Original Article

Prevention of Cardiovascular Events with Pitavastatin is Associated with Increased Serum Lipoprotein Lipase Mass Level: Subgroup Analysis of the TOHO-LIP

Daiji Nagayama[1, 2], Atsuhito Saiki[2], Yasuhiro Watanabe[2], Takashi Yamaguchi[2], Masahiro Ohira[2],
Naoko Sato[3], Mizuho Kanayama[3], Masao Moroi[4], Yoh Miyashita[5], Kohji Shirai[6] and Ichiro Tatsuno[2]

[1]Nagayama Clinic, Tochigi, Japan
[2]Center of Diabetes, Endocrine and Metabolism, Toho University Sakura Medical Center, Chiba, Japan
[3]Pharmaceutical Unit, Toho University Sakura Medical Center, Chiba, Japan
[4]Division of Cardiovascular Medicine (Ohashi), Department of Internal Medicine, Faculty of Medicine, Toho University, Tokyo, Japan
[5]Medical Correction Center in East Japan, Tokyo, Japan
[6]Department of Internal Medicine, Mihama Hospital, Chiba, Japan

Aim: To clarify the mechanism by which pitavastatin reduced cardiovascular (CV) events more effectively than atorvastatin in the TOHO Lipid Intervention Trial Using Pitavastatin (TOHO-LIP), the changes in (Δ) non-heparinized serum level of lipoprotein lipase mass (LPL mass) during administration of the respective statins were investigated.

Methods: From TOHO-LIP data, 223 hypercholesterolemic patients with any CV risks followed at Toho University Sakura Medical Center were analyzed. The patients were randomized to pitavastatin (2 mg/day) group ($n=107$) or atorvastatin (10 mg/day) group ($n=116$), and followed for 240 weeks. In this subgroup study, the primary and secondary end points were the same as those in TOHO-LIP, and 3-point major adverse cardiovascular events (3P-MACE) was added. The relationship between ΔLPL mass during the first year and the incidences of each end point was analyzed.

Results: The lipid-lowering effect was not different between the two statins. Cumulative 240-week incidence of each end point was significantly lower in pitavastatin group (primary: 1.9% vs. 10.3%, secondary: 4.7% vs. 18.1%, 3P-MACE: 0.9% vs. 6.9%). Mean LPL mass (64.9 to 69.0 ng/mL) and eGFR (70.1 to 73.6 ml/min/1.73m^2) increased in pitavastatin group, but not in atorvastatin group during the first year. Cox proportional-hazards model revealed that ΔLPL mass (1 ng/mL or 1SD) contributed to almost all end points.

Conclusions: Pitavastatin administration reduced CV events more efficaciously than atorvastatin despite similar LDL cholesterol-lowering effect of the two statins. Increased LPL mass during the first year by pitavastatin treatment may be associated with this efficacy.

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Introduction

The significance of lowering low-density lipoprotein cholesterol (LDL-C) level in the reduction of cardiovascular (CV) disease in both primary and secondary prevention has been reported[1, 2]. Hydroxymethylglutaryl (HMG)-CoA reductase inhibitors, also known as statins, inhibit the

production of mevalonic acid from HMG-CoA, leading to upregulation of hepatic LDL receptor and resulting in potent LDL-C-lowering effect[3]. Atorvastatin, one of the most used statins all over the world, was established to be efficacious in primary and secondary prevention of CV events in hypercholesterolemic patients[4, 5].

Pitavastatin is a statin with a chemical structure and pharmacokinetic profile different from those of atorvastatin, and has been clinically available in Japan since 2003 for the treatment of primary hyperlipidemia or mixed dyslipidemia[6]. Study showed greater increases in high-density lipoprotein cholesterol (HDL-C) and apolipoprotein A-1 levels in hypercholesterolemic patients treated with pitavastatin than with atorvastatin[7]. Several pleiotropic effects of pitavastatin may lead to the reduction of CV risk. For example, pitavastatin treatment was shown to improve endothelial function[8], anti-oxidative and anti-inflammatory condition[9] and coronary plaque stabilization[10]. Besides, compared with atorvastatin, favorable effects of pitavastatin on glucose metabolism[11-13] and lipid metabolism irrespective of obesity-related parameters[7, 14] were observed. However, it remained unclear whether pitavastatin was as effective as atorvastatin in preventing CV events.

We therefore conducted a multicenter, open-label, randomized controlled trial of head-to-head comparison between pitavastatin and atorvastatin, named the TOHO Lipid Intervention Trial Using Pitavastatin (TOHO-LIP)[15]. This trial, which enrolled 664 hypercholesterolemic patients with one or more CV risks, revealed that pitavastatin (2 mg/day) therapy was superior to atorvastatin (10 mg/day) therapy in the prevention of primary end point which was a composite of CV death, sudden death of unknown origin, nonfatal myocardial infarction (MI), nonfatal stroke, transient ischemic attack and heart failure requiring hospitalization [pitavastatin: 2.9%, atorvastatin : 8.1%, hazard ratio (HR): 0.366], despite similar LDL-C-lowering effect. In this trial, C-reactive protein (CRP) level only in the limited population of pitavastatin group was reduced during the first year. Additionally, in a subgroup analysis of TOHO-LIP, Saiki *et al.*[16] reported that the reduction in CV events by pitavastatin treatment tended to be associated with decreased arterial stiffness. However, the mechanism by which pitavastatin preferentially prevents CV events is not fully elucidated.

