Possible Involvement of Minor Lysophospholipids in the Increase in Plasma Lysophosphatidic Acid in Acute Coronary Syndrome

Makoto Kurano,* Akiko Suzuki,* Asuka Inoue, Yasunori Tokuhara, Kuniyuki Kano, Hirotaka Matsumoto, Koji Igarashi, Ryunosuke Ohkawa, Kazuhiro Nakamura, Tomotaka Dohi, Katsumi Miyauchi, Hiroyuki Daida, Kazuhsa Tsukamoto, Hitoshi Ikeda, Junken Aoki, Yutaka Yatomi

Objective—Lysophosphatidic acids (LPA) have important roles in the field of vascular biology and are derived mainly from lysophosphatidylcholine via autotaxin. However, in our previous study, only the plasma LPA levels, and not the serum autotaxin levels, increased in patients with acute coronary syndrome (ACS). The aim of this study was to elucidate the pathway by which LPA is increased in patients with ACS.

Approach and Results—We measured the plasma lysophospholipids species in 141 consecutive patients undergoing coronary angiography (ACS, n=38; stable angina pectoris, n=71; angiographically normal coronary arteries, n=32) using a liquid chromatography-tandem mass spectrometry analysis. Among the ACS subjects, notable increases in the 22:6 LPA, 18:2 LPA, and 20:4 LPA levels were observed. The in vitro experiments revealed that serum incubation mainly increased the 18:2 LPA level, whereas platelet activation increased the 20:4 LPA level. Minor lysophospholipids other than LPA were also elevated in ACS subjects and were well correlated with the corresponding LPA species, including 22:6 LPA. A multiple regression analysis also revealed that lysophosphatidylinositol, lysophosphatidylcholine, lysophosphatidylethanolamine, and lysophosphatidylglycerol were independent explanatory variables for several LPA species.

Conclusions—Specific LPA species, especially long-chain unsaturated LPA, were elevated in ACS patients, along with the corresponding minor lysophospholipids. The elevation of these LPA species might be mainly caused by presently unidentified LPA-producing pathway(s). Minor lysophospholipids might be involved in the generation of LPA, especially 22:6 LPA, and in the pathogenesis of ACS. (Arterioscler Thromb Vasc Biol. 2015;35:463-470. DOI: 10.1161/ATVBAHA.114.304748.)

Key Words: autotaxin ■ coronary disease ■ lysophosphatidic acids ■ lysophospholipids ■ molecular species

Lysophosphatidic acid (LPA) has been demonstrated to play important roles in the field of vascular biology, and the involvement of LPA and its receptors in the pathogenesis of several diseases, including fibrosis, autoimmune diseases, and cancer, has been elucidated. Further, basic studies of LPA have suggested the presence of both thrombogenic and atherogenic properties of this bioactive lipid. LPA induces platelet shape changes, the aggregation of platelets, platelet-monocyte aggregation, and an increase in the expression of tissue factor in plaques. Therefore, LPA is strongly speculated to be involved in plaque rupture, which is a characteristic of acute coronary syndrome (ACS). LPA has also been proposed to be involved in the development of atheromatous plaques: LPA induces the expression of adhesion molecules and chemokines in endothelial cells and the migration and secretion of inflammatory cytokines in smooth muscle cells. Therefore, these findings obtained from in vitro studies and from animal studies strongly suggest that LPA may have several roles in both the formation and rupture of atheromatous plaques. Hence, LPA and its receptors have been proposed as causative factors of ischemic heart disease and have been considered as possible pharmacological targets.

