INTRAVASCULAR FREE TISSUE FACTOR PATHWAY INHIBITOR (TFPI) IS INVERSELY CORRELATED WITH HDL CHOLESTEROL AND POST-HEPARIN LIPOPROTEIN LIPASE (LPL), BUT PROPORTIONAL TO APOLIPOPROTEIN A-II (APO A-II)

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ABSTRACT

The distribution of tissue factor pathway inhibitor (TFPI) consisting of free, lipoprotein-bound and endothelial anchoring forms, was studied in 156 patients with coronary artery disease (average age 61.2±9.1: range 32 to 78 years-old) by heparin infusion (50 IU/kg), and compared with the pre-heparin TFPI levels of 229 healthy subjects (average age 59.6±9.4 : range 41 to 80 years-old). In the pre-heparin state, the patients had lower free TFPI and lower high-density lipoprotein cholesterol (HDL.c) levels than those of the healthy subjects with equivalent lipoprotein-bound forms (free TFPI; 15.9±6.5 vs. 19.2± 8.1 ng/ml). In a partial correlation analysis, the free TFPI levels were shown to be inversely correlated with the HDL.c concentrations in both the patients (r=.-454:P<.001) and the healthy subjects (r=.-136:P<.05). In the post-heparin state, the patients generally showed free form levels less than 10%, lipoprotein-bound form levels at 30% and endothelial TFPI at 60%. The patients were divided into four categories by their low density lipoprotein cholesterol (LDL.c:130 mg/dl) and HDL.c (40 mg/dl) levels to specify their coronary risks. The low HDL.c groups had significantly increased intravascular free TFPI levels (including endothelial TFPI) and decreased post-heparin LPL levels, while the high LDL.c groups showed increased levels of lipoprotein-bound TFPI. As a result, in a partial correlation analysis, we found a proportional relation between intravascular free TFPI (including endothelial TFPI) and apo lipoprotein A-II (r=.5327), and between HDL.c and LPL (r=.4906). Intravascular free TFPI was inversely correlated with HDL.c (r=-.4280) and post-heparin LPL (r=-.4791). These findings suggested that increased intravascular free TFPI in patients with low HDL.c may be a compensatory response of the endothelium to prevent atherothrombotic processes.

Key Words

(1) TFPI, (2) HDL cholesterol, (3) Apolipoprotein A-II,

(4) Lipoprotein lipase (lipoprotein lipase), (5) coronary artery disease

Abbreviation

TFPI	Tissue Factor Pathway Inhibitor
Lp-TFPI	Lipoprotein-bound TFPI
LDL.c	Low-density Lipoprotein Cholesterol
HDL.c	High-density Lipoprotein Cholesterol
CAD	Coronary Artery Disease
LPL	Lipoprotein Lipase
HL	Hepatic Lipase
Apo A-II	Apolipoprotein A-II

INTRODUCTION

Tissue factor pathway inhibitor (TFPI) plays an important role in the anti-thrombotic properties of vessel walls by inhibiting extrinsic coagulation processes **(1-2).** Intravascular TFPI consists of three major pools except in platelets **(3)**, i.e., the free form without carrier, lipoprotein-bound form **(4)** and endothelial anchoring form **(5)**. An exogenous administration of recombinant TFPI to experimental animals prevents thrombosis **(6)** while increased TFPI levels have been observed in patients with acute myocardial infarction **(7)**. Although the alteration of serum lipids by cholesterol lowering therapy influences lipoprotein-bound TFPI profiles **(8-11)**, little is known about the clinical implications of the TFPI level and its regulation in relation to lipid risk profiles.

In the present study, we compared TFPI subfractions in pre-heparin plasmas of normal healthy subjects and patients with coronary artery disease (CAD). Significant negative correlations were observed between the free TFPI and high density lipoprotein cholesterol (HDL.c) concentrations in both of these groups. Accordingly, we studied the TFPI distribution including endothelial TFPI levels in CAD patients divided according to their different phenotypes of dyslipidemias, especially hypercholesterolemia (high low density lipoprotein cholesterol: LDL.c) and low HDL cholesterolemia to evaluate the patients' coronary risks. Although lipoprotein-bound TFPI constitutes the major part of circulating TFPI, endothelial TFPI released by heparin is physiologically the most important form. Because the amount of endothelial TFPI is the still greater than that of lipoprotein-bound form in vessels and mediates the major antithrombotic activity as free form **(12-13)**. Endothelial TFPI is believed to bind to heparan sulfate on the cell surface, as does lipoprotein lipase (LPL) **(14)**, which regulates the LDL and HDL fractions by hydrolyzing triglyceride-rich lipoproteins (TGRL) and by producing LDL particles or a surface coat of

HDL particles **(15)**. Consequently, we found that the level of intravascular free TFPI (including endothelial TFPI) was increased in the patients with low HDL.c concentration, and significantly proportional to apolipoprotein A-II (apo A-II) levels and negatively correlated with post-heparin LPL mass. These results suggest the close relation between antithrombotic aspect and lipid metabolism on vascular beds, giving new insight into risk profiles for atherothrombosis in vivo.