Lipoprotein lipase (LPL), which is thought to be anchored on the surface of endothelial cells and hydrolyzes triglyceride (TG) in the blood, is mainly produced in adipocytes and skeletal muscle cells[17]. Generally, the measurement of LPL activity and LPL mass are conducted using plasma after a heparin injection, which releases LPL from the surface of endothelial cells into blood[18]. On the other hand, a sensitive immunoassay system using a specific monoclonal antibody against LPL can detect LPL mass in serum without heparin injection, although the LPL mass in preheparin serum has no activity[19, 20]. The preheparin serum level of LPL mass (abbreviated as LPL mass hereinafter) correlates negatively with BMI and intra-abdominal visceral fat area evaluated by computed tomography[21]. Additionally, LPL mass also correlated negatively with the severity of metabolic syndrome, type 2 diabetes[22-25] and coronary atherosclerosis[26, 27]. These findings indicate that low LPL mass reflects insulin resistance and risk of coronary artery disease. Furthermore, insulin-sensitizing therapies including body weight reduction[28], insulin therapy[29] and administration of troglitazone, a PPARγ enhancer[30], also increased LPL mass in patients with metabolic syndrome. Especially, pitavastatin is known to enhance LPL expression in both 3T3L1 preadipocyte[31] and L6 skeletal muscle cells[32]. Moreover, it has been reported that pitavastatin was more effective than atorvastatin in increasing LPL mass in Japanese patients[33]. With these backgrounds, we posed the research questions of whether pitavastatin increases LPL mass, and whether the change in (Δ) LPL mass is associated with the incidence of CV event in hypercholesterolemic patients.

Therefore, we investigated the relationship between the incidences of CV events and annual Δ LPL mass during the first year in a subgroup of subjects in TOHO-LIP, who were followed at the Toho University Sakura Medical Center.

Subjects and Methods

The rationale, study design, inclusion criteria, and other details of the TOHO-LIP are described elsewhere[15]. This multicenter randomizes open-label trial compared head-to-head two lipid-lowering drugs; pitavastatin and atorvastatin, regarding the prevention of CV events in middle-aged or older patients with hypercholesterolemia at high risk of atherosclerotic CV disease.

From April 1, 2006 to May 31, 2011, a total of 664 patients were enrolled from 3 medical centers of Toho University (Japan) and randomized to receive either pitavastatin or atorvastatin. The dose of pitavastatin used was 2 mg/day, which is known to exert LDL-C-lowering effect comparable to that of atorvastatin 10 mg/day[10]. The optimal treatment target was set at LDL-C < 100 mg/dL. The duration of treatment with the statins was 240 weeks. The

estimated glomerular filtration rate (eGFR) was calculated by the following equation:

eGFR $(mL/min/1.73m^2) = 194 \times Creatinine^{-1.094} \times age^{-0.287} \times (0.739$ if female$)$.

In the present subgroup analysis, we studied the subjects in TOHO-LIP recruited from a single center. From the database of TOHO-LIP, 390 outpatients who attended the Center of Diabetes, Endocrine and Metabolism, Toho University Sakura Medical Center (Chiba, Japan) were included. All participants in this study were outpatients, and blood samplings were taken sitting in a chair. Among them, patients with LPL mass data obtained both at baseline and one year later were enrolled. Eventually, 223 patients were analyzed (pitavastatin group: 107 patients, atorvastatin group: 116 patients).

LDL-C was calculated using Friedewald formula: LDL-C = total cholesterol (TC) − (HDL-C) − (TG/5). Since this formula is not valid for patients with TG ≥ 400 mg/dL[34], subjects with TG ≥ 400 mg/dL (3.1% of all participants) were excluded from the analysis of LDL-C. The LPL mass in serum was measured by a sandwich enzyme-linked immunosorbent assay using a specific monoclonal antibody against LPL (Sekisui Medical, Japan), as described by Kobayashi *et al.*[19]. The linearity of this assay system was observed from 5 to 400 ng/mL. The within-run coefficient of variation was 2.8%. Between-day coefficient of variation was 4.3%. Interference by serum TG from 50 to 1,500 mg/dL and serum HDL-C from 5 to 120 mg/dL was not observed[24, 26].

The protocol of the present subgroup study was prepared in accordance with Declaration of Helsinki and approved by the Ethics Committee of Toho University Sakura Medical Center (S18069). Informed consent was obtained in the form of opt-out on the website.

End Points

The primary and secondary end points used in TOHO-LIP[15] were adopted in this subgroup study. The primary end point was a composite of CV death, sudden death of unknown origin, nonfatal acute MI, nonfatal stroke, transient ischemic attack, or heart failure requiring emergency hospitalization. Secondary end point was a composite of the primary end point plus coronary revascularization for stable angina. Additionally, in the present subgroup analysis, 3-point major adverse cardiovascular events (3P-MACE: nonfatal stroke, nonfatal MI, or CV death) was assessed as another composite end point.

Note that LPL mass at one year after randomization were measured in all participants, even if any endpoint occurred during the first year.

Statistical Analysis

The results are expressed as mean ± standard error (SE) only for ΔLPL mass or mean ± standard deviation (SD) for other variables. Wilcoxon signed-rank test was used to compare all parametric data between baseline and after one year of treatment. Mann-Whitney *U*-test or Fisher's exact test was performed to compare the characteristics between two different groups. Kaplan–Meier survival analysis was employed to estimate the time to end point, and log-rank test was used between two groups. Spearman rank correlation coefficient (Spearman's ρ) was used to determine the relation between LPL mass and coronary risks. Cox proportional-hazards analysis was performed to examine the contribution of ΔLPL mass to each end point, and the result is expressed as hazard ratio with 95% confidence interval. A two-sided *P* value of 0.05 was considered statistically significant. Statistical analyses were performed with EZR (Version 1.40, Saitama Medical Center, Jichi Medical University, Saitama, Japan)[35].