Recently, we measured the plasma LPA levels in subjects undergoing coronary angiography and found that the plasma levels of LPA were significantly elevated in patients with ACS, compared to those with stable angina pectoris or angiographically normal coronary arteries. These findings suggest that LPA may have a role in the pathogenesis of ACS.
LPA levels were elevated in patients with ACS but not in those with stable angina pectoris (SAP). Concordant with our previous report, an analysis of the plasma total LPA levels using LC-MS/MS revealed that the plasma total LPA levels were significantly elevated in the ACS group, compared with the normal coronary arteries or SAP group (Figure IA). Regarding the LPA species, the levels of 14:0 LPA, 16:0 LPA, 18:0 LPA, 18:3 LPA, 20:3 LPA, and 20:5 LPA were unchanged, whereas several unsaturated long chain LPAs (18:1 LPA, 18:2 LPA, 20:4 LPA, 22:5 LPA, and 22:6 LPA) were significantly elevated in ACS (Figure 1B–1D; Figure IA–IC in the online-only Data Supplement). Considering the absolute values of the LPA species, 22:6 LPA, 18:2 LPA, and 20:4 LPA, in particular, were increased in the order shown (Figure 1E). This result suggested that 22:6 LPA, 18:2 LPA, and 20:4 LPA are characteristic LPA species that are elevated in patients with ACS.

Because several clinical factors (sex, presence of diabetes mellitus, and regular medication with aspirin [although all the subjects who had not taken aspirin regularly took aspirin before they entered the catheter laboratory], β blocker, renin–angiotensin system inhibitor, or statin before administration) differed significantly among the 3 groups, as shown in a previous report, we performed a stratified analyses to exclude possible confounding factors (Tables I–IV in the online-only Data Supplement). The results from a stratified analysis of subjects showed that the increase of the total LPA and specific LPA molecular species (18:2 LPA, 20:4 LPA, and 22:6 LPA) was observed essentially in patients who did not regularly take aspirin, β blockers, renin–angiotensin system inhibitor, or statin. Regarding the effects of medication, several significant differences were observed only in the ACS group: the total LPA and 18:2 LPA levels were lower than those in patients who regularly took aspirin; the total LPA, 18:2 LPA, and 20:4 LPA levels were lower than those in patients who regularly took β blockers, and the 20:4 LPA level was lower than that in patients who regularly took statins.

**Autotaxin-Mediated Pathway Might not be Involved in the Production of 22:6 LPA**

To elucidate the sources of the elevated LPA levels in ACS, we first investigated the autotaxin-mediated pathway because LPA is thought to be mainly produced from LPC via autotaxin. The correlations between the serum autotaxin level and each LPA species are shown in Figure 1F–1H. The 18.2 LPA (r=0.397; P<0.001) and 20:4 LPA (r=0.277; P=0.001) levels were more strongly correlated with the autotaxin level than with the 22:6 LPA level (r=0.218; P=0.009). 16:0 LPA is a major LPA species, but its level remained unchanged in ACS, whereas the 18:1 LPA and 22:5 LPA levels were significantly elevated in ACS; the 16:0 LPA and 18:1 LPA levels were significantly correlated with the autotaxin level (r=0.277; P=0.001 and r=0.336; P<0.001, respectively), whereas the 22:5 LPA level was not (Figure ID–IF in the online-only Data Supplement).

To confirm these results in vitro, we incubated the serum for 24 hours to investigate which LPA species increased under in vitro conditions where autotaxin is mainly involved in LPA production; in serum, the substrate LPC and the enzyme autotaxin coexist and are involved in LPA production. Although all the LPA species were elevated and the increases were inhibited by the autotaxin inhibitor HA130, the 18:2 LPA level increased to the largest degree among all the species (Figure 2), which was
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Figure 1. Increased lysophosphatidic acid (LPA) species in acute coronary syndrome (ACS) subjects and their correlation with autotaxin levels. A–D, Total and molecular species of LPA in normal coronary arteries (NCA), stable angina pectoris (SAP), and ACS groups. *P<0.01. A, Total LPA; B, 18:2 LPA; C, 20:4 LPA; and D, 22:6 LPA. The bar represents the mean values and the box represents ±SD. E, Amount of increased LPA species in the ACS group, compared with the normal coronary arteries (NCA) group. F–H, Correlations of plasma concentrations of LPA species with serum autotaxin levels. F, 18:2 LPA; G, 20:4 LPA; and H, 22:6 LPA.