METHODS

Subjects

One–hundred and fifty-six patients with CAD, aged 32 to 78 years, were recruited for the study. These patients were referred to and admitted to the National Cardiovascular Center Hospital (Osaka, Japan) because of stable symptoms or suspected CAD. All patients had no hepatic or renal dysfunction, but anti-anginal drugs had been administered such as nitrates (47.8%), calcium antagonists (46.6%), β-blockades (29.2%) and aspirin (44.1%). The patients who had been administered lipid-lowering drugs, those with a triglyceride (TG) level more than 4.5 mmol/L (400 mg/dl), and those with familial hypercholesterolemia or type III hyperlipidemia were excluded from this study. All patients were examined by coronary angiography to evaluate the severity of coronary atherosclerosis. Blood samples were obtained during the diagnostic catheterization procedure in the early morning. Pre-heparin plasma samples were collected from a femoral artery just after the insertion of the sheath for catheter manipulation. Post-heparin plasma samples were obtained 10 minutes after an intravenous injection of unfractionated standard heparin (Novo-Nordisk A/S, Denmark) with a bolus of 50 IU/kg body weight. The blood samples were drawn into evacuated tubes containing 0.38% trisodium citrate (1:9 vol/vol) and disodium EDTA (0.1%), immediately placed in refrigerator and centrifuged at 3,000g at 4 ℃ within 1 hour to obtain platelet-poor plasma. Aliquots were stored at –80 ℃ until assay.

As control subjects, 229 normal volunteers, aged 40 to 81years, were selected from the population-based prospective cohort in the district of Ise (Kisei-cho, Mie Prefecture in Japan). Serum lipids and TFPI were measured without heparin infusion. Blood samples were obtained after the subjects had fasted overnight, from an anticubital vein into evacuated tubes. Informed consent was obtained from all patients and healthy subjects for the blood sampling and measurements.

Classification

To evaluate their coronary risk according to their lipid profiles, the CAD patients were divided into four categories according to their LDL cholesterol (LDL.c) and HDL cholesterol (HDL.c) levels **(Fig. 1)**: the normal group (a), low HDL.c group (b), low HDL.c and high LDL.c group (c), and high LDL.c group (d). The cut-off values for the LDL.c and HDL.c levels were 3.34 mmol/L (130 mg/dl) and 1.03 mmol/L (40 mg/dl), respectively. HDL.c was plotted as reciprocal values (1/HDL.c) as a negative risk factor. This classification according to lipid risk can be used to evaluate the independent contributions of the two confounding variables of HDL.c and LDL.c to atherosclerosis. A simple factorial analysis of variance (two-way ANOVA) was applied to determine the separate abilities of HDL.c and LDL.c levels to affect the dependent variables.

Measurements of lipids and risk

The serum total cholesterol (TC) and triglyceride (TG) levels of pre- and post-heparin plasma samples were determined by enzymatic methods **(16-17)** with the reagents commercially supplied by Daiichi Kagaku (Tokyo, Japan) for cholesterol and by Nihon Shoji (Osaka) for TG using an auto-analyzer (COBAS MIRA plus, Nihon Roche, Tokyo). HDL.c was determined in the supernatant after heparin-manganese precipitation of the apo B-containing lipoproteins **(18)**. Apolipoproteins A-1, A-II, B, C-II, C-III and E were measured immunoturbidimetrically with the use of kits (Daiichi Kagaku) **(19)**. LDL.c was calculated by Friedewald's formula **(20)**. In the patients, blood pressure and body-mass index (BMI : body weight divided by height in square meters) were measured, and alcohol and smoking habits were also documented based on a standard questionnaire.

TFPI and LPL antigen determination

Both the total and free TFPI concentrations were measured by a one-step sandwich enzyme-linked immunosorbent assay (ELISA) method using "total" and "free" TFPI reagent kits (Kaketsuken, Kumamoto, Japan) in both pre-heparin and post-heparin plasma **(21)**. Monoclonal anti-TFPI antibodies for Kunitz 3 domain (K9) and for the tertiary structure of first and second Kunitz domains (K270) were prepared to detect the free and total TFPI levels, respectively. The amount of lipoprotein–bound TFPI and endothelial TFPI were calculated by subtracting free TFPI concentration from total TFPI concentration and by subtracting the total TFPI concentration in the pre-heparin plasma from total TFPI concentration in the post-heparin plasma, respectively. Intravascular free TFPI consists of endothelial TFPI released by heparin and circulating free TFPI in pre-heparin plasma, because endothelial anchoring TFPI is detected as "free" form after a heparin infusion. However, post-heparin free TFPI is different from intravascular free TFPI, because part of the heparin releasable TFPI from endothelium newly binds to lipoprotein as described in the result.

The plasma LPL mass was measured by a sandwich enzyme immunoassay (EIA) using commercial reagent kits (Daiichi Kagaku) **(22)**. The endothelial LPL level was calculated by subtraction of the pre-heparin LPL concentration from the post-heparin LPL concentration. Regarding these LPL levels, the intravascular LPL concentration was equal to the post-heparin LPL concentration.

Statistical analysis

Statistical analysis was performed using SPSS software (SPSS Inc, Chicago, IL). Two-tailed Student's t test was applied for the differences between the control subjects and CAD patients, except for the gender distribution (chi square test). Comparisons of the

dyslipidemic categories were made by a simple factorial analysis of variance (two-way ANOVA) to determine the differences in clinical parameters, where HDL and LDL cholesterol were the two independent factors. Significant differences of the main effect of each factor was examined after that of their interaction. Partial correlation coefficients was calculated for the pre- and post-heparin TFPI, LPL, lipids and apolipoprotein levels. The TG concentrations were logarithmically transformed because of their skewed distribution. P values less than 5 % were considered significant.