Results

Participants Characteristics at Baseline and after One Year of Treatment in Pitavastatin and Atorvastatin Groups

Participant characteristics of the two statin groups at baseline and after one year are shown in **Table 1**. No significant differences in all baseline continuous variables were detected between two groups when analyzed by Mann-Whitney *U*-test. After one year of treatment, TC, TG and LDL-C decreased, and HDL-C increased in both groups. In all participants, mean LDL-C decreased from 161.5 to 99.7 mg/dL, and 59.4% of the participants (pitavastatin: 58.5%, atorvastatin: 61.2%) achieved the target of LDL-C < 100 mg/dL. On the other hand, LPL mass increased significantly from baseline to one year after treatment only in pitavastatin group (pitavastatin: 64.9 to 69.0; *P* < 0.01, atorvastatin: 65.9 to 65.0 ng/mL). Additionally, pitavastatin group also showed significant increase in eGFR (70.1 to 73.6 ml/min/1.73m²).

There were no significant differences in prevalence of macrovascular disease, smoking status, risk factors and medications between two groups when analyzed by Fisher's exact test. In both groups, over 90% of the participants had diabetes, almost 70% had hypertension, and less than 10% had a history of ischemic heart disease. Additionally, no significant change in the frequency of use of each medication after one year was observed in both groups.

The adverse event rate during the 240-week

Table 1. Subjects characteristics at baseline and after one year of treatment in pitavastatin and atorvastatin groups

	Pitavastatin (*N*=107)		Atorvastatin (*N*=116)	
	Baseline	After one year	Baseline	After one year
Male (%)	42.1	-	41.4	-
Age (years)	63.9 ± 10.6	-	66.0 ± 8.4	-
Smoking status (%)				
Never	72.0	-	67.2	-
Former	21.5	-	28.5	-
Current	6.5	-	4.3	-
BMI (kg/m^2)	24.6 ± 4.0	24.2 ± 4.0	24.5 ± 3.1	24.4 ± 3.2
Systolic BP (mmHg)	133 ± 16	133 ± 12	137 ± 17	131 ± 15
Diastolic BP (mmHg)	77 ± 10	77 ± 8	79 ± 10	76 ± 9
HbA1c (%)	7.0 ± 1.1	7.1 ± 1.4	7.1 ± 1.2	7.3 ± 1.4
LPL mass (ng/mL)	64.9 ± 23.6	69.0 ± 22.3*	65.9 ± 20.5	65.0 ± 21.6
TC (mg/dL)	253 ± 39	190 ± 37*	245 ± 39	176 ± 30*
TG (mg/dL)	157 (111 – 225)	115 (84 – 178)*	136 (103 – 180)	109 (82 – 142)*
HDL-C (mg/dL)	54 ± 13	57 ± 14*	56 ± 13	57 ± 14*
LDL-C (mg/dL)	164 ± 35	103 ± 30*	159 ± 34	97 ± 27*
Creatinine (mg/dL)	0.78 ± 0.26	0.75 ± 0.27*	0.79 ± 0.21	0.79 ± 0.22
eGFR (ml/min/1.73m^2)	70.1 ± 17.2	73.6 ± 18.5*	67.8 ± 15.3	67.9 ± 15.9
Prevalence of diabetes (%)	92.2	-	94.4	-
Prevalence of hypertension (%)	69.0	-	69.2	-
History of ACS and/or coronary revascularization (%)	7.5	-	9.5	-
Stroke (%)	5.6	-	6.0	-
Peripheral artery disease (%)	1.9	-	3.4	-
Medication (%)				
Antihypertensive drugs				
ACE-I and/or ARB	46.7	48.6	42.4	45.7
Calcium channel blocker	43.0	44.9	39.7	39.7
Diuretics	12.1	16.8	15.5	14.7
Beta-receptor antagonist	16.8	17.8	8.6	9.5
Antidiabetes				
Insulin	21.5	24.3	22.4	28.4
Sulfonylurea	52.3	48.6	50.9	52.6
Biguanide	30.8	31.8	27.6	34.5
Thiazolidinedione	15.0	16.8	14.7	12.1

Data are presented as mean ± SD or percentage, or median (inter quartile range). *P<0.01 vs. baseline, Wilcoxon signed-rank test.
BMI, body mass index; BP, blood pressure; HbA1c, glycated hemoglobin; LPL, lipoprotein lipase; TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; eGFR, estimated glomerular filtration rate; ACS, acute coronary syndrome; ACE-I, angiotensin converting enzyme inhibitor; ARB, angiotensin II receptor blocker; SD, standard deviation.

study period of this subgroup analysis was 1.9% in pitavastatin (1 muscle pain and 1 cholecystitis), and 4.3% in atorvastatin group (4 muscle pain and 1 Alzheimer's disease). Rhabdomyolysis was not observed in all participants.

Kaplan-Meier Survival Analysis to Estimate Differences between Pitavastatin and Atorvastatin Group

In the TOHO-LIP main study, pitavastatin significantly reduced the risk of the primary and secondary end points compared with atorvastatin[15]. In this subgroup analysis of 223 participants, we performed Kaplan-Meier survival analysis to reexamine the difference between two groups as shown in **Fig. 1**.