Figure 3A, all the LPA species that were specifically elevated in ACS instead decreased in a manner dependent on the CuSO₄ concentration, whereas saturated LPA dramatically increased during lipoprotein oxidation under the same conditions. Accordingly, it seems unlikely that LDL oxidation is involved in the elevation of unsaturated long chain LPAs observed in ACS.

Platelet Activation Produced 20:4 LPA in Particular

Next, we challenged the possibility that platelet activation, which is provoked after plaque rupture, might produce LPAs characteristic of ACS. As shown in Figure 3B and 3C, 20:4 LPA was increased during platelet activation by thrombin to the greatest degree, followed by 18:0 LPA and 18:1 LPA, whereas 18:2 LPA and 22:6 LPA were only marginally increased. Platelets are reportedly associated with autotaxin, and autotaxin has been proposed to be involved in platelet-derived LPA production.²⁶ Consistently, the elevation of 20:4 LPA was inhibited by the autotaxin inhibitor HA130, demonstrating that ≥20:4 LPA seems to be derived from the autotaxin-associated LPA-producing pathway, as reported previously.²⁵

We performed similar experiments in the presence of corresponding platelet-poor plasma; however, we did not observe any specific modulations by thrombin of the LPA species that were characteristic of ACS (Figure II in the online-only Data Supplement).

Accordingly, platelet activation might be involved in the elevation of 20:4 LPA in ACS, but not in the elevation of other unsaturated long chain LPAs.

Oxidation of Low-Density Lipoprotein

Increased Saturated LPA and Decreased Unsaturated LPA Levels

Next, we investigated the possible involvement of lipoprotein oxidation because the oxidation of low-density lipoprotein (LDL) is deeply related to atherosclerosis, and as previously reported,²¹,²⁵ LPA increased when lipoproteins were oxidized. As shown in Figure 2, changes in each lysophosphatidic acid (LPA) species from serum samples after a 24-hour incubation period. Serum samples (n=4) were incubated for 24 hours at 37°C with the vehicle or 50 µM HA130. The data shows the mean±SD. *P<0.01, †P<0.05.
Possible Involvement of Minor LPLs in Elevated LPA Levels in Human Subjects

In our study using LC-MS/MS, we were able to measure not only the LPA species, but also the species of other LPLs: LPC, LPG, LPI, LPE, and lysophosphatidylserine. Although LPA is mainly produced from LPC by autotaxin, other minor LPLs might have the potential to be converted into LPA, considering their structures. So, to evaluate the possible involvement of minor LPLs in LPA synthesis, we next analyzed the relation between LPA and other LPLs using statistical analyses.

Interestingly, as shown in Figure 4, the total LPE, LPG, LPI, and lysophosphatidylserine levels were elevated in addition to the LPA level in patients with ACS. Regarding the molecular species, almost every unsaturated long chain species of LPL, including LPC, was elevated in patients with ACS (Tables V–IX in the online-only Data Supplement).

We examined the correlation between LPA species and other LPLs of corresponding species. As shown in Figure 5 and Figure III in the online-only Data Supplement, 18:2 LPA, 20:4 LPA, and 22:6 LPA possessed significant correlations with the corresponding LPC, LPE, LPI, lysophosphatidylserine, and LPG species, with stronger correlations observed for LPC, LPE, and LPI. Such correlations were not clearly observed for other LPA species, especially saturated or short-chain LPA (14:0 LPA, 16:0 LPA, 18:0 LPA, and 18:3 LPA; Table X in the online-only Data Supplement).

To analyze which factors might be independent explanatory variables for LPA, we further performed stepwise multiple regression analyses using the LPA species as objective variables and the corresponding LPL species and serum autotaxin levels as explanatory variables. The explanatory variables for LPA seemed to differ among these LPA species: for 18:2 LPA, autotaxin and 18:2 LPC were selected as important variables (Table), consistent with the in vitro experiments (Figure 2). These results suggested that 18:2 LPA was derived mainly from the autotaxin-mediated pathway. For 20:4 LPA, 20:4 LPI was selected as the most important variable, followed by 20:4 LPC and autotaxin (Table). Considering that LPI is generated from phosphatidylinositol in activated platelets, this result might be reasonable because 20:4 LPA was generated to a greater degree by platelet activation in our in...
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Discussion