RESULTS

Comparison of serum lipid, apolipoprotein and TFPI levels in control subjects and patients

Figure 1 shows a scatter gram of the LDL.c and HDL.c levels of the healthy subjects and CAD patients. The HDL.c levels of the patients were clearly shifted to the lower range characterizing lipid profiles of patients at risk for atherosclerosis ; 80 patients (51%) had HDL cholesterol levels in the range less than 1.03mmmol/L (40 mg/dl). The CAD patients had significantly lower serum lipids and apolipoprotein levels compared to those of the control subjects, except for the apo C-II levels **(Table 1)**. However, the apo A-II level divided by HDL.c was higher in the patients group than in the healthy subjects, indicating relatively increased apo A-II rich HDL particles in the CAD patients.

Both the free and total TFPI concentrations were significantly lower in the patients than in the control subjects. The lipoprotein-bound TFPI concentrations were not significantly different between the patients and control subjects. The free (TFPI) / total (TFPI) ratios and Lp-bound (TFPI) / total (TFPI) ratios showed that the TFPI in the control group was about one-fourth (25%) the free form and 75% the lipoprotein-bound form. The TFPI of the patients was about one-fifth (20%) the free form and 80% the lipoprotein-bound form.

Partial correlation coefficients in control subjects and patients

The free TFPI levels were inversely correlated with the HDL.c levels (**Table 2**) in both the healthy subjects (r=-.1358; P<.001: **Fig. 2a**) and the CAD patients (r=-.4540; P<.001: **Fig.2b**). In the control subjects, HDL.c was correlated with apo A-I (r=.7895: P<.001) and apo A-II (r=.3054: P<.001), while in the patients, HDL.c was weakly correlated with apo A-I (r=.1898: P=.026), but no longer correlated with the apo A-II level (r=-.0638). Despite the

negative correlation of free TFPI with HDL.c, the free TFPI concentration was positively correlated with the apo A-II level in both groups, more so in the patients. Although the circulating free TFPI was related to apo A-II rather than apo A-I in both the patients and the control subjects, the patients who had low HDL.c levels had a stronger correlation between free TFPI and apo A lipoproteins (apo A-I and apo A-II). In only the control group, free TFPI was correlated with the triglyceride level (r=.254; P<.001) and LDL.c level (r=.1669; P<.01).

The lipoprotein-bound TFPI level was found in LDL fraction as the major part in the control subjects (r=.4587; P<.001) and the patients (r=.4623;<.001). In the control group, the HDL fraction (r=.3152;P<.001) significantly contributed to the binding of lipoprotein-bound TFPI. In the patients, in contrast, an explanatory linkage of lipoprotein-bound TFPI and the HDL fraction was not observed.

Different forms of TFPI associated with dyslipidemic profiles

The patients were divided by their LDL.c and HDL.c levels into four categories (**Table 3**). The low HDL.c groups (b and c) had higher BMI and lesser alcohol consumption values than those of the patients without low HDL.c (a and d). There was no significant difference in smoking status among the groups. LDL.c and HDL.c contributed to the significant differences of TC, apo A-I and apo B between the patients with low HDL.c (b and c) and high LDL.c (c and d) without any secondary interaction. Although the apo A-II levels were lower in the high LDL.c groups, the apo A-II / HDL.c ratio was significantly elevated in the low HDL.c groups. Apo C-II levels were also higher in the low HDL.c patients than in the patients without low HDL.c.

The heparin infusion (50 IU/kg) caused marked increases in the circulating free TFPI, from 7.7- to 11.1-fold and LPL concentrations from 12- to 16- fold in the pre-heparin plasma **(Table 4)**.

The low HDL.c groups were characterized by significantly higher free TFPI and reduced LPL levels in both pre-heparin and post-heparin plasma compared to the high LDL.c groups. Accordingly, the endothelial TFPI values calculated were also increased in the low HDL.c groups compared to the high LDL.c groups, while the endothelial LPL levels were significantly lower in the low HDL.c groups (P<.005) and higher in the high LDL.c groups (P<.01) compared to those of the other groups.

As expected, the lipoprotein-bound TFPI levels in both the pre-heparin and post-heparin plasma were increased in the groups with high LDL.c. However, the total TFPI level in post-heparin plasma was higher in the low HDL.c groups compared to that in the high LDL.c groups, because of the higher endothelial TFPI released by heparin infusion in the low HDL.c groups. Interestingly, the lipoprotein-bound form in the post-heparin plasma was increased by about 10 to 20 ng/ml compared to that in the pre-heparin plasma in all groups. Newly detected lipoprotein-bound TFPI represented by δ-Lp-TFPI was significantly higher in the high LDL.c groups (P<.005). Consequently, the intravascular TFPI in the patients was less than 10% circulating free TFPI, 30% lipoprotein-bound form and 60% endothelial anchoring form, regardless of dyslipidemias. The circulating free TFPI levels (in pre-heparin plasma) was about 10 to 14% of the endothelial TFPI.

Partial correlation coefficients among TFPI, LPL and lipids in the patients group

Based on the partial correlation analysis of the patients **(Table 5**), the free TFPI levels in pre-heparin plasma were shown to be significantly related to the intravascular free TFPI concentration (r=.6748; P<.001), while pre-heparin LPL showed a weak association with post-heparin LPL levels (r=.3473; P<.001). Similar to the results shown by the dyslipidemic classification, the HDL.c concentration was inversely correlated with the free TFPI levels and positively correlated with LPL in both pre-heparin and post-heparin plasmas.

Although the apo A-I and apo A-II levels were well correlated with each other (r=.6458; P<.001), only apo A-I was associated with the HDL.c level (r=.2009; P<.01). These two types of apolipoproteins were positively correlated with free TFPI levels (circulating and intravascular) and were negatively correlated with intravascular LPL, where apo A-II was much more strongly correlated than was apo A-I in both cases.