The cumulative 240-week incidence of each end point was: pitavastatin 1.9% (2/107), atorvastatin 10.3% (12/116) (*P*=0.009) for primary end point **(Fig. 1a)**; pitavastatin 4.7% (5/107), atorvastatin 18.1% (21/116) (*P*=0.002) for secondary end point **(Fig. 1b)**. Furthermore, the risk of 3P-MACE was also
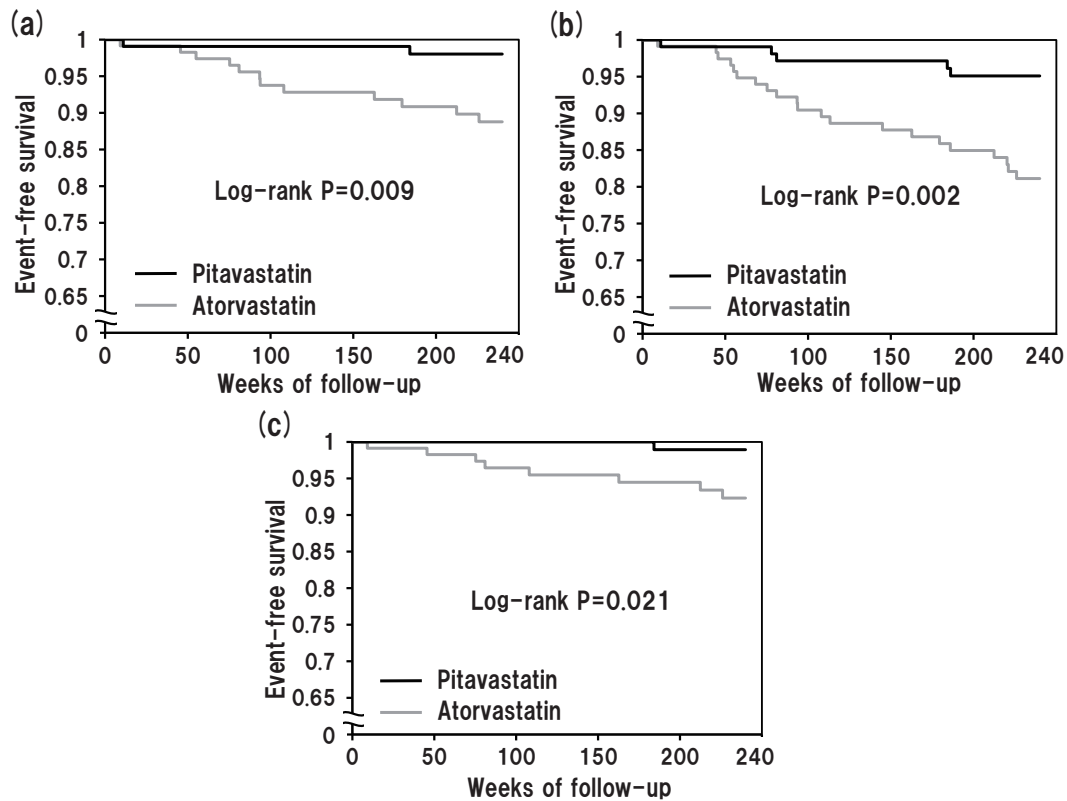
Fig. 1. Kaplan-Meier curves for the primary end point (a), secondary end point (b) and 3P-MACE (c) for pitavastatin and atorvastatin group

Primary end point was a composite of cardiovascular death, sudden death of unknown origin, nonfatal acute myocardial infarction, nonfatal stroke, transient ischemic attack, and heart failure requiring hospitalization. Secondary end point was a composite of the primary end point plus coronary revascularization. 3P-MACE was a composite of nonfatal stroke, nonfatal myocardial infarction, and cardiovascular death. 3P-MACE, 3-point major adverse cardiac event.
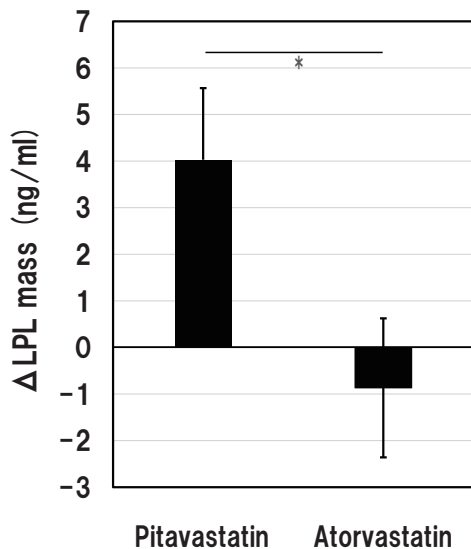

Fig. 2. Comparison of change in LPL mass during the first year between pitavastatin and atorvastatin group

Data are expressed as mean ± standard error. *$P < 0.05$ using Mann-Whitney's U test. LPL, lipoprotein lipase.

reduced in pitavastatin group pitavastatin 0.9% (1/107), atorvastatin 6.9% (8/116), $P = 0.021$ **(Fig. 1c)**. The results of primary and secondary end points were almost equal to those in the main TOHO-LIP study.

In this subgroup analysis, the incidence of heart failure requiring hospitalization was pitavastatin 1.9% (2/107) and atorvastatin 2.6% (3/116). Of the two patients in the pitavastatin group, only one was initially receiving a beta-receptor antagonist, and none was receiving a diuretic. Also, of the three patients in the atorvastatin group, none were initially receiving a beta-receptor antagonist and only two were receiving a diuretic. These results therefore suggest that the frequency of diuretics/beta-receptor antagonist administration had little effect on the difference in end points between the two groups in this study.

Comparison of Characteristics at Baseline and Changes during the First Year in Subjects with or without Secondary End Point

Next, we investigated the characteristics of

subjects who had vascular events during the 240-week study period. We examined the secondary end point because the number of cases was the largest among the three end points. All subjects were divided into two groups based on the presence or absence of the secondary end point. In addition, each group was divided into pitavastatin and atorvastatin groups. The differences in baseline characteristics and their changes during the first year between are compared in **Table 2**.