LPA has been demonstrated to have proatherosclerotic properties, and this bioactive lipid and its receptors have been proposed to be potential pharmacological targets for

Figure 5. Correlations between lysophosphatic acid (LPA) and other lysophospholipids. A-C, Correlation between 18:2 LPA and other lysophospholipids: 18:2 LPC (µM) and 18:2 LPI (µM) (A), 18:2 LPC (µM) and 18:2 LPE (µM) (B), 18:2 LPC (µM) and 18:2 LPG (µM) (C). The lysophospholipids of the corresponding molecular species and autotaxin were used as the possible explanatory factors.

Table. Multiple Regression Analyses for Plasma LPA Species

<table>
<thead>
<tr>
<th>B</th>
<th>95% CI</th>
<th>Standardized β</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autotaxin</td>
<td>0.126 (0.082–0.170)</td>
<td>0.309</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LPC</td>
<td>0.003 (0.001–0.004)</td>
<td>0.301</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LPI</td>
<td>0.064 (0.025–0.103)</td>
<td>0.289</td>
<td>0.002</td>
</tr>
<tr>
<td>LPE</td>
<td>0.014 (0.000–0.028)</td>
<td>0.217</td>
<td>0.047</td>
</tr>
<tr>
<td>LPC</td>
<td>0.116 (0.093–0.139)</td>
<td>0.581</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LPC</td>
<td>0.006 (0.004–0.008)</td>
<td>0.292</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Autotaxin</td>
<td>0.043 (0.021–0.066)</td>
<td>0.203</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LPI</td>
<td>−3.136 (−5.369 to −0.903)</td>
<td>−0.143</td>
<td>0.006</td>
</tr>
</tbody>
</table>

Multiple regression analyses for plasma 18:2 LPA (A), 20:4 LPA (B), and 22:6 LPA (C). The lysophospholipids of the corresponding molecular species and autotaxin were used as the possible explanatory factors.

CI indicates confidence interval; LPA, lysophosphatic acid; LPC, lysophosphatidylcholine; LPE, lysophosphatidylethanolamine; LPG, lysophosphatidylglycerol; and LPI, lysophosphatidylinositol.

atherosclerotic diseases. Recently, we reported that the plasma LPA level is elevated in ACS subjects, suggesting the possible involvement of LPA in plaque instability and rupture. LPA is mainly produced via an autotaxin-mediated pathway, and in healthy subjects and patients with several diseases, the plasma LPA level is closely correlated with the serum autotaxin level. In patients with ACS, however, the serum autotaxin level was not elevated, compared with the LPA level.

In this study, we aimed to reveal the sources of LPA elevation in patients with ACS and to determine the species of LPA using LC-MS/MS. We found that compared with the normal coronary arteries and SAP patients, the levels of 22:6 LPA, 18:2 LPA, 20:4 LPA, 18:1 LPA, and 22:5 LPA were increased in patients with ACS in the order shown (Figure 1A–1E; Figure 1A–1C in the online-only Data Supplement). According to a previous report by others, the species of acyl LPA in the lipid-rich core regions of human atherosclerotic plaques are composed mainly of 18:0 LPA, followed by 18:1 LPA, 16:0 LPA, and 20:4 LPA, in this order, which is different from the molecular species of LPA that were elevated in the plasma of the ACS patients in our study. Therefore, this discrepancy suggests that the elevated LPA levels in the plasma of ACS patients did not derive directly from the ruptured plaques, but from some secondary reactions, such as platelet activation, occurring after plaque rupture. Alternatively, subjects with larger amounts of unsaturated long chain LPA might be more prone to have plaque rupture. To our knowledge, our article demonstrated for the first time that specific LPA species were elevated in the plasma of ACS subjects.