Significant correlations were observed between intravascular free TFPI and apo A-II (r=.5327; P<.001) **(Fig. 3a)** and between post-heparin LPL and HDL.c (r=.4906; P<.001), whereas significant inverse correlations were documented between intravascular free TFPI and post-heparin LPL (r=-.4791; P<.001) **(Fig. 3b)**, between intravascular free TFPI and HDL cholesterol (r=-.4280; P<.001), and between post-heparin LPL and apo A-II (r=-.3678; P<.001).

DISCUSSION

To elucidate the intravascular TFPI distribution in the present study, it was necessary to discriminate the free form and lipoprotein-bound form of TFPI, because of their different anticoagulant properties **(7-8).** The heparin infusion enable to measure the total intravascular TFPI including the endothelial anchoring form, which was 7.7 to 11.2 times more than the free TFPI level in the pre-heparin plasma, as previously reported **(5)**. The intravascular TFPI of the present CAD patients generally consisted of less than 10% of the circulating free form (6-9%), 30% of the lipoprotein-bound form (27-32%) and 60% of the endothelial anchoring form (60-64%) regardless of patients' dyslipidemic profiles. In addition, intravascular free TFPI was strongly correlated with pre-heparin free TFPI (r=.6748), indicating a certain physiological equilibrium between the endothelial TFPI and circulating free TFPI. In contrast, the pre-heparin LPL concentration was not a good predictor of the intravascular LPL levels (r=.3709).

In our study, the patients with CAD had serum lipid levels that were lower than those of the control subjects, suggesting that the stable diet control during hospitalization might have affected the lipid profiles of the patients. However, a critical difference between the healthy subjects and patients was present in the HDL.c level, which may be the best indicator for CAD rather than hypercholesterolemia in the Japanese population. We observed the inverse correlation between free TFPI and HDL cholesterol in the pre-heparin plasma (r=-.1358) of the healthy subjects (Fig. 2a), and in the pre-heparin (r=.-4547) and post-heparin plasma of the CAD patients (r=-.4280: Fig. 2b). In the dyslipidemic classification, the pre-heparin free TFPI and endothelial TFPI levels were significantly increased in the groups of the patients with low HDL.c concentration compared with the other groups (Table 4). Hansen et al. **(10)** reported that pre-heparin plasma TFPI activity of 14 patients with familial

hypercholesterolemia was not associated with HDL.c or apo A-I. But they did not discriminate between TFPI activity and antigen, and mixed data at base-line and after different lipid-lowering therapy. Cella et al. **(23)** reported a positive relation between HDL.c and total TFPI antigen in post-heparin plasma (20 IU/kg of heparin), analyzing a small number of heterogeneous subjects (12 hospitalized patients with marked obesity (mean BMI 41.4) and 14 normal subjects). Although the reason for the difference in findings between that study and ours is unclear, it may be associated with the discrimination of free and lipoprotein-bound forms and the interpretation of TFPI response dependent on the study population. We demonstrated that symptomatic CAD patients had free TFPI levels lower than those of normal healthy subjects. This result indicates a reduction of intravascular free TFPI in the atherogenic vascular milieu, except for the lipoprotein-bound form which is dependent on cholesterol levels. However, the negative correlation between the free TFPI and HDL.c indicated different aspects of the regulation of TFPI concentration, i.e. a compensatory augmentation of free TFPI levels produced by endothelial cells against the atherogenic vascular milieu. Increased TFPI levels in patients with acute myocardial infarction **(7)** may be evidence of such an endothelial compensation.

Lesnik et al. **(24)** reported elevated TFPI activity in small dense LDL and HDL fractions. Moor et al. **(25)** also described that HDL_{3b} was associated with TFPI in patients with CAD. A low HDL.c concentration is generally associated with the presence of small and dense HDL particles, because of the shrinkage of HDL cycles between HDL_2 and HDL_3 **(13)**. Small and dense HDL particles are enriched in apo A-II. Although apo A-I was positively correlated with HDL.c in the present CAD patients, apo A-II was no longer correlated with HDL.c in them. Among the patients with low HDL.c (80 patients), apo A-II was inversely correlated with HDL.c levels (r=-.291; P=.009), indicating that the lower the HDL.c is, the more enriched apo A-II is present in the CAD patients with low HDL.c. This idea may be

supported by the findings that the apo A-II / HDL.c ratio was significantly higher in the CAD patients compared to the healthy subjects (Table 1), and the apo A-II/HDL.c ratio of the patients with low HDL.c was higher than that of the patients without low HDL.c (Table 3). TFPI bound to HDL particles is thought to covalently bind to apo A-II which was cleaved by reduction, suggesting a disulfide-bond **(26-27)**. However, we found that the apo A-II levels were significantly correlated with free TFPI, not with the lipoprotein-bound form. In addition, the apo A-II and free TFPI levels were in complete opposition to the HDL.c levels in the control subjects and the patients, especially in the post-heparin plasma of the patients **(Fig. 2b)**.

Since HDL particles have several protective functions for vessels against injury **(28-29)**, anti-atherogenic properties should be reduced in a low HDL.c milieu. When the augmentation of anti-thrombotic activity is required, endothelial cells may be forced to generate TFPI and compensate for the consumption of free TFPI in response to certain signals such as thrombin **(30)** or other inflammatory mediators **(31)**. From this point of view, elevated free TFPI in a certain population could be a risky aspect as indicated by the inverse relationship between HDL.c and free TFPI in the present CAD patients. The negative correlation between free TFPI and HDL.c was stronger in the symptomatic patients than in the healthy subjects, and in the patients with low HDL.c than in the patients without low HDL.c.