Subjects without secondary end point had lower prevalences of hypertension, male ratio and history of ACS and/or coronary revascularization, and higher prevalence of diabetes. These confounders required adjustment when the association of ΔLPL mass with each end point was analyzed by Cox proportional-hazards model (to be shown below).

Subjects with secondary end points showed higher frequency of diuretics ($P=0.064$) and biguanide ($P=0.020$). These medications were not recruited as the adjusting factor for ΔLPL in **Table 4** because of their intraclass correlations to history of ACS and/or coronary revascularization. Note that the mean ΔLPL mass during the first year in subjects without secondary end point tended to be higher than that in subjects with secondary end points (2.2 vs. -5.7, $P=0.065$), even though their mean baseline LPL mass were equal.

There was no difference in change in BP between pitavastatin and atorvastatin groups as shown in **Table 1**. However, in subjects without secondary end point, atorvastatin group showed greater decrease in BP. Besides, the greater increase in eGFR was observed also in pitavastatin group without secondary end point.

Association of Baseline and Annual Change in LPL Mass with Risk Factors

The association of LPL mass with clinical characteristics are shown in **Table 3**.

Baseline LPL mass correlated positively with age ($\rho=0.172$) and HDL-C ($\rho=0.393$), and negatively with BMI ($\rho=-0.246$) and TG ($\rho=-0.356$) at baseline. Significantly lower LPL mass was observed in subjects with TG ≥ 150 mg/dL (mean value, yes vs. no: 60.0 vs. 70.2 ng/mL), BMI ≥ 25 kg/m^2 (59.5 vs. 68.2 ng/mL), history of ACS and/or coronary revascularization (54.8 vs. 66.1 ng/mL), and in men (56.5 vs. 71.6 ng/mL).

Next, in each statin group, we compared the annual change in LPL mass between various two categorical groups. Resultantly, higher increase in annual change in LPL mass was observed in hypertensive subjects (6.5 vs. 5.7) or subjects with TG ≥ 150 mg/dL (6.6 vs. 3.8 ng/mL) in pitavastatin

group. Additionally, annual ΔLPL mass during the first year did not correlate with changes in any metabolic parameters in both groups.

Saiki *et al.* reported in another subgroup analysis of TOHO-LIP[16] that systemic arterial stiffness assessed by cardio-ankle vascular index (CAVI) decreased only in pitavastatin group. On the other hand, in the present study, CAVI did not significantly decrease in pitavastatin group (9.34 to 9.30, $P=0.176$). However, ΔLPL was significantly correlated with ΔCAVI in all participants (Spearman's $\rho=-0.352$, $P<0.001$) (data not shown).

Comparison of Changes in LPL Mass during the First Year between Pitavastatin and Atorvastatin Group

Furthermore, we compared ΔLPL mass between pitavastatin and atorvastatin groups. As shown in **Table 1**, LPL mass increased during the first year only in pitavastatin group. Besides, mean ΔLPL mass in pitavastatin group was significantly higher compared with that in atorvastatin group (4.03 vs. -0.87 ng/mL, $P=0.023$) **(Fig. 2)**.

Association of Change in LPL Mass during the First Year with Each End Point

Next, the contribution of ΔLPL mass to each end point was examined using Cox proportional-hazards model **(Table 4)**. After adjusting for confounders of gender, diabetes, hypertension and history of ACS and/or coronary revascularization **(Table 2)** in Model 1, ΔLPL mass (1 ng/mL or 1 SD increase) contributed roughly to the primary, secondary and 3P-MACE end points. However, only the contribution of ΔLPL mass (1SD increase) to the primary end point was not significant ($P=0.082$).

In Model 2, we added pitavastatin administration as a confounder. Resultantly, the significant contributions of ΔLPL for primary end point (1 ng/mL and 1 SD increase) and for 3P-MACE (1 SD increase) partially disappeared, indicating the intraclass correlation between pitavastatin administration and ΔLPL.

Discussion

In this subgroup analysis of TOHO-LIP, we reconfirmed that pitavastatin therapy was superior to atorvastatin therapy in the prevention of CV events, and compared ΔLPL mass during the first year between the two statin groups. LPL mass and eGFR increased only in pitavastatin group, and the annual increase in LPL mass during the first year contributed to the reduction of cumulative 240-week incidence of

Table 2. Comparison of characteristics at baseline and changes during the first year in subjects with or without secondary end points