To elucidate the sources of the elevations in LPA species in ACS (22:6 LPA, 18:2 LPA, and 20:4 LPA), we first investigated the possible origins of these LPA species using in vitro experiments. As mentioned previously, LPA is mainly produced by autotaxin. Hence, we determined which LPA species were produced by autotaxin by incubating serum. As shown in vitro experiment than the other LPA species (Figure 3B and 3C). However, for 22:6 LPA (the most dominant LPA species elevated in ACS), 22:6 LPC, 22:6 LPE, and a negative relation for 22:6 LPG (although the absolute amount of 22:6 LPG was rather small) were selected as independent explanatory factors, but not autotaxin. These results suggested that 22:6 LPA might originate mainly from an unknown pathway during ACS. Considering the correlation between LPA species and the corresponding minor LPL species and the results from multiple regression analyses, minor LPLs might also be involved in this unknown LPA-producing pathway. Actually, factors other than the classical molecules involved in the autotaxin-mediated, LPA-producing pathway (autotaxin and LPC) were selected as significant explanatory factors for other LPA species, as follows. For 18:1 LPA, 18:1 LPI and 18:1 LPE were selected as positive explanatory factors along with autotaxin, whereas LPC was not selected as a positive factor. On the other hand, for 16:0 LPA, 16:0 LPI was selected as a positive explanatory factor along with autotaxin but not 16:0 LPC (rather, 16:0 LPC was selected as a negative explanatory factor). Moreover, for 22:5 LPA, only 22:5 LPI was selected, and neither autotaxin nor lysophosphatidylcholine was selected (Table XI in the online-only Data Supplement).
in Figure 2, the level of 18:2 LPA, in particular, increased followed by 16:0 LPA and 20:4 LPA, although all the LPA species were elevated and the increases were inhibited by an autotaxin inhibitor. This result was consistent with the correlation between the LPA species and the serum autotaxin levels (Figure 1C–E); compared with the significant positive correlations observed between 18:2 LPA or 20:4 LPA and autotaxin, the correlation between 22:6 LPA and autotaxin was relatively weak. Of note, although 16:0 LPA increased in vitro, the 16:0 LPA level was not elevated in ACS subjects (Figure IA in the online-only Data Supplement). One possible explanation for this discrepancy is that the serum incubation experiment could not completely reproduce the in vivo conditions, at least for 16:0 LPA, the reasons for which remain unknown. Actually, 16:0 LPA was not correlated positively with 16:0 lysophosphatidylcholine in vivo (Table X in the online-only Data Supplement). Further studies are needed to elucidate the involvement of autotaxin in the production of LPA in vivo.

LPA is also known to increase during lipoprotein oxidation, which is believed to accumulate in atherosclerotic lesions; consequently, we evaluated the increase in LPA species in oxidized LDL. As shown in Figure 3A, saturated LPA species, such as 18:0 LPA and 16:0 LPA, were mainly increased. This result suggests that the modification of LPA species during lipoprotein oxidation could not explain the increase in LPA in patients with ACS, although we cannot exclude the possibility that the in vitro experiments failed to reproduce the conditions in vivo; unsaturated LPA might be produced locally at the atherosclerotic lesions and then rapidly escaped into the blood stream from the oxidizing situations in vivo, whereas lipoproteins continued to be exposed to the oxidizing circumstance in this experiment.

ACS is characterized by plaque rupture, which activates platelets, and LPA is known to increase in response to platelet stimulation. So, we next investigated which LPA species increased during platelet activation. As shown in Figure 3B and 3C, the ranking of the LPA species that increased during platelet activation was 20:4 LPA>18:0 LPA>18:1 LPA>16:0 LPA=22:6 LPA=18:1 LPA, which was somewhat different from the results of previous reports. Sano et al showed that the ranking of LPA species secreted from thrombin-stimulated platelets was 18:0 LPA>20:4 LPA>18:1 LPA>16:0 LPA>18:2 LPA, whereas Gerard et al reported a ranking of 16:0 LPA>18:0 LPA>20:4 LPA>18:1 LPA>18:2 LPA. This inconsistency remains to be resolved, but individual variations might be involved. In fact, platelets from some subjects secreted more 18:0 LPA than 20:4 LPA. In either case, we can safely conclude that among the LPA species that were elevated in ACS, 20:4 LPA increased to the greatest degree during platelet activation. We further performed similar experiments in the presence of corresponding platelet-poor plasma; however, the presence of platelet-poor plasma obscured the increases of LPAs by platelet stimulation, probably because of a great LPA production by autotaxin using the substrates, such as lysophosphatidylcholine, in the plasma. On the contrary, LPA production via autotaxin might be in an equilibrium state in human subjects.

