but this effect was opposite for each gender: a positive relation in females and a negative relation in males. Plasma apo F exists in two forms, one active form bound to LDL and the other inactive form bound in a 470 kDa lipoprotein complex. Both lecithin: cholesterol transferase (LCAT) and CETP activities drive the redistribution of apo F from inactive 470 kDa complex to LDL. Increased plasma triglyceride also enhances the redistribution of apo F from LDL to VLDL and/or remodeling by CETP-mediated lipid transfer processes, resulting in increased plasma clearance of apo F. Taken together, our observation was that the negative association of apo F with apo B in healthy male subjects occurred as a result of the redistribution of plasma apo F by increased plasma triglyceride levels, suggesting that the apo F level may reveal the condition of reverse cholesterol transport. In addition, correlations between triglyceride or LDL cholesterol and apo F levels were also inconsistent in this study, and further study of gender differences or the dyslipidemic status is needed in a larger general population. In these patients, the apo F level may reveal the condition of reverse cholesterol transport.

Furthermore, we observed a positive relationship between apo F and PLTP mass levels in healthy and hypertriglyceridemic male subjects. As described above, plasma apo F in human exists predominantly in the HDLs fraction. In humans, most plasma PLTP associated with HDLs particles exists as an inactive form. The mechanism of inactivation of PLTP remains unclear. By the quantitative trait locus (QTL) analysis in mice, ApoF has been found to be a candidate gene affecting PLTP activity. As both proteins are quite similar in particle size and complex with the same lipoprotein particles, apo F has an inhibitory effect on not only CETP activity but also PLTP activity. It is hypothesized that apo F may interact and complex with PLTP-containing particles, resulting in inactivation of PLTP activity. The precise mechanism should be examined further.

We have developed a reliable and accurate sandwich ELISA for the concentration of plasma apo F. Our ELISA system might be a useful tool to determine the clinical significance in subjects with dyslipidemia, metabolic syndrome and lipoprotein metabolism, including reverse cholesterol transport.

**Conflicts of Interest**

None.

**References**

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