	Subjects without secondary end point			Subjects with secondary end point		
	Total ($N=197$)	Pitavastatin ($N=102$)	Atorvastatin ($N=95$)	Total ($N=26$)	Pitavastatin ($N=5$)	Atorvastatin ($N=21$)
Male (%)	39.6	40.2	38.9	57.7*	80.0	52.4
Age (years)	64.8±9.8	63.7±10.9	66.1±8.5	65.5±7.3	66.6±3.4	65.2±8.0
Age ≥ 65 years (%)	56.3	52.0	61.1	57.7	80.0	2.4
Baseline BMI (kg/m^2)	24.7±3.5	24.7±3.9	24.6±3.2	23.6±2.9	23.7±3.0	23.6±2.9
BMI ≥ 25 kg/m^2 (%)	33.0	28.5	38.5	26.9	25.5	27.5
ΔBMI (kg/m^2)	-0.23±0.90	-0.4±1.3	-0.2±3.2	-0.05±0.88	-0.07±0.83	-0.05±0.90
Baseline systolic BP (mmHg)	135±16	134±16	136±16	136±17	137±8	137±18
ΔSystolic BP (mmHg)	-4±17	-1±16	-7±17†	0±12	-4±5	1±13
Baseline diastolic BP (mmHg)	78±11	78±11	79±10	77±11	74±26	77±9
ΔDiastolic BP (mmHg)	-2±9	0±9	-4±8†	0±11	-11±15	1±13
Hypertension (%)	66.0	67.6	64.2	92.3**	100.0	90.5
Baseline HbA1c (%)	7.1±1.2	7.0±1.1	7.1±1.2	7.2±1.3	6.7±1.4	7.3±1.3
ΔHbA1c (%)	0.1±0.9	0.1±1.0	0.1±0.8	0.1±1.3	-0.1±0.4	0.2±1.4
Diabetes (%)	95.9	96.1	95.8	73.1**	60.0	76.,2
Baseline LPL mass (ng/mL)	65.7±22.4	65.5±23.9	65.9±20.8	62.0±17.7	49.6±2.8	65.6±18.6
ΔLPL mass (ng/mL)	2.2±15.6	3.7±15.5	0.7±15.8	-5.7±16.5*	13.6±23.3	-11.2±9.3
Baseline TC (mg/dL)	250±37	254±38	246±36	237±49	225±39	241±51
ΔTC (mg/dL)	-66±38	-64±36	-69±39	-63±50	-51±29	-66±54
Baseline TG (mg/dL)	148 (105 – 203)	158 (109 – 225)	138 (104 – 189)	133 (99 – 168)	134 (121 – 157)	131 (97 – 169)
TG ≥ 150 mg/dL (%)	48.7	54.9	42.1	42.3	40.0	42.9
ΔTG (mg/dL)	-27 (-63 – 5)	-69 (-560 – -20)	-59 (-540 – -36)	-22 (-39 – 3)	-37 (-65 – -16)	-19 (-31 – 4)
Baseline HDL-C (mg/dL)	55±13	54±13	57±13	52±11	49±13	52±11
HDL-C < 40 mg/dL (%)	8.1	12.7	3.2†	11.5	20.0	9.5
ΔHDL-C (mg/dL)	2±7	3±8	1±7	3±9	6±12	2±8
Baseline LDL-C (mg/dL)	162±34	165±35	158±32	159±43	143±33	163±45
ΔLDL-C (mg/dL)	-62±33	-63±31	-62±36	-55±47	-40±18	-59±52
eGFR (ml/min/1.73m^2)	69.5±16.4	70.7±17.3	68.2±15.5	64.4±14.1	57.8±10.1	65.9±14.6
ΔeGFR (ml/min/1.73m^2)	1.7±9.3	3.4±10.3	-0.1±7.8†	1.8±8.9	5.0±12.0	1.1±8.3
History of ACS and/or coronary revascularization (%)	5.1	4.9	5.3	34.6**	60.0	28.6
Medication (%)						
Antihypertensive drugs						
ACE-I and/or ARB	42.6	47.1	37.9	57.7	40.0	61.9
Calcium channel blocker	39.6	43.1	35.8	36.4	40.0	57.1
Diuretics	12.2	11.8	12.6	26.9*	20.0	28.6
Beta-receptor antagonist	12.2	15.7	8.4	15.4	40.0	9.5
Antidiabetes						
Insulin	21.8	19.6	24.2	23.1	60.0	14.3
Sulfonylurea	52.3	53.9	50.5	46.2	20.0	52.4
Biguanide	26.4	29.4	23.2	50.0**	60.0	47.6
Thiazolidinedione	15.7	15.7	15.8	7.7	0.0	9.5

Data are presented as mean ± SD, or median (inter quartile range). *$P<0.10$ and **$P<0.05$, comparison between total subjects with and without secondary end point groups. †$P<0.05$, comparison between pitavastatin and atorvastatin groups. Mann-Whitney's U test or Fisher's exact test. BMI, body mass index; BP, blood pressure; HbA1c, glycated hemoglobin; LPL, lipoprotein lipase; TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; eGFR, estimated glomerular filtration rate; ACE-I, angiotensin converting enzyme inhibitor; ARB, angiotensin II receptor blocker; SD, standard deviation.

Table 3. Association between baseline / annual change in LPL mass and risk factors