Next, we tried to elucidate the origins of the increase in LPA in ACS patients using statistical methods. Although the main precursor of LPA is lysophosphatidylcholine, other minor LPLs could be a precursor of LPA in theory, considering their structures. Using LC-MS/MS, we were able to measure the species of other minor LPLs. Therefore, we investigated which LPLs might best explain the LPA levels using stepwise multiple regression analyses. These statistical analyses supported the hypothesis that the 3 LPA species that are characteristic of ACS might be mainly derived from different pathways (Table): 18:2 LPA might be mainly derived from an autotaxin-mediated pathway, whereas 20:4 LPA might be mainly derived from a platelet-related pathway. However, the main origins of 22:6 LPA might differ from the presently known pathways, and LPE and LPG might be involved, in addition to lysophosphatidylcholine, in this LPA-producing pathway. These results suggest that minor LPLs can be important determinants, in addition to autotaxin, in the formation of LPA in human subjects, or these minor LPLs may increase under conditions where LPA increases.

The roles of minor LPLs, especially in the field of atherosclerotic diseases, remain unknown at present, and our study is the first to demonstrate the association of minor LPLs with ACS. LPE can be generated from phosphatidylethanolamine, which is a component of the cell membrane. In the present study, LPE increased more markedly in ACS patients than LPA. Although we have not elucidated the reason for this marked elevation of LPE, phosphatidylethanolamine reportedly is degraded more preferably from the ischemic rat heart than other phospholipids, and hence LPE is concomitantly formed. Hence, ischemia of cardiac myocytes in ACS patients can be one possible explanation for the LPE increase. Although only a few papers have reported the physiological function of LPE to date, LPE might modulate several biological responses, possibly through G-protein-coupled receptor.

On the other hand, LPI is thought to be produced from phosphatidylinositol in activated platelets, and its involvement in the migration of smooth muscle cells has been proposed. Recently, a series of elegant studies have demonstrated that the state of cell activation and the autotaxin levels may determine the production of LPA. Therefore, minor LPLs might be associated with the state of cell activation. Further studies may elucidate novel unknown properties of these minor LPLs.

Our study suggested the importance of measuring LPA species because different species of LPA increased in patients with ACS, and these species might have different origins. Supporting this proposal, several differences in the properties of LPA species have been demonstrated in previous papers. For example, 20:4 LPA has been proposed to be a more potent activator of platelets and PPARγ compared with other LPA species. It was also demonstrated that unsaturated LPA, rather than saturated LPA, might be capable of inducing vascular remodeling. In addition to these proposed differences, our study suggested, for the first time, that long chain unsaturated LPAs, but not saturated LPAs, might be deeply involved in the pathogenesis of ACS, such as in platelet aggregation and plaque instability, although whether these species of LPA have proatherosclerotic or antiatherosclerotic properties remains to be elucidated.

A limitation of this study is that the backgrounds of the subjects, especially the use of regular medication, were not homogeneous among the groups. A stratified analysis revealed...
that the total LPA, 18:2 LPA, 20:4 LPA, and 22:6 LPA levels were elevated in the ACS group when subjects were confined to those without specific medication, whereas regular medication with aspirin, β blockers, and statins might have had suppressive effects on the elevation of plasma LPA levels in patients with ACS, suggesting possible novel roles of these drugs in suppressing the LPA levels. Another limitation is that the ACS patients tended to undergo emergency catheterization, whereas the normal coronary arteries and SAP patients more often underwent scheduled catheterization. The length of fasting before sampling was thus more varied among the ACS patients, and this might be a potential confounding factor. Even among the subjects who underwent scheduled catheterization, the length of fasting varied from 4 to 16 hours. Because this study was performed in a Japanese population, which was considered to consume more n-3 fatty acids than Western populations, whether similar results can be obtained in Western populations remains unclear. Further study in different population(s) is expected. Finally, because this study was an observational study, we cannot conclude whether these LPA species increased as a result of ACS or individuals with higher levels of these LPA species were prone to suffer ACS.