The metabolic state of the low HDL.c level was associated with an increased clearance of apo A-I and apo A-II **(32)**, presumably due to an overdriving reverse cholesterol transport system. Since the HDL.c concentration was shown to be proportional to the LPL levels as in a previous study **(33)**, LPL may also be processed in the low HDL.c situation, reflected by the negative correlation of LPL with intravascular TFPI. It is possible that increased TFPI shares the binding sites of heparan sulfate proteoglycans, where LPL is replaced by TFPI,

resulting in the negative correlation between the two parameters **(Fig. 3b)**.

Apo A-II has been reported to have the ability to activate hepatic lipase (HL) **(34)**, another key enzyme of triglyceride hydrolysis, and HL was shown to be inversely correlated with HDL_2 **(35).** It is also conceivable that free TFPI from endothelium binds to apo A-II enriched HDL particles on the way of HDL metabolism. However, the correlation between intravascular free TFPI and apo A-II indicates some interrelation between TFPI and apo A-II or some function of apo A-II beyond that as a binding mediator of TFPI to HDL particles, since it correlates with free TFPI.

The present study also revealed the novel finding that about 10% (8-15%) of endothelial TFPI (10 to 20 ng/ml) was bound to lipoproteins, a level which was significantly higher in the patients with high LDL.c, suggesting the instant interaction between TFPI and lipoproteins. TFPI synthesized in the endothelium is believed to bind to heparan sulfate proteoglycans on the cell surface, where LPL also enhances the binding of lipoproteins **(36)**. It is possible that part of the endothelial TFPI is physiologically processed to lipoproteins mediated by LPL on the cell surface, forming lipoprotein-bound TFPI. Although the lipoprotein-bound TFPI was significantly correlated with LDL.c levels in both the healthy and CAD patient groups of the present study, it was no longer linked with the HDL fraction in the patients **(Table 2)**, because of their reduced HDL.c concentrations. The pre-heparin free TFPI levels were positively correlated with the triglyceride concentrations in only the control group **(37)**, not in the patients with CAD as previously reported **(15).** This means that the contribution of triglycerides to TFPI levels is not ubiquitous and depends on the lipid regulation in a given study population.

Taken together, the present findings indicate that the levels of intravascular TFPI (including lipoprotein-bound form) is influenced by lipid metabolism as presented by a characteristic constellation involving the TFPI, apo A-II, HDL.c, LDL.c and LPL levels. The

lower the HDL cholesterol concentration is, the higher the intravascular free TFPI level is. However, the intravascular free TFPI level should be interpreted as a biphasic phenomenon, whether it is decreased or not, and whether it is compensated or not when TFPI is not reduced. Although the event leading to this constellation of high TFPI and low HDL.c remain to be elucidated, it appears that increased intravascular free TFPI in a risky state may be a compensatory response of the endothelium, which clearly plays a crucial role in the regulation of not only anti-thrombotic properties, but also the lipoprotein metabolism mediated by heparan sulfate proteoglycans.

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Table 1 Comparison between the patients with coronary artery disease and the healthy control subjects

	Patients	Control subjects
Number (M/F§)	156(122/34)	229(70/159)
Age (yr)	61.2±9.1	59.6±9.4
BMI (kg/m^2)	23.7±3.0	23.3±3.1
TC (mmol/L) §	4.85±1.00	5.99±1.01
TG (mmol/L)	1.25±0.65	1.35±0.86
LDL.c (mmol/L) §	3.18±0.87	3.93±0.96
HDL.c (mmol/L) §	1.08±0.36	1.45±0.32
Apo A-I (mg/dl) §	135.0±32.1	182.6±38.9
Apo A-II (mg/dl) *	33.9±10.7	36.3±6.5
Apo B (mg/dl) *	116.6±32.0	124.9±30.0
Apo C-II (mg/dl)	5.2±2.7	5.8±2.5
Apo C-III (mg/dl) §	10.4±4.6	13.2±4.6
Apo E (mg/dl) §	4.8±1.4	6.2±1.8
Apo A-II/HDL.c ratio §	0.92±0.44	0.67±0.15
Free TFPI (ng/ml) §	15.9±6.5	19.2±8.1
Lp-bound TFPI (ng/ml)	61.2±18.7	62.0±19.2
Total TFPI (ng/ml) *	77.1±20.5	81.2±19.1
Free/total ratio (%) ‡	21.0±7.7	24.5±10.8
Lp-bound/total ratio (%) ‡	79.0±7.7	75.5±10.8

Data are mean ± SD. Two-tailed Student's t test was applied to determine the difference of parameter averages except for the gender distribution (chi square test). TG was transformed logarithmically at analysis. *<.05, ‡ <.005, §<.001.

Abbreviations: M , male; F, female; yr; year-old; BMI; body mass index, TC; total cholesterol, TG; triglyceride, HDL.c; high-density lipoprotein cholesterol, LDL.c; low-density lipoprotein cholesterol, Apo; apolipoprotein. Free (Lp-bound) / total ratio means the free (lipoprotein-bound) TFPI/total TFPI ratio.

Table 2 Partial correlation coefficients

	Control subjects		Patients with CAD	
	Free TFPI	**Lp-bound TFPI**	**Free TFPI**	**Lp-bound TFPI**
LDL.c	.1669 †	.4587 §	-.0930	.4623 §
Log [TG]	.2540 §	.0029	.0076	-.0530
HDL.c	-.1358 *	.3152 §	-.4540 §	.0030
Apo A-I	.0840	.1315	.2544 ‡	-.1310
Apo A-II	.1635 †	.1550 *	.4275 §	-.1608

Partial correlation coefficients among circulating free TFPI, lipoprotein-bound TFPI, lipids (LDL.c, HDL.c and logarithmically transformed TG), apo A-I,A-II, B, C-II, C-II and E in pre-heparin plasma were calculated. *<.05, †<.01, ‡ <.005, §<.001.