vs.	Coefficient of correlation with baseline LPL mass	
Age (years)	0.172*	
BMI (kg/m^2)	-0.246*	
Systolic BP (mmHg)	-0.082	
HbA1c (mg/dL)	-0.007	
TC (mg/dL)	0.008	
TG (mg/dL)	-0.356*	
HDL-C (mg/dL)	0.393*	
LDL-C (mg/dL)	0.015	
eGFR (ml/min/1.73m^2)	-0.062	
Categorical variable	**Comparison of baseline LPL mass (ng/mL)**	
Pitavastatin / Atorvastatin	64.9 ± 23.6 / 65.9 ± 20.5	
Sex, males/females	56.5 ± 20.1 / 71.6 ± 21.3[†]	
Hypertension, Yes/No	64.2 ± 22.9 / 67.9 ± 19.9	
TG >150 mg/dL, Yes/No	60.0 ± 23.8 / 70.2 ± 19.2[†]	
Diabetes, Yes/No	65.3 ± 22.1 / 67.3 ± 21.2	
eGFR <60 ml/min/1.73m^2, Yes/No	65.3 ± 22.2 / 65.4 ± 22.0	
BMI ≥ 25kg/m^2, Yes/No	59.5 ± 23.7 / 68.2 ± 20.7[†]	
History of ACS and/or Coronary revascularization, Yes/No	54.8 ± 16.7 / 66.1 ± 22.2[†]	
Categorical variable	**Annual change in LPL mass**	
	Pitavastatin	Atorvastatin
Sex, males/females	5.9 (-1.3 – 14.0) / 5.6 (-8.3 – 14.3)	-1.1 (-12.0 – 7.8) / -1.2 (-11.0 – 10.0)
Hypertension, Yes/No	6.5 (-1.4 – 15.7) / 5.7 (-12.7 – 11.0)[†]	-0.4 (-9.9 – 10.3) / -5.7 (-12.0 – 6.4)
TG >150 mg/dL, Yes/No	6.6 (-3.7 – 14.6) / 3.8 (-2.8 – 11.0)[†]	-1.1 (-11.1 – 7.3) / -1.9 (-13.4 – 8.0)
Diabetes, Yes/No	5.9 (-3.7 – 14.4) / 4.9 (-2.0 – 10.4)	-0.9 (-11.5 – 9.4) / -5.2 (-12.9 – -0.1)
eGFR <60 ml/min/1.73m^2, Yes/No	4.9 (-5.9 – 12.1) / 6.6 (-2.4 – 14.4)	1.1 (-7.2 – 10.6) / -5.2 (-11.7 – 6.8)
BMI ≥ 25kg/m^2, Yes/No	7.1 (-0.4 – 13.2)/ 5.7(-3.9 – 14.2)	-0.6 (-10.7 – 7.9) / -3.2 (-12.0 – 8.9)
History of ACS and/or Coronary revascularization, Yes/No	10.4 (0.8 – 17.6) / 5.8 (-5.1 – 14.2)	7.8 (-0.1 – 22.3) / -2.6 (-11.7 – 7.7)
vs.	**Coefficient of correlation with annual change in LPL mass**	
	Pitavastatin	Atorvastatin
ΔBMI (kg/m^2)	-0.007	-0.120
ΔSystolic BP (mmHg)	-0.073	-0.088
ΔHbA1c (mg/dL)	-0.010	0.154
ΔTC (mg/dL)	0.013	-0.098
ΔTG (mg/dL)	-0.147	-0.097
ΔHDL-C (mg/dL)	0.036	0.135
ΔLDL-C (mg/dL)	0.068	-0.087
ΔeGFR (ml/min/1.73m^2)	-0.174	-0.042

*$P < 0.05$, Spearman's coefficient of correlation (ρ). [†]$P < 0.05$, Mann-Whitney's U test to compare two categorical groups. Data of baseline or annual change in LPL mass are presented as mean ± SD or median (inter quartile range).

LPL, lipoprotein lipase; eGFR, estimated glomerular filtration rate; BMI, body mass index; BP, blood pressure; HbA1c, glycated hemoglobin; TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; ACS, acute coronary syndrome.

primary, secondary end points and 3P-MACE. Furthermore, baseline LPL mass correlated positively with age and HDL-C, and negatively with BMI and TG. Relatively low LPL mass was associated with male gender, obesity and coronary artery disease. These findings of LPL mass are consistent with previous reports[22-25], reconfirming low LPL mass as a CV risk. Additionally, the contribution of increased LPL mass

Table 4. Association of change in LPL mass during the first year with each endpoint

Model 1	Primary end point		Secondary end point		3P-MACE	
	HR (95% CI)	*P* value	HR (95% CI)	*P* value	HR (95% CI)	*P* value
ΔLPL mass (1 ng/mL increase)	0.960 (0.922 – 1.000)	0.0497	0.952 (0.925 – 0.980)	< 0.001	0.940 (0.896 – 0.986)	0.011
ΔLPL mass (1SD increase)	0.521 (0.250 – 1.085)	0.082	0.495 (0.293 – 0.834)	0.008	0.375 (0.143 – 0.982)	0.046
Model 2	Primary end point		Secondary end point		3P-MACE	
	HR (95% CI)	*P* value	HR (95% CI)	*P* value	HR (95% CI)	*P* value
ΔLPL mass (1 ng/mL increase)	0.961 (0.916 – 1.008)	0.100	0.951 (0.920 – 0.983)	0.003	0.929 (0.877 – 0.985)	0.013
ΔLPL mass (1SD increase)	0.589 (0.270 – 1.285)	0.184	0.532 (0.311 – 0.909)	0.021	0.416 (0.150 – 1.150)	0.091

Cox proportional-hazards analyses.
Model 1: adjusted for gender, diabetes, hypertension and history of ACS and/or coronary revascularization.
Model 2: adjusted for gender, diabetes, hypertension and history of ACS and/or coronary revascularization and pitavastatin administration.
The primary end point, cardiovascular death, sudden death of unknown origin, nonfatal acute myocardial infarction, nonfatal stroke, transient ischemic attack and heart failure requiring hospitalization. Secondary end point, the primary end point plus coronary revascularization. 3P-MACE, nonfatal stroke, nonfatal myocardial infarction and cardiovascular death.
LPL, lipoprotein lipase; HR, hazard ratio; CI, confidence interval; 3P-MACE, 3-point major adverse cardiovascular events; SD, standard deviation.

to the reduction of each end point was independent of gender, diabetes, hypertension and coronary artery disease. These findings about LPL mass may partially explain the mechanism by which pitavastatin reduces CV events.

In the main TOHO-LIP study, CRP level decreased during the first year only in pitavastatin group[15]. Additionally, as previously mentioned, improved systemic arterial stiffness, assessed by CAVI, due to pitavastatin might result in reduced CV events in the other subgroup analysis of TOHO-LIP[16]. CAVI essentially reflects the stiffness of the arterial tree from the origin of the aorta to the ankles, and may be a potential therapeutic target for cardiometabolic complications. These findings may indicate that pitavastatin reduced CV events through the amelioration of inflammation and arterial stiffening. So what is the newly supplied pleiotropic effect of pitavastatin in this subgroup analysis? Regarding the mechanism for the superiority of pitavastatin in preventing CV events, we propose the following hypotheses.