In summary, we demonstrated that specific LPA species, especially long-chain unsaturated LPA, increased in ACS patients, along with minor LPLs. The elevations of these LPA species increased in ACS patients, and this might be a potential confounding factor. Even among the subjects who underwent scheduled catheterization, the length of fasting varied from 4 to 16 hours.

Because this study was performed in a Japanese population, which was considered to consume more n-3 fatty acids than Western populations, whether similar results can be obtained in Western populations remains unclear. Further study in different population(s) is expected. Finally, because this study was an observational study, we cannot conclude whether these LPA species increased as a result of ACS or individuals with higher levels of these LPA species were prone to suffer ACS.

Sources of Funding
This work was supported by Grants-in-Aid for Scientific Research (KAKENHI) from Japan Society for the Promotion of Science (JSPS) Grant Number 22249017 and 25253040 (Y. Yamotani) and Grant Number 25860740 (M. Kurano), and CREST from JST.

Disclosures
None.

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**Significance**

Lysophosphatic acid (LPA) plays important roles in the field of vascular biology, and the involvement in acute coronary syndrome (ACS) is no exception. We previously reported that the plasma LPA levels, but not the serum autotaxin levels, increased in patients with ACS, which is not consistent with the established idea that LPA is derived mainly from lysophosphatidylcholine by the lysophospholipase D activity of autotaxin. Here, we have found that (1) specific LPA species (22:6 LPA, 18:2 LPA, and 20:4 LPA) are notably increased in ACS subjects and (2) minor lysophospholipids are also elevated in ACS subjects and are well correlated with the corresponding LPA species. These results suggest that minor lysophospholipids might be involved in the generation of LPA and in the pathogenesis of ACS. We believe that our present analysis will contribute greatly to our understanding of the involvement of LPA and other lysophospholipids in ACS.
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Arterioscler Thromb Vasc Biol. 2015;35:463-470; originally published online November 25, 2014;
doi: 10.1161/ATVBAHA.114.304748

Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1079-5642. Online ISSN: 1524-4636

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Material and Methods

Possible Involvement of Minor Lysophospholipids in the Increase in Plasma Lysophosphatidic Acid in Acute Coronary Syndrome

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Methods

Samples from patients who underwent coronary angiography

The samples obtained from the subjects who underwent coronary angiography have been previously described. Briefly, subjects who underwent coronary angiography at Juntendo University Hospital (J-Bacchus trial) between July and December 2009 were enrolled according to previously mentioned entry and exclusion criteria. Patients without significant stenosis were placed in a group with normal coronary arteries (NCA), whereas those with significant stenosis were placed in groups with acute coronary syndrome (ACS) or stable angina pectoris (SAP). Patients with acute myocardial infarction and unstable angina were included in the ACS group. The ethics review committee at Juntendo University Hospital approved the study, all the participants signed informed consent forms, and the study was registered in the UMIN protocol registration system (#UMIN000002103). This study was also approved by the institutional review boards of both the University of Tokyo and Juntendo University School of Medicine.

Arterial blood samples were obtained from the arterial sheaths of all the patients prior to the coronary angiography procedures. Blood samples were directly collected into glass vacutainer tubes with or without EDTA to obtain plasma and serum samples, respectively. The samples were immediately placed on ice. The anticoagulated samples were centrifuged at 1000 × g for 10 min, and the supernatant comprising the plasma was then carefully collected to avoid contamination with cell components. Whole blood samples collected without EDTA-2Na were left to clot, and the serum was then separated by centrifugation at 1000 × g for 10 min. Both the plasma and serum samples were stored at -80°C, and the freeze-thaw treatment was limited to once before the measurement of the LPL and ATX levels.