Abbreviations: Lp-bound TFPI; lipoprotein-bound TFPI, TG; triglyceride, HDL.c; high-density lipoprotein cholesterol, LDL.c; low-density lipoprotein cholesterol, Apo; apolipoprotein.

Table 3. Clinical characteristics of the CAD patients by dyslipidemic classification

	a	b	c	d		
	Normal	**Low HDL.c**	**High LDL.c Low HDL.c**	**High LDL.c**	**HDL.c (sig. P)**	**LDL.c (sig. P)**
No.	39	54	26	37		
Age (yr)	60.1±9.7	63.5±7.9	57.1±10.5	62.0±8.5	Ns	Ns
BMI (kg/m^2)	23.3±2.9	24.4±3.2	24.3±2.3	22.8±2.7	<.05	Ns
Cigarettes (/day)	28.6±18.0	27.1±18.5	26.8±15.9	21.7±19.3	Ns	Ns
Alcohol (mg/day)	29.0±35.8	8.5±17.6	14.6±19.4	34.7±58.9	<.01	Ns
TC (mmol/L)	4.62±0.61	4.05±0.69	5.69±0.95	5.68±0.60	<.05	<.001
TG (mmol/L)	1.16±0.71	1.31±0.80	1.22±0.40	1.26±0.49	Ns	Ns
HDL.c (mmol/L)	1.40±0.33	0.79±0.15	0.83±0.12	1.34±0.25	<.001	Ns
LDL.c (mmol/L)	2.69±0.46	2.61±0.38	4.30±0.92	3.76±0.46	Ns	<.001
Apo A-1 (mg/dl)	146.0±39.	133.5±29.6	122.6±23.6	134.2±29.0	<.05	<.05
Apo A-II (mg/dl)	35.4±11.7	34.7±10.6	32.0±11.7	32.4±8.9	Ns	<.05
Apo B (mg/dl)	101.8±26.	113.4±30.3	143.8±40.4	117.9±20.1	<.001	<.001
Apo C-II (mg/dl)	4.5±2.9	5.8±3.0	5.6±2.12	5.00±2.25	<.05	Ns
Apo C-III (mg/dl)	10.4±4.9	9.4±5.0	10.5±3.9	11.7±3.9	Ns	Ns
Apo E (mg/dl)	4.88±1.33	4.50±1.85	5.15±1.13	5.06±0.60	Ns	Ns
Apo II/HDL.c ratio	0.70±0.26	1.20±0.46	1.04±0.48	0.64±0.20	<.001	Ns

Data are mean ± SD. See the text for the method of classification. A simple factorial analysis of variance (two-way ANOVA) was performed to determine differences in parameters. Significant differences were expressed by the main effects of HDL.c and LDL.c as the independent variables. The triglyceride values were logarithmically transformed. Cigarettes; cigarettes consumption per day, alcohol; alcohol consumption per day.

Abbreviations: TC; total cholesterol, TG; triglyceride, BMI; body mass index, HDL.c; high-density lipoprotein cholesterol, LDL.c; low-density lipoprotein cholesterol, Apo; apolipoprotein.

Table 4. TFPI profile of the pre-heparin and post-heparin plasma of the CAD patients by dyslipidemic classification

Group :	a	b	c	d		
	Normal	Low HDL.c	High LDL.c Low HDL.c	High LDL.c	HDL.c (sig. P)	LDL.c (sig. P)
Pre-heparin						
Free TFPI (ng/ml)	14.2±7.1	19.2±5.9	17.0±6.7	15.7±20.8	<.001	<.05
Lp-bound TFPI (ng/ml)	60.1±16.4	52.9±12.2	73.7±28.9	64.5±16.5	Ns	<.001
Total TFPI (ng/ml)	74.4±20.7	72.1±14.3	90.6±30.1	80.2±20.6	<Ns	<.005
Free/total ratio (%)	19.0±5.3	26.9±7.2	19.7±8.6	18.0±12.5	<.001	<.001
Lp/total ratio (%)	81.1±5.4	73.4±7.2	80.3±8.3	84.0±5.0	<.005	<.005
LPL (ng/ml)	13.4±3.0	11.7±3.0	12.3±3.4	13.2±2.9	<.05	Ns
Post-heparin						
Free TFPI (ng/ml)	122.7±47.8	152.9±52.3	139.8±38.9	118.2±32.4	<.005	Ns
Lp-bound TFPI (ng/ml)	71.4±20.1	61.9±18.3	89.7±29.9	86.7±24.8	Ns	<.001
Total TFPI (ng/ml)	194.1±5.3	214.2±57.4	229.5±50.3	205.0±38.1	<.05	Ns
Free/total ratio (%)	61.8±8.8	69.7±10.6	60.8±9.1	56.4±9.3	<.001	<.001
Lp/total ratio (%)	38.3±9.1	30.7±10.4	39.4±9.0	43.6±6.9	<.005	<.001
LPL (ng/ml)	192.4±78.8	130.1±65.9	175.7±68.4	204.7±59.1	<.001	<.05
Difference between pre- & post-heparin plasma						
δ-Lp-TFPI(ng/ml)	9.30±18.6	14.2±14.4	22.7±20.4	20.0±17.6	Ns	<.005
EC-TFPI (ng/ml)	119.8±49.1	144.8±51.0	138.9±49.1	124.8±31.6	<.05	Ns
EC-LPL (ng/ml)	179.5±78.9	123.6±61.0	164.4±69.0	192.2±59.0	<.005	<.01

Data are mean ± SD. A simple factorial analysis of variance (two-way ANOVA) was applied to determine differences in parameters, as in Table 3.