First, we propose the pharmacological action of pitavastatin on vascular endothelial function as an explanation for the superiority of this statin. Pitavastatin has been reported to restore impaired endothelial function in *in vivo* and *vitro* studies, suggesting its pharmacological action related to the reduction of pro-inflammatory response and

enhancing nitric oxide production[9, 36]. Besides, Hitsumoto *et al.*[26] reported the implication of low LPL mass as a marker of coronary vasospasm in early stage atherosclerosis, which is characterized by endothelial dysfunction. Additionally, we previously found the association of CAVI with endothelial function assessed by flow-mediated dilation[37], and the fact that appropriate therapeutic approaches in diabetic patients simultaneous improved CAVI and LPL mass[25, 38]. We therefore speculate that, in TOHO-LIP, pitavastatin-induced improvement of endothelial function was expressed as decreased CAVI and increased LPL mass. Kobayashi *et al.* also reported that atorvastatin administration did not change LPL mass in Japanese hyperlipidemic subjects[39]. In contrast, in subjects with low LPL mass (less than 50 ng/mL), atorvastatin has been reported to increase LPL mass[40]. There were few patients with low LPL mass in the present study, which may have led to the different result. Furthermore, our previous *in vitro* study revealed that atorvastatin enhanced LPL expression in skeletal muscle, but not in adipose tissue. On the contrary, pitavastatin enhances LPL expression also in adipose tissue, resulting in stronger increase in LPL mass in whole body than atorvastatin[31, 32].

Second, elimination of insulin resistance by pitavastatin may exert a direct or indirect anti-arteriosclerotic effect. LPL, which degrades circulating

TG in the bloodstream, is produced mainly in adipocytes and skeletal muscle cells stimulated by insulin, and LPL production is inhibited when insulin function is obstructed[17]. Moreover, we have reported the significance of low LPL mass as an indicator of insulin resistance[22, 41]. On the other hand, unlike atorvastatin, pitavastatin is speculated to have insulin-sensitizing effect[42], and improved insulin sensitivity is associated with the suppression of systemic oxidative stress independent from glycemic control[41, 43]. Accordingly, in the present subgroup study with over 90% of the subjects having diabetes, pitavastatin therapy may have contributed to CV event reduction through improvement of insulin sensitivity as indicated by increased LPL mass. However, we observed no remarkable improvement in severity of diabetes in this study. A large-scale and long-term cohort study is needed to clarify the effect of pitavastatin on diabetes control.

Third, LPL production enhanced by pitavastatin may promote the hydrolysis of circulating atherogenic triglyceride rich lipoproteins (remnant or intermediate lipoproteins)[44]. In CHIBA study, pitavastatin but not atorvastatin reduced TG and increased HDL-C in patients with metabolic syndrome[14]. It has been reported that pitavastatin stimulated *in vitro* LPL activity in 3T3-L1 preadipocytes more potently than atorvastatin, which may facilitate the increase in HDL through efficient metabolism of TG-rich lipoproteins[31]. Additionally, we previously reported that all of the conventional lipid parameters contributed independently to systemic arterial stiffening in logistic regression models, although TG showed the largest area under the receiver-operating-characteristic curve for predicting high CAVI[45]. However, in the TOHO-LIP, there was no difference in TG-lowering effect between pitavastatin and atorvastatin during the 240-week observation period, suggesting that this mechanism of TG-lowering effect potentially resulting from enhanced LPL production may not be greatly involved in CV event prevention by pitavastatin.

It seems strange that the prevalence of diabetes was lower in subjects with secondary end point compared with those without secondary end points (Table 2). This result is difficult to explain because diabetes is generally a contributing factor to cardiovascular events. A plausible explanation is that insulin-resistant diabetics might benefit more from pitavastatin compared with non-diabetics. However, in a Cox model that included diabetes as a confounder, ΔLPL mass was identified as an independent contributor to the cardiovascular event. In addition, in the TOHO-LIP main study[15], the presence or absence of diabetes was not associated with the event-preventing effect of pitavastatin. Further validation is warranted.

There are several limitations in this study. First, the TOHO-LIP main study was conducted as an open-label trial with inherent limitations. However, the independent event committee adjudicated all end point events while blinded to the assigned group. Moreover, to compensate slightly for the open-label trial design, the primary end point did not include coronary revascularization procedures because the decision for coronary revascularization is made by physicians who are aware of the assigned treatment group. Second, unlikely the main study, this subgroup analysis was a retrospective, single-center study using the TOHO-LIP database. The sample size was small, which could be a significant obstacle in showing a significant relationship. Third, when this study was designed, the LDL-C target level was set at 100 mg/dL, which is inadequate according to current guideline[46]. Fourth, the limitation of this study includes the lack of data on some potential confounders such as proteinuria and smoking status. In addition, CRP, which was mentioned as a supplemental file in the main manuscript, was excluded from the analysis in this study due to the small amount of data (7.6% in all participants). The end point of this study was CV disease, but serum BNP, a marker of heart failure, was not included in the evaluation. Finally, the change in LPL mass for the first year only was analyzed. This short duration was chosen in order to ensure high follow-up rate and include as many subjects as possible for this analysis with a small sample size.

Conclusions

Pitavastatin administration reduced CV events more efficaciously than atorvastatin despite similar LDL cholesterol-lowering effect of the two statins. Increased LPL mass during the first year by pitavastatin treatment may be associated with this efficacy.

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