Measurement of LPL species using LC-MS/MS

Quantification of the LPLs was performed as previously described by us; the validation of this method was also described here. Briefly, the plasma samples were mixed and sonicated with a 10-fold volume of methanol and an internal standard. After centrifugation at 21,500 × g, the resulting supernatant was recovered and used for the LC-MS analysis. Then, 20 μL of methanol extract was separated using Nanospace LC (Shiseido) equipped with a C18 CAPCELL PAK ACR column (1.5 × 250 mm; Shiseido) using a gradient of solvent A (5 mM ammonium formate in water) and solvent B (5 mM ammonium formate in 95% [v/v] acetonitrile). Elution was sequentially ionized using an ESI probe, and the parent ion (m/z 380.2) and the
fragment ion (m/z 264.2) were monitored in the positive mode using a Quantum Ultra Triple Quadrupole Mass Spectrometer (Thermo Fisher Scientific). For each lysophospholipid class, 12 acyl chains (14:0, 16:0, 16:1, 18:0, 18:1, 18:2, 18:3, 20:3, 20:4, 20:5, 22:5, and 22:6) were monitored. In the present study, we extracted lipids under a neutral condition, and we did not separate 1-acyl-2-lyso- and 2-acyl-1-lyso-phospholipids.

Measurement of serum ATX levels

The ATX antigen levels in the serum were determined using a two-site immunoenzymetric assay with an ATX assay reagent and the TOSOH AIA system (TOSOH, Tokyo, Japan)\(^3\).

In vitro experiments

For serum incubation, the serum samples were frozen immediately or after incubation for 24 hours at 37°C with the vehicle (DMSO) or 50 \(\mu\)M HA130 (Echelon Biosciences Inc., Salt Lake City, UT), an inhibitor of the lysoPLD activity of ATX.

For the oxidation of LDL, LDL was isolated from the plasma using standard ultracentrifugation followed by dialysis against PBS. LDL at 100 mg/dL was oxidized with 0 \(\mu\)M, 0.1 \(\mu\)M, 1 \(\mu\)M, or 10 \(\mu\)M of CuSO\(_4\) for 4 and 20 hours, referring to previous reports\(^4,5\).

For platelet stimulation, washed platelet concentrates at 5 \(\times\) 10\(^8\)/mL were stimulated with 1 U/mL of thrombin (Sigma-Aldrich Co., St. Louis, MO) and then incubated for 10 min and 30 min with the vehicle or 50 \(\mu\)M of HA130. The supernatants were then collected.

Statistical Analysis

All the data were statistically analyzed using SPSS (Chicago, IL). The results were expressed as the mean ± SD. In the clinical studies, the values obtained from three groups were compared using the Kruskal-Wallis test followed by the Games Howell test as a post-hoc test, since normality or equality of variance had been rejected with the Kolmogorov-Smirnov test or the Levene test for most of the parameters or analyses. Correlations were sought using the Spearman correlation test. The independent effects of LPLs and ATX on LPA were evaluated using a stepwise multiple regression analysis. For the in vitro experiments on serum incubation and platelet activation in the presence of platelet poor plasma (PPP), the differences among paired groups were evaluated using a one-way ANOVA for repeated measures. For the in vitro experiments on the
activation of washed platelets, the differences among the LPA species were evaluated using the Friedman test followed by the Wilcoxon signed rank test. $P$-values less than 0.05 were regarded as statistically significant in all the analyses.

References
2. Okudaira M, Inoue A, Shuto A, Nakanaga K, Kano K, Makide K, Saigusa D, Tomioka Y, Aoki J. Separation and quantification of 2·acyl·1·lysophospholipids and 1·acyl·2·lysophospholipids in biological samples by LC-MS/MS. *J Lipid Res*. 2014
Supplemental Figure I. Increased LPA species in ACS subjects and their correlations with ATX levels.

(A-C) LPA levels in NCA, SAP, and ACS groups. *P < 0.01. A, 16:0 LPA; B, 18:1 LPA; and C, 22:5 LPA. The