δ Lp-bound TFPI means newly lipoprotein-bound TFPI after heparin infusion.

Abbreviations ; LPL: lipoprotein lipase, EC-TFPI (LPL); endothelial TFPI (LPL).Other abbreviations are as in Table 3.

Table 5 Partial correlation coefficients among TFPI, LPL, lipids and apolipoproteins

	Intravasc. free TFPI	Pre-free TFPI	PHP LPL	Pre-LPL	HDL.c	LDL.c	Apo A-I
Pre-free TFPI	.6748 §						
PHP-LPL	-.4791 §	-.3984 §					
Pre-free LPL	-.1658	-.2125 *	.3473 §				
HDL.c	-.4280 §	-.4547 §	.4906 §	.3709 §			
LDL.c	-.0923	-.0901	.1962 *	-.0057	-.0325		
Apo A-I	.3311 §	.2613 †	-.2937 ‡	.0185	.2009 *	-.0782	
Apo A-II	.5327 §	.4357 §	-.3678 §	-.0785	-.0568	-.0923	.6458 §

A partial correlation coefficient analysis was applied to the parameters of pre-heparin free TFPI, intravascular free TFPI, pre-heparin LPL, post-heparin (PHP) LPL, pre-heparin lipoprotein-bound TFPI, LDL cholesterol, HDL cholesterol, and apolipoproteins A-I, A-II, B, C-II, C-III, and E. “Intravasc. free TFPI “ means the circulating free TFPI in pre-heparin plasma and endothelial TFPI. “Pre-free TFPI” means the pre-heparin free TFPI concentration. *<.05, †<.01, ‡ <.005, §<.001.

LEGENDS

Fig 1: Scatter diagram shows the relation between LDL cholesterol and 1/HDL cholesterol in 229 healthy subjects (○) and 156 patients with coronary artery disease (▲). All subjects were divided according to their LDL and HDL cholesterol levels into four categories: **(a)** normal lipid profile, **(b)** low HDL cholesterolemia, **(c)** low HDL cholesterolemia and high LDL cholesterolemia and **(d)** high LDL cholesterolemia. The cut-off levels (dotted lines) of LDL cholesterol and HDL cholesterol were 130 mg/dl (3.34 mmol/L) and 40 mg/dl (1.03 mmol/L), respectively.

Fig 2: (a) Scatter diagram of HDL cholesterol and pre-heparin free TFPI in the 229 healthy subjects. An inverse relation (r=-.1358) was observed between the HDL cholesterol concentration and circulating free TFPI level in pre-heparin plasma (Table 2). **(b)** Scatter diagram of HDL cholesterol and pre-heparin free TFPI in the 156 CAD patients. An inverse relation (r=-.4587) was revealed between the HDL cholesterol concentration and circulating free TFPI level in pre-heparin plasma (Table 2).

Fig 3: (a) Relation between apo A-II and free TFPI in intravascular free TFPI in the 156 CAD patients (r=.5327) (Table 5) **(b)** : Inverse relation between post-heparin LPL and intravascular free TFPI in the 156 CAD patients (r=-.4791) (Table 5).

Figure 1:

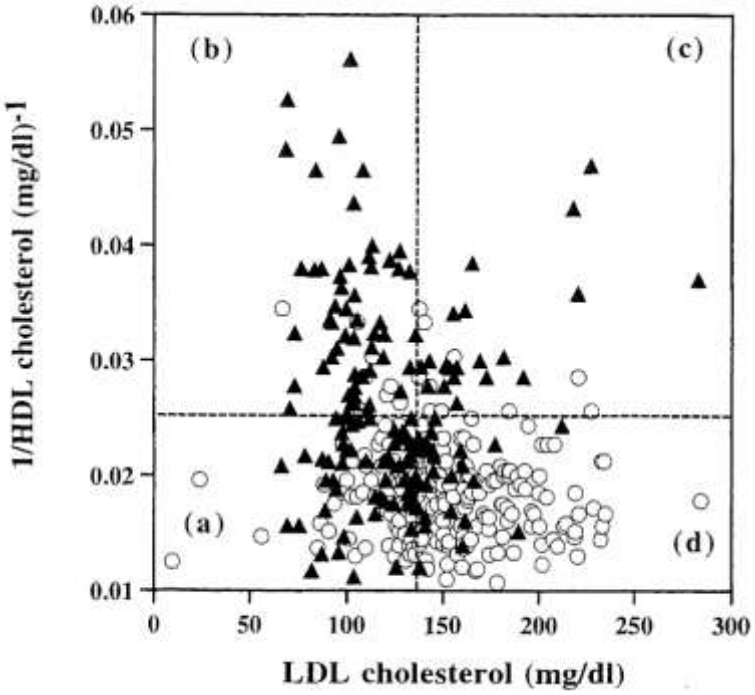


Figure 2:

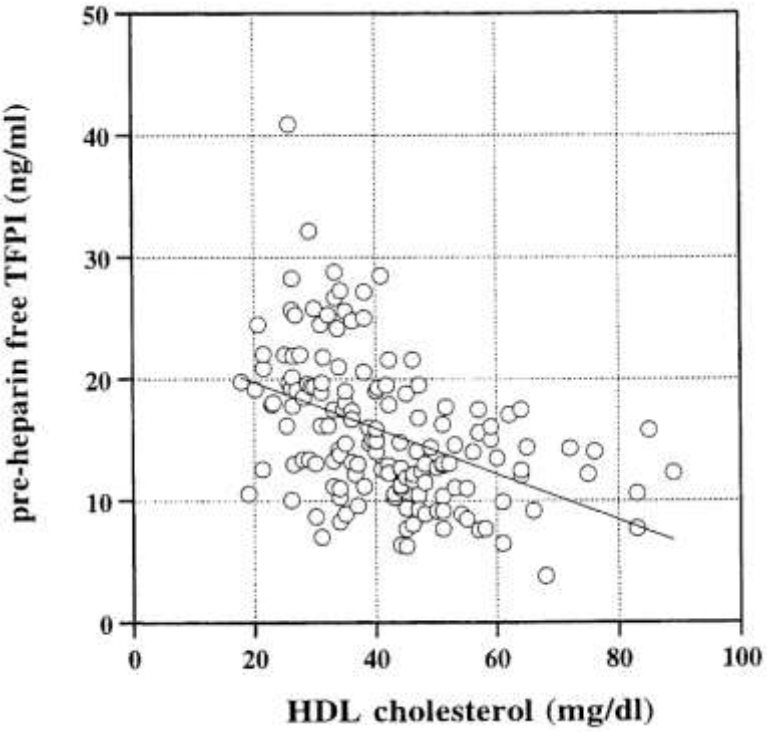
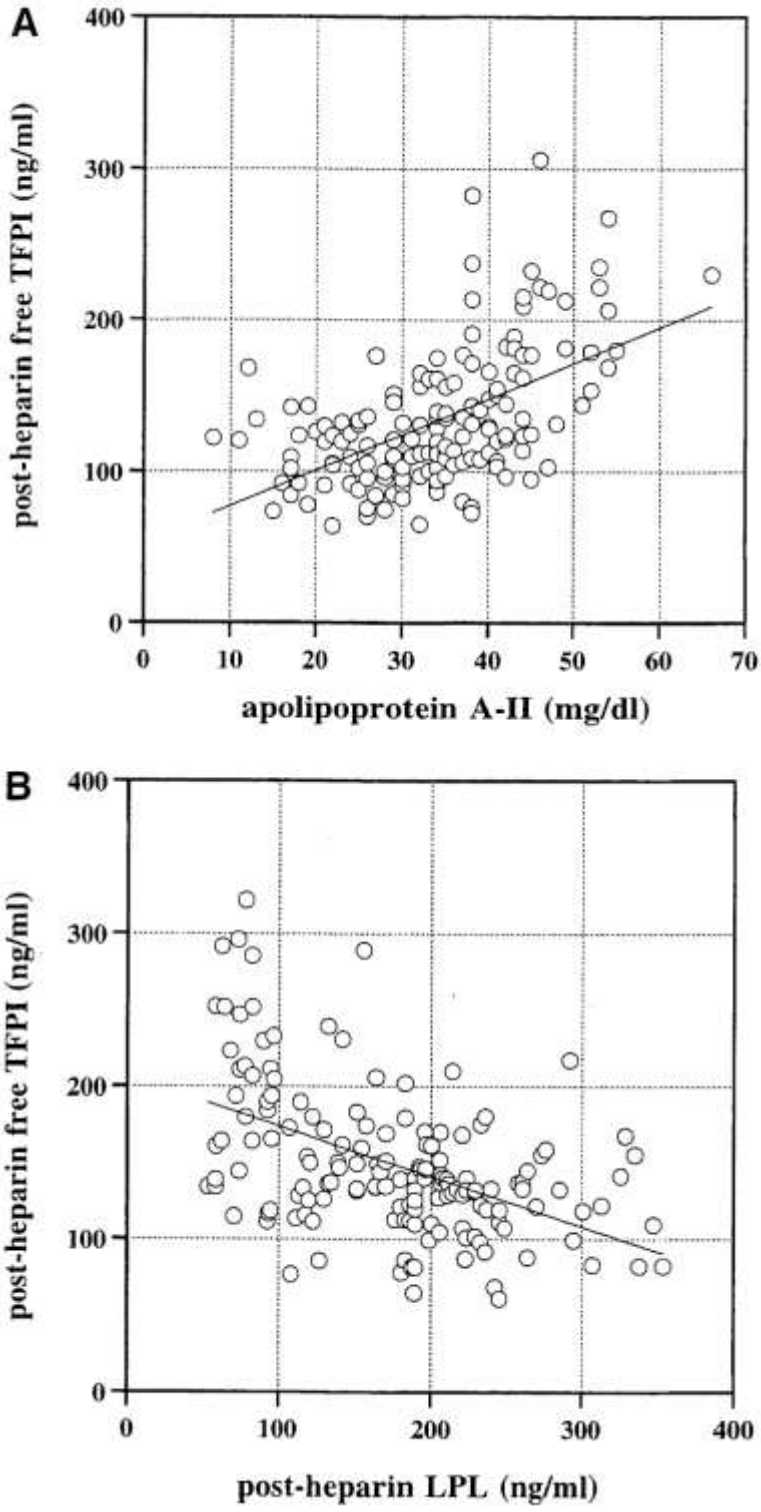


Figure 3:


A
post-heparin free TFPI (ng/ml)
400
300
200
100
0
0
10
20
30
40
50
60
70
apolipoprotein A-II (mg/dl)
B
post-heparin free TFPI (ng/ml)
400
300
200
100
0
0
100
200
300
400
post-heparin LPL (ng/ml)