Altered Serum Lysophosphatidylethanolamine Species Profile in Patients with Autoimmune Pancreatitis

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A short running title: serum lysoPE species of AIP patients

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(Abstract)

Lysophosphatidylethanolamine (LPE) are hydrolysis metabolites of polyunsaturated fatty acids and the alkenyl group-rich phosphatidylethanolamine (PE). It was reported that LPE functions as a lipid mediator in vivo and it is involved with an inflammatory process of the chronic autoimmune disease. LPE is present in not only cells but also serum, but the pathophysiological role and the metabolic details of serum LPE are not known. In this study, the characterization of the serum LPE species in the autoimmune pancreatitis (AIP) was analyzed by mass spectrometry. Serum samples from patients with AIP (n = 7) and healthy subjects (n = 9) were examined for fatty acid compositions using gas chromatography mass spectrometry. LPE species were analyzed using matrix-assisted laser desorption/ionization–time of flight mass spectrometry. Blood lipid tests, cholesterol and triglyceride, were almost in the criteria range in patients with AIP. Serum arachidonic acid (C20:4) levels were higher in patients with AIP than in healthy subjects, but eicosapentaenoic acid(C20:5) and docosahexaenoic acid(C22:6) concentrations were not the significant difference. In patients with AIP, serum total LPE levels were lower than those in healthy subjects, and LPE 20:4 levels were positively correlated with LPE 20:5 and LPE 22:6 levels. LPE p16:0 (plasmalogen-type) level was negatively correlated with LPE 20:4, LPE 20:5 and LPE 22:6 levels. This study showed that though serum lipids and the fatty acid compositions of patients with AIP resembled healthy subjects, serum LPE level of patients were significantly low and some serum LPE species were fluctuated in a correlated way.

I. Introduction

Phosphatidylethanolamine (PE) is found with a cell membrane and serum lipoprotein and occupies 17-25% of total phospholipid with mammalian cells[1]. PE is associated with the cell functions with various cellular processes namely the precursor of phosphatidylcholine (PC) biosynthesis, autophagy and oxidative phosphorylation reaction[2-4]. It was reported that the disorder of the PE metabolism is associated with chronic disease and an infection[5-7]. PE can be a diacyl- or alkenylacyl-type (plasmalogen-type)[8] (Fig. 1). Plasmalogen-type PE (PE-pl) bound an alkenyl group at *sn-1* position (a vinyl-ether bond) and is including much polyunsaturated fatty acids (PUFAs) at *sn-2* position[9]. PE-pl is distributed through the whole of the body of the organism, particularly in cranial nerve cellular tissue and inflammatory immunologic cellular (lymphocytes, a macrophage and neutrophil) [10-11]. PE has many kinds of molecular species by the combination of acyl group and alkenyl group. As an analysis of molecular species, mass spectrometry is used, but the combinatorial molecular species of multiple substituents may be present in the same mass electric charge ratio (*m/z*). Therefore, the analysis of PE species is difficult to apply clinically with complicatedness.

Lysophosphatidylethanolamines (LPEs) are lyso-type metabolites of PE by phospholipase A (PLA) activity. LPE is an intercellular signaling molecule[12]. It has been reported that LPE was bioactivity lipids in an important process of the life in cells such as an inflammatory cascade reaction and a cell proliferation or the cell death [13]. On the other hand, it was reported that the protracted and uncontrolled immune responses can lead to chronic inflammation, irreparable tissue damages, and chronic diseases[14]. Uncontrolled immune

responses also occur in many common autoimmune diseases[15]. The endogenous bioactive lipids induce immunity in an inflammatory process and adjust it and are played an important role to set a limit.

LPEs including acyl group (LPE-acyl) and alkenyl groups (PE-plasmalogen; LPE-pl) are generated by a response of the PLA to PE. LPEs including the PUFA and alkenyl group are produced from PE primarily by a response of phospholipase A_1 (PLA1) and phospholipase A2 (PLA2), respectively. Because the side chain (acyl group or alkenyl group) of LPE is one, it is readily-soluble molecule, and approximately several hundred ng/mL is present in human serum[16]. In LPE, the identification of molecular species by the mass spectrometry is easier than the analysis of PE. It was reported that LPE including the polyunsaturated fatty acid provides anti-inflammatory[17]. Recently, it was reported that the decrease in serum PE of the NASFLD patients is associated with decrease in serum LPE[18]. The serum LPE level may reflect PE metabolism. However, a pathophysiological role of serum LPE and the metabolic details are not known. The analysis of the serum LPE species by liquid chromatography-tandem mass spectrometry (LC-MS/MS) was reported[18], but it is hard to handle operations. Matrix-assisted laser desorption/ionization–time of flight mass spectrometry (MALDI-TOF MS) is simple analytical procedure for analysis of LPE species.

Immunoglobulin4 related disease (IgG4-RD) is characterized by high IgG4 serum levels as well as growth, infiltration, and fibrosis of lymphocytes and IgG4-positive plasma cells in organs throughout the body. In organs such as the pancreas and bile ducts, IgG4-RD manifests as chronic lymphoproliferative disease with fibrotic and mass lesions, hyperplastic lesions, and cellular infiltration[19]. Autoimmune pancreatitis (AIP) is an IgG4-RD, and its characteristics include obstructive jaundice, pancreatic calculi, ductus pancreatic stenosis, high serum IgG4 levels, and a distended spleen[20]. The levels of the inflammatory marker, such as serum C-

reactive protein (CRP), are not always elevated in patients with AIP[21]. The lysophospholipids are involved as bioactive lipids for an inflammatory process of various chronic autoimmune disease, but the details are unknown. To the best of our knowledge, no study has examined whether AIP is associated with a LPE metabolism disorder. In this study, we analyzed serum fatty acids using gas chromatography mass spectrometry, and the serum LPE species from the patients with AIP using MALDI-TOF MS.

II. Materials and Methods

A Chemical reagents

Chloroform, methanol, dichloromethane, isopropanol, and acetonitrile for HPLC were obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Strata-XL-AW tubes for solid-phase extraction (SPE) were obtained from Phenomenex (Torrance, CA, USA). The matrix reagent, 9-aminoacridine (9-AA) and heptadecanoic acid standard (C17:0) were obtained from Sigma-Aldrich (St. Louis, MO, USA). The LPE-C13:0 internal standard was obtained from Avanti Polar Lipids, Inc. (Alabaster, AL, USA).

B Study subjects

The study enrolled 9 healthy subjects (five males and four females; the average age: 53.1±11.7 years old, mean ± SD) and 7 patients with AIP (five males and two females; 69.1±10.6 years old). All patients with AIP had been on steroid drugs (prednisone: PSL of 1-5mg/day in six patients, and Celestamine of

5mg/day in one patients) for treatment of AIP. There were not the dieting and the use of the eicosapentaenoic acid (EPA) drugs for patients with AIP. The blood glucose level that three patients in AIP had a past medical history of diabetes mellitus was controlled to 121-131 mg/dL. The study protocol was approved by the Ethical Review Board of the Shinshu University School of Medicine (approved number: No. 3954).

C Preparation of serum

Participants fasted for 12 h, after which blood was collected. For serum samples, blood was collected in tubes containing a blood coagulation accelerant (Venoject II, TERUMO, Tokyo, Japan). After 15 min at room temperature, the samples were centrifuged for 10 min at 2,500 × g, and the supernatants (serum) were collected. Serum samples were stored at −80° C for a maximum of 1 week prior to analysis.

D Lipid extraction

To a 200 μL sample, 10 μL LPE C13:0 internal standard (240 μmol/L) and 2 mL chloroform/methanol (C/M: 2/1 v/v) were added. The solution was mixed vigorously for 1 min, after which 400 μL distilled water was added. Following inversion mixing, the sample was centrifuged for 10 min at 2,500 × g. The bottom layer was separated and dried using a centrifugal concentrator. The resultant solid product was dissolved in 600 μL dichloromethane/isopropanol (DM/IP: 9/1 v/v) to prepare a lipid extract sample.

E. Solid-phase extraction (SPE)

Lipid extract samples were applied to Strata-XL-AW tubes equilibrated with DM/IP (9/1 v/v), and the unbound fraction was eluted using 4 column volumes of DM/IP (6/1 v/v). Bound material was then eluted using one column volume of methanol, and the samples were dried using a centrifugal concentrator.

F MALDI-TOF mass spectrometry

Solid-phase extraction (SPE) samples were dissolved in 25 μL isopropanol/acetonitrile (IP/AN: 3/2 v/v); then, 25 μL 9-AA (10 mg/mL in IP/AN: 3/2 v/v) was added. Following mixing, 0.25 μL was placed in a sample well plate and allowed to dry naturally. Samples were then analyzed using a MALDI-TOF/TOF MS 5800 system (AB SCIEX, Framingham, MA, USA) in the negative ion mode. LPE-acyl and LPE-pl peaks were detected in the region *m/z* 400–600. The peak molecular species were determined using product ion analysis. MS data analysis was performed by quantifying the peaks using image analysis software (Data Explore, AB SCIEX).

G Gas chromatography mass spectrometry (GC MS)

Total FA in serum was analyzed using GC MS. Serum (200 μL), the internal standard (C17:0 FA), and a C/M mixed solution (2/1 v/v, 2 mL) were added to a glass tube and mixed. Subsequently, 5 times the quantity of water was added to the sample mixture. After centrifugal separation for 10 min at 2,500 × g, the lower layer liquid was dispensed, and the lipid sample was dried. The lipid sample was subjected to trans-methyl esterification of all FAs using the method described by Taketomi et al[22]. The GC MS device used was GC-MS-QP2010 (Shimadzu, Kyoto). FA methyl esters were analysed using the temperature rise method (70–

280 °C), with a cyanopropyl polysilphenylene-siloxane GC capillary column (BPX-90; film thickness: 0.25 μm; length: 60 m; inside diameter: 0.25 mm; SGE Analytical Science Pvt., Ltd., Melbourne, Australia).

H Statistical analysis

Data are expressed as mean ± standard deviation (SD). The Student's *t*-test was used for a statistical evaluation of significant difference between the two groups. Correlation was assessed by linear regression analysis. Statistical analysis was performed using Stat Flex Ver.6 software (Artech Co., Ltd. Osaka)

III Results

A Clinical laboratory data

Table 1 shows the clinical characteristics of the seven patients with AIP and the nine healthy subjects. Except for one patient, the serum IgG4 levels were 10 times higher (from 1.5) than the upper limit of the reference value. The lipid data, total cholesterol (TC), high-density lipoprotein–cholesterol (HDL-C), low-density lipoprotein–cholesterol (LDL-C), and triglyceride (TG) were within the normal range. Other clinical laboratory data were also within the normal range.

B Total FA compositions in serum

Serum total FA levels in patients with AIP and healthy subjects were measured using GCMS. AIP patient serum was found to have lower stearic acid (SA; C18:0) and higher arachidonic acid (ARA; C20:4) levels than those of healthy subjects ($p = 0.048$ and $p \leqq 0.001$, respectively) (Fig. 2). Other serum fatty acids did not have the significant difference in both groups.

C Serum LPE species level

We measured the LPE molecular species levels in serum from patients with AIP and healthy subjects (Table 2). Patients with AIP showed lower serum total LPE, LPE-pl, and LPE-acyl levels compared to those in healthy subjects. Furthermore, LPE species analysis showed that patients with AIP had significantly lower levels of LPE p16:0, LPE p18:1, LPE p18:0, LPE 16:0, LPE 18:0, LPE 20:5, and LPE 20:4 than those of healthy subjects (Table 2).

D Correlation between LPE species

We examined the correlations between LPE species in serum from healthy subjects and patients with AIP. In healthy subjects, LPE 20:4 levels positively correlated with LPE 20:5 levels (r = 0.67), but showed no correlation with LPE 22:6 levels (r = 0.12) (Fig. 3-A). In patients with AIP, there was a positive correlation between LPE 20:4 levels and LPE 20:5 and LPE 22:6 levels (r = 0.61 and 0.91, respectively) (Fig. 3-B).

We also examined the correlations between the three LPE-pls, LPE p16:0, LPE p18:1and LPE p18:0, and LPE-PUFAs, LPE 20:4, LPE 20:5, and LPE 22:6, respectively. In healthy subjects, LPE p16:0 levels positively correlated with LPE 22:6 levels (r = 0.60), but not with other LPE-PUFA levels (Fig. 4-A). LPE p18:1 and

LPE p18:0 in healthy subjects did not have a correlation with other LPE-PUFAs (Fig. 4-B, C). In patients with AIP, LPE p16:0 levels showed a strong negative correlation with LPE 20:4, LPE 20:5, and LPE 22:6 levels (r = 0.72, 0.63, and 0.68, respectively) (Fig. 4-D). LPE p18:1 level positively correlated with LPE 20:4 and LPE 22:6 levels (r = 0.59 and 0.55, respectively), but did not correlate with LPE 20:5 levels (r = 0.26) (Fig. 4-E). There was no correlation between LPE p18:0 and other LPE-PUFA levels (Fig. 4-F).

IV Discussion

This study showed that though serum lipids and the fatty acid compositions of patients with AIP resembled healthy subjects, serum LPE level were significantly low and serum LPE species linked and fluctuated. LPE 20:4 was positively correlated with LPE 20:5 and LPE 22:6, and LPE p16:0 (plasmalogen type) was negatively correlated with LPE-PUFAs. It was reported that lysophospholipid functions as endogenous bioactivity lipids for the life activity in cells such as an inflammatory cascade and a cell death[23]. The bioactive lipids are related to immune responses, and the association with the common autoimmune disease is found[14]. It is reported that LPE-PUFA and LPE-pl (plasmalogen type) provide anti-inflammatory functions[17]. Water-soluble LPE is present in serum, but pathophysiological roles and the metabolic details of serum LPE are not known. The analysis of the serum LPE species may contribute to explaining a mechanism of the lipid metabolism of the patients with AIP.

PEs are glycerophospholipids with two types, diacyl-type and a plasmalogen-type. The former is included two ester bonded fatty acid chains (acyl group) at *sn-1* and *sn-2* positions, and the latter is included a vinyl-ether and an ester bond at the *sn-1* and *sn-2* position, respectively. Particularly, PUFAs are included in *sn-2* position of the PE[24]. PUFAs and plasmalogens are known to play those roles in an inflammatory process. Because a difference of the side chain influences a function of the PE, it is necessary to identify molecular species. However, multiple PE molecular species are present in number of the same mass electric charge ratio (*m/z*). It is difficult to identify PE species using mass spectrometry. LPE is hydrolyzed metabolite by the phospholipase A (PLA) of PE. LPE is isolated from a cell membrane easily and is present in serum; the side chain of the glycero-frame is one of the acyl group or alkenyl group. LPE has an important function as bioactive lipids in an inflammatory process, but a pathophysiological role of serum LPE and the metabolic details are not known. Recently, the analysis of serum LPE species by LC-MS/MS was reported[17] but the clinical application is difficult, because the operation is cumbersome. MALDI-TOF MS is a simple and easy analysis for the LPE species. In this study, the characterization of the serum LPE species in the autoimmune pancreatitis (AIP) was analyzed by MALDI-TOF MS.

It was reported that the serum LPE level was associated with a serum PE level, because that PE and LPE decreased together in the NASH patients[19]. Serum LPE may be provided an insight into PE metabolism. The lower LPE-pl (plasmalogen-type) levels in patients with AIP may reflect lower PLA2 activity, and the lower LPE 20:4, LPE 20:5 and LPE 22:6 levels may reflect lower PLA1 activity. Phospholipase isozyme, sPLA2-IID, is secreted by the dendritic cells of lymph nodes, and shows high selectivity to PE[25]. It promotes selective hydrolysis of PUFA from PE; this has been reported to control inflammatory reactions by suppressing

the Th1 reply of lymph nodes[26]. It is not clear whether PLA2 and PLA1 activities are mediated by the same or different enzyme proteins. PLA1, which can act upon PE, is intracellular, and it has been found in cytosolic fractions and microsomes[27]. Pancreatic lipase (carboxylesterase) of the pig pancreas and rat liver cytosol exhibits PLA1 activity, in addition to acylglycerol and carboxylesterase activity[28]. There are also PS-specific PLA1 (PS-PLA1) activity and PA-selective PLA1α and PLA1β (PA-PLA1α and PA-PLA1β) with only PLA1 activity[29]. PS-PLA1 activity in stimulated rat platelets has been reported. Intracellular PLA1α and PLA1γ (iPLA1α and iPLA1γ) develop by progress among intracellular PLA_1 (iPLA1) and exhibit PLA1 activity that uses phospholipids such as PE as substrates[30]. However, sufficient information on PLA1 for PE is lacking.

The LPE 20:4 concentrations of patients in AIP correlated significantly with LPE 22:6 and LPE 20:5. However, LPE 22:6 did not correlate with the LPE 20:4 concentrations in healthy subjects. Furthermore, LPE 20:4, LPE 20:5 and LPE 22:6 of patients in AIP correlated negatively with LPE p16:0. These results suggested that the hydrolysis of LPE-PUFA by PLA1 may be associated with the PLA2 which is specific for LPE p16:0 generation in patients with AIP. It was suggested that the decrease in the plasmalogen levels of the patients with AIP induces or worsens oxidative stress, because plasmalogen has antioxidant properties[31]. Kurano et al reported that LPE-PUFA of the patients with acute coronary syndrome was increased[32]. Arachidonyl-LPE (LPE 20:4) and LPE 22:6 by zymosan A-induced peritonitis model provide anti-inflammatory action[33], but the details are not known. The change that LPE C20:4 and LPE C22:6 tuned to may be associated with anti-inflammatory action. Recently Yamamoto et al. reported that LPE molecular species decreased in NAFLD[19]. It was reported that the inflammation of the liver in the NASH patients may increase lysophosphatidylcholine transferase three or four (respectively, LPCAT3, 4), expression of lysophosphatidylethanolamine transferase

1 (LPEAT1), and subsequently the serum LPE level was reduced. However, it is unknown of the metabolism mechanism of cells.

A low dose of steroid (six patients: prednisone of 5mg/day, one patient; celestamine of 0.5mg/day) was given to patients with AIP. By the steroid therapy, side reaction of the disorder of lipid metabolism is reported, but is increase of serum cholesterol and the triglyceride primarily. In the patients in AIP, the serum lipids were almost all criteria ranges. It was reported that arachidonate is inhibited to be released by steroid reagent[34]. In this study, serum ARA level of the patients with AIP was significantly high than a healthy subject, and, as for serum fatty acid composition of patients with AIP, as for other PUFAs, there was not the significant difference. The effect by low dose steroid may be low in this study. However, we have not enough evidence whether or not the change of phospholipid molecular species is caused by a steroid, and it is a future problem.

V Conclusion

This study showed that though serum lipids and the fatty acid compositions of patients with AIP resembled healthy subjects, serum LPE level of patients were significantly low and some serum LPE species were fluctuated in a correlated way. The analysis of the serum LPE species of MALDI-TOFMS may contribute to reveal the molecular mechanism and the pathophysiological role of the LPE metabolism in the inflammatory disease.

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Conflict of interest

The authors declare that they have no conflicts of interest.

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Figure Legends

Fig. 1 Structures of diacyl (A) and alkenylacyl (B) phosphatidylethanoamine.

R': saturated fatty acid (SFA), monounsaturated fatty acid (MUFA); R": poly unsaturated fatty acid (PUFA); *sn-1*, *sn-2* and *sn-3* glycerol position; PLA1: phospholipase A_1; PLA2: phospholipase A_2; dotted line: breakpoint of the phospholipase A.

Fig. 2 Concentration of total fatty acid in serum

Open column: healthy subjects; closed column: patients with AIP. *$p < 0.05$; **$p < 0.01$.

Fig 3. Correlation of LPE 20:4 versus LPE 20:5 and LPE 22:6 in serum of healthy subjects and patients with AIP

A: healthy subjects; B: patients with AIP. Open circle (dotted line): LPE 20:4 vs LPE 20:5; Closed circle (solid line): LPE 20:4 vs LPE 22:6. LPE: lysophosphatidylethanolamine.

Fig 4. Correlation between LPE-pl (plasmalogen-type) and LPE-PUFA species in serum of healthy subjects and patients with AIP.

A–C: healthy subjects; D–F: patients with AIP. A, D: LPE p16:0 vs LPE-PUFA; B, E: LPE p18:1 vs. LPE-PUFA; C, F: LPE p18:0 vs LPE-PUFA. Open triangle (dotted line): LPE 20:4; open circle: LPE 20:5 (dashed dotted line); closed circle (solid line), LPE 22:6. LPE p16:0, LPE p16:1 and LPE p18:1. LPE-PUFA: LPE 20:4, LPE 20:5, and LPE 22:6.

Table 1 Clinical characteristics of the patients with AIP and the healthy subjacts

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Table 2 Concentration of LPE species in serum

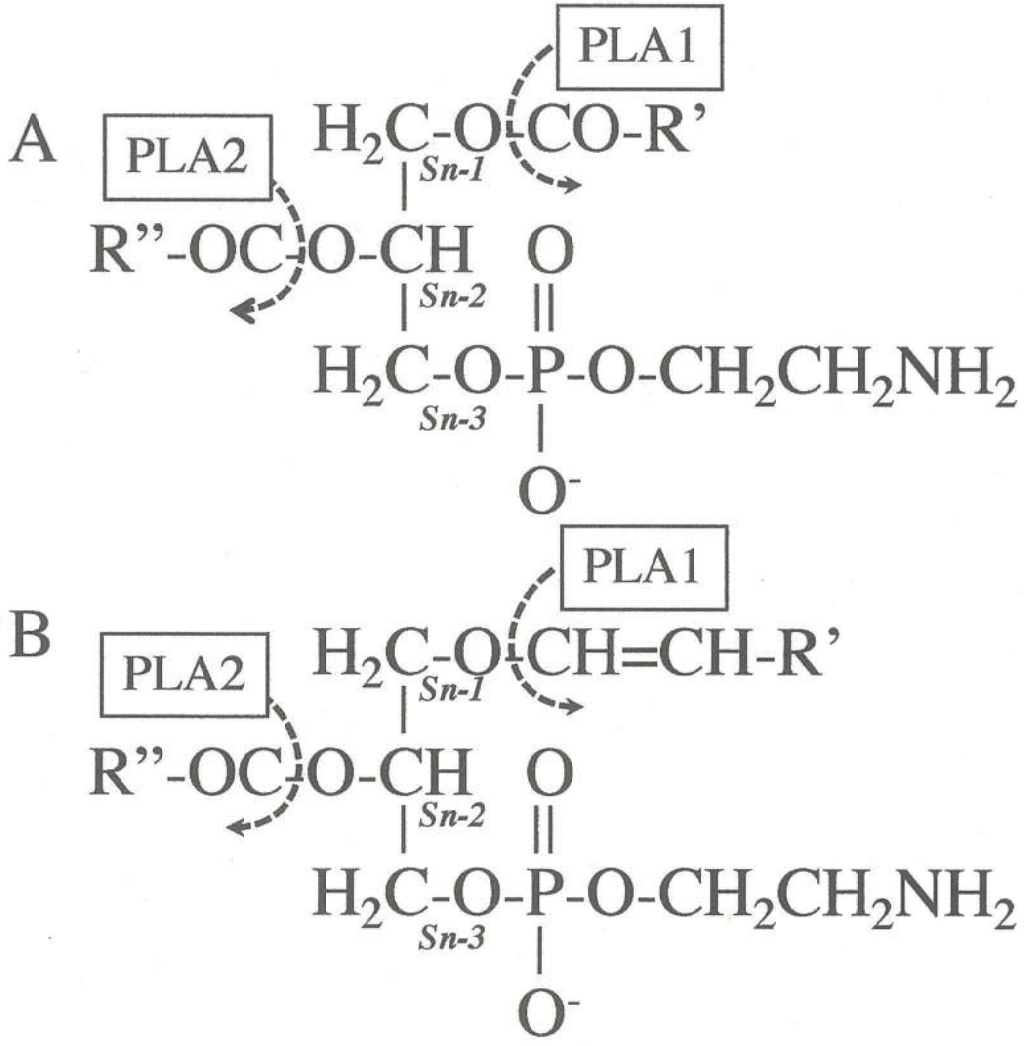
LPE: lysophosphatidylethanolamine, plasmalogen-type: LPE p16:0, LPE p18:1 and LPE p18:0, acyl-type: LPE 16:0, LPE 18:2, LPE 18:1, LPE 18:0, LPE 20:5, LPE 20:4 and LPE 22:6.

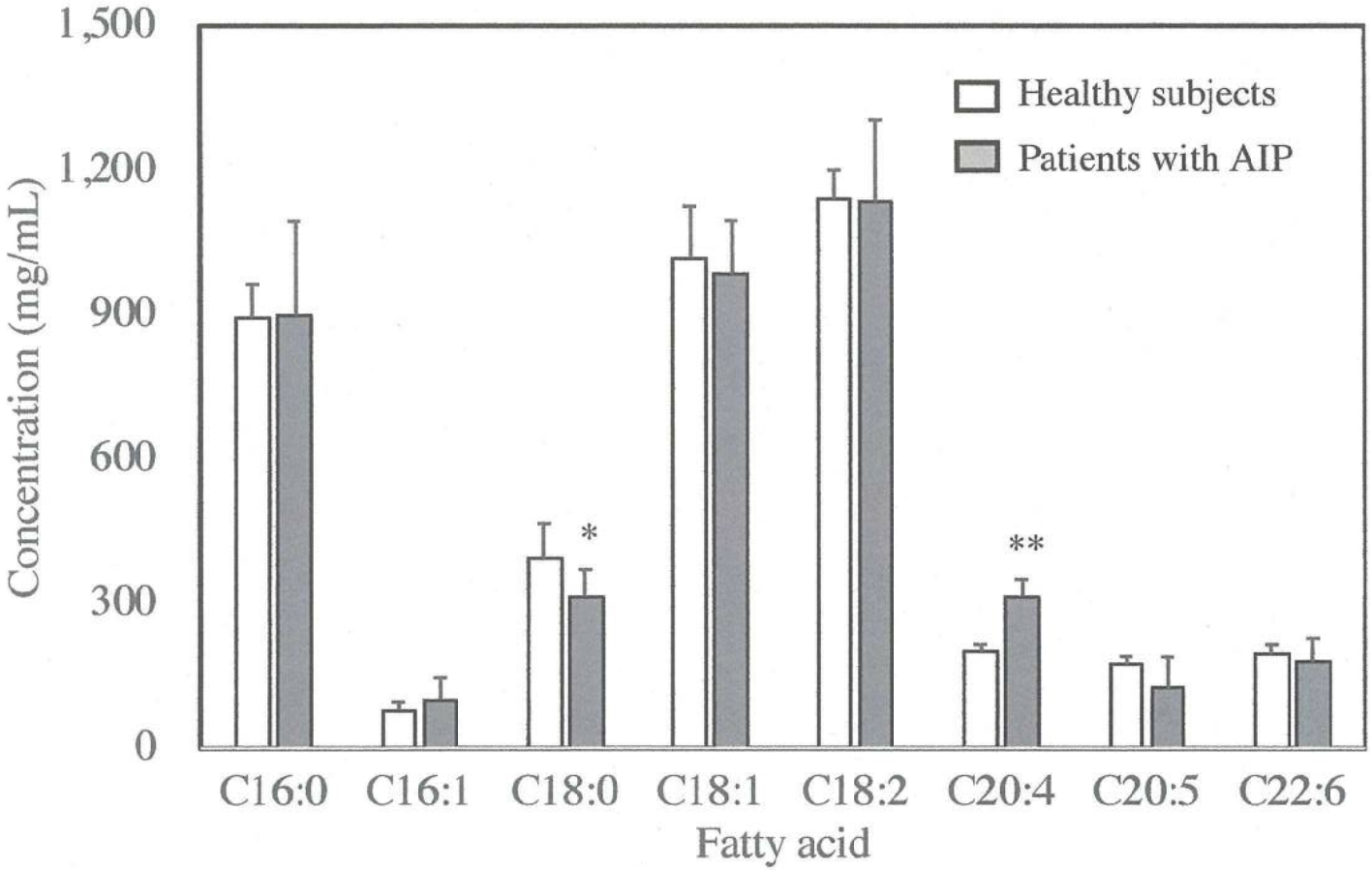
Table 1 Clinical characteristics of the patients with AIP and healthy subjects

	Normal range	Unit	Healthy subjects	AIP patietns
Age		years	53.1 ± 11.7	69.1 ± 10.6
BMI	18.0 – 25.0 [1)]		–	23.7 ± 3.6
Glucose	73 – 109	(mg/dL) [2)]	100.6 ± 27.8	114.5 ± 10.6
TC	142 – 248	(mg/dL) [2)]	202.0 ± 26.9	184.1 ± 30.6
HDL-C	≧ 40	(mg/dL) [3)]	66.6 ± 9.8	67.1 ± 18.2
LDL-C	≦ 140	(mg/dL) [3)]	115.4 ± 22.0	90.6 ± 17.5
TG	≦ 150	(mg/dL) [3)]	100.0 ± 36.8	115.6 ± 40.3
CRP	≦ 0.14	(mg/dL) [2)]	< 0.10	0.090 ± 0.107
IgG4	≦ 135	(mg/dL) [2)]	53.7 ± 24.6	493.9 ± 410.9
UA	< 7.8	(mg/dL) [2)]	5.18 ± 1.28	6.01 ± 0.82
AST	13 – 30	(IU/L) [2)]	19.2 ± 4.5	22.1 ± 6.5
ALT	7 – 42	(IU/L) [2)]	15.7 ± 6.0	18.6 ± 8.1
γ-GT	9 – 32	(IU/L) [2)]	18.4 ± 5.1	19.1 ± 12.9

Table 2 Concentration of LPE species in serum

	Healthy subjects		Patients with AIP		
LPE	mean	SD	mean	SD	*P*-value
p16:0	2.51	0.49	1.87	0.30	0.011
p18:1	1.85	0.36	1.23	0.46	0.010
p18:0	3.06	0.70	2.15	0.47	0.002
16:0	3.19	0.88	2.17	0.97	0.060
18:2	3.55	0.74	2.26	1.11	0.022
18:1	2.54	0.67	1.63	1.20	0.094
18:0	4.50	0.68	3.08	0.77	0.003
20:5	2.68	1.31	1.15	1.27	0.047
20:4	2.16	0.30	1.34	0.76	0.012
22:6	2.05	0.42	1.69	1.26	0.462
	(μmol/ L)		(μmol/ L)		


A
PLA1
PLA2
H2C-O-CO-R'
Sn-1
R''-OC-O-CH
Sn-2
O
H2C-O-P-O-CH2CH2NH2
Sn-3
O-
B
PLA1
PLA2
H2C-O-CH=CH-R'
Sn-1
R''-OC-O-CH
Sn-2
O
H2C-O-P-O-CH2CH2NH2
Sn-3
O-


1,500
1,200
900
600
300
0
Concentration (mg/mL)
Healthy subjects
Patients with AIP
*
**
C16:0
C16:1
C18:0
C18:1
C18:2
C20:4
C20:5
C22:6
Fatty acid

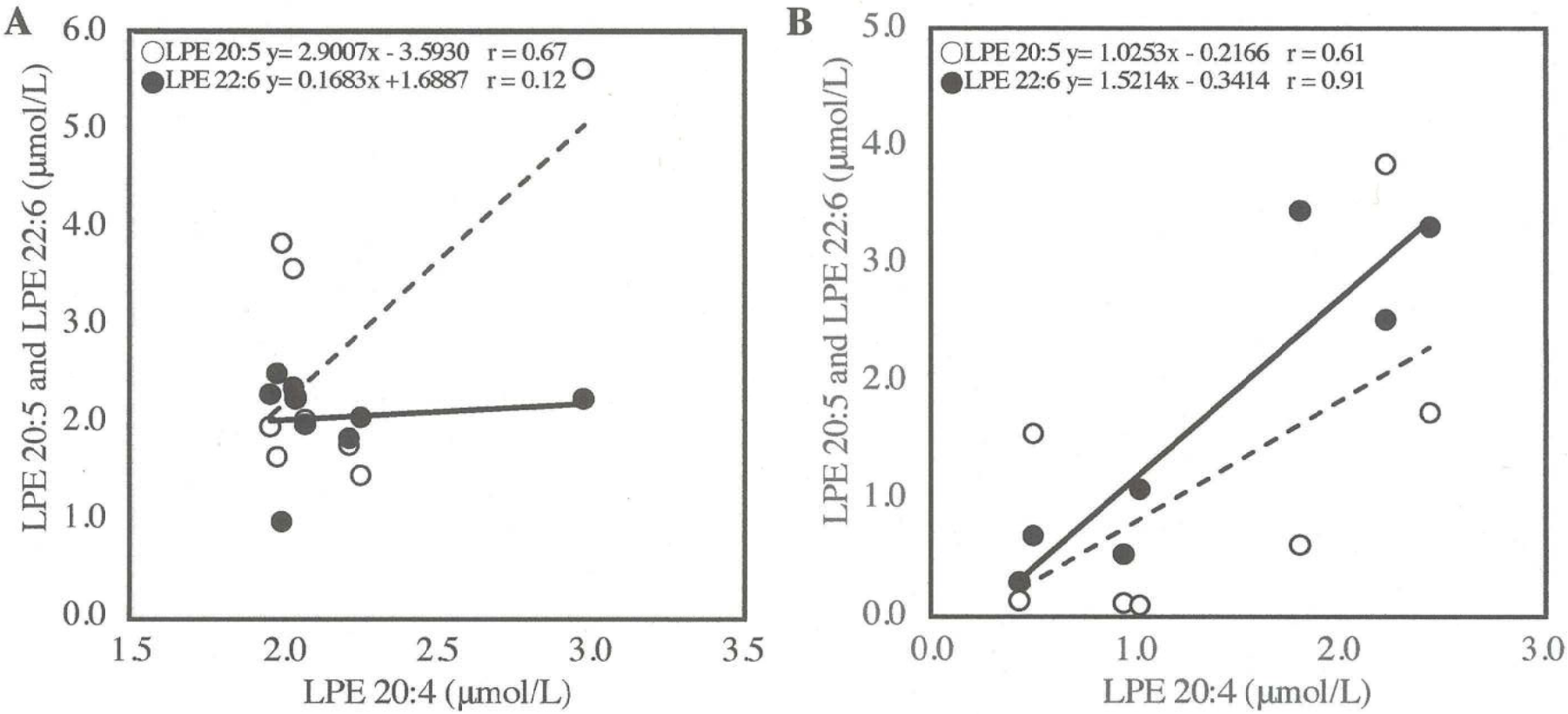
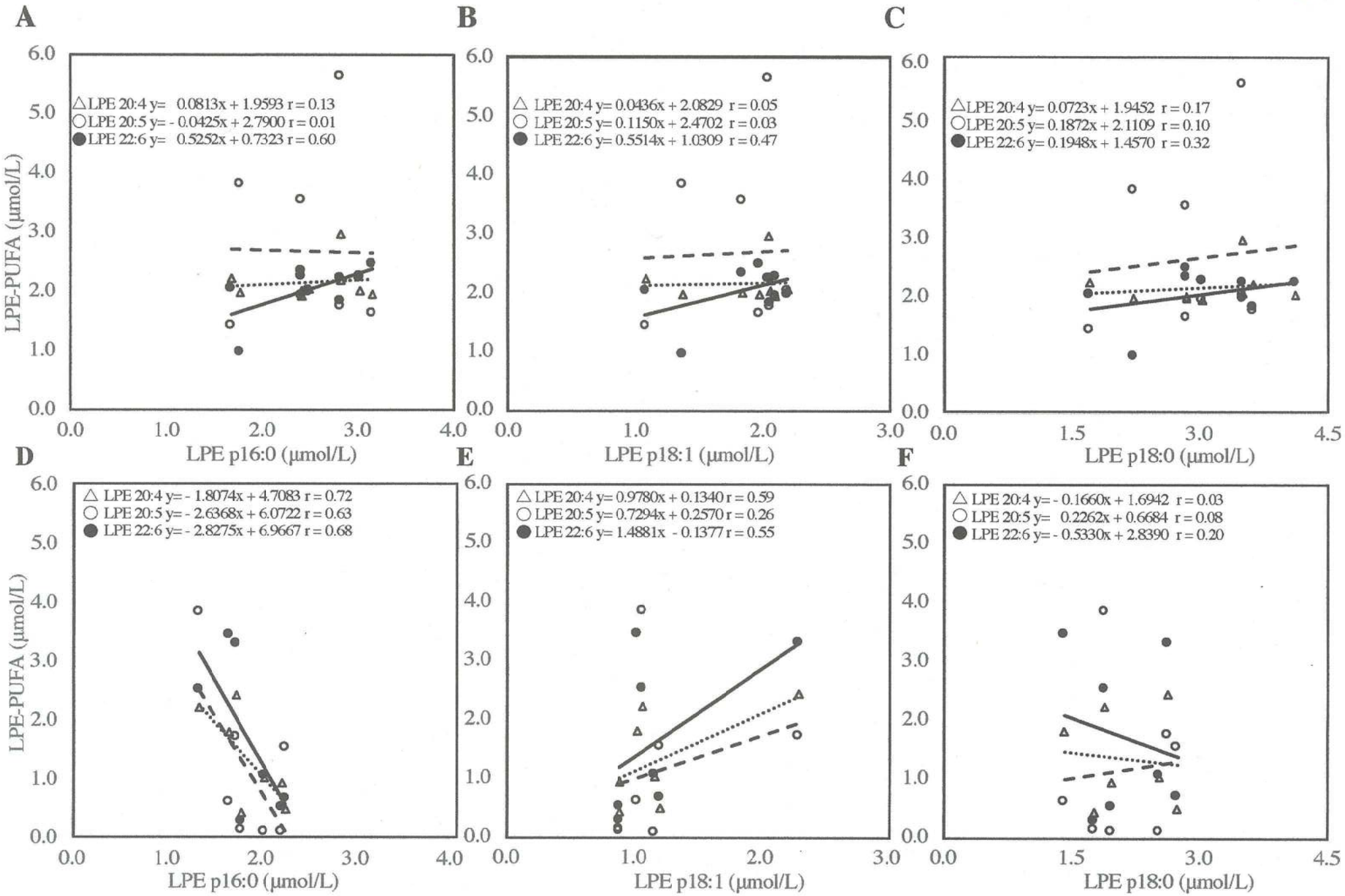

A
○LPE 20:5 y= 2.9007x - 3.5930 r = 0.67
●LPE 22:6 y= 0.1683x +1.6887 r = 0.12
LPE 20:5 and LPE 22:6 (μmol/L)
LPE 20:4 (μmol/L)
6.0
5.0
4.0
3.0
2.0
1.0
0.0
1.5
2.0
2.5
3.0
3.5
B
○LPE 20:5 y= 1.0253x - 0.2166 r = 0.61
●LPE 22:6 y= 1.5214x - 0.3414 r = 0.91
LPE 20:5 and LPE 22:6 (μmol/L)
LPE 20:4 (μmol/L)
5.0
4.0
3.0
2.0
1.0
0.0
0.0
1.0
2.0
3.0

Fig.4


A
△LPE 20:4 y= 0.0813x + 1.9593 r = 0.13
○LPE 20:5 y= - 0.0425x + 2.7900 r = 0.01
●LPE 22:6 y= 0.5252x + 0.7323 r = 0.60
LPE-PUFA (μmol/L)
LPE p16:0 (μmol/L)
B
△LPE 20:4 y= 0.0436x + 2.0829 r = 0.05
○LPE 20:5 y= 0.1150x + 2.4702 r = 0.03
●LPE 22:6 y= 0.5514x + 1.0309 r = 0.47
LPE p18:1 (μmol/L)
C
△LPE 20:4 y= 0.0723x + 1.9452 r = 0.17
○LPE 20:5 y= 0.1872x + 2.1109 r = 0.10
●LPE 22:6 y= 0.1948x + 1.4570 r = 0.32
LPE p18:0 (μmol/L)
D
△ LPE 20:4 y= - 1.8074x + 4.7083 r = 0.72
○ LPE 20:5 y= - 2.6368x + 6.0722 r = 0.63
● LPE 22:6 y= - 2.8275x + 6.9667 r = 0.68
LPE-PUFA (μmol/L)
LPE p16:0 (μmol/L)
E
△ LPE 20:4 y= 0.9780x + 0.1340 r = 0.59
○ LPE 20:5 y= 0.7294x + 0.2570 r = 0.26
● LPE 22:6 y= 1.4881x - 0.1377 r = 0.55
LPE p18:1 (μmol/L)
F
△ LPE 20:4 y= - 0.1660x + 1.6942 r = 0.03
○ LPE 20:5 y= 0.2262x + 0.6684 r = 0.08
● LPE 22:6 y= - 0.5330x + 2.8390 r = 0.20
LPE p18:0 (μmol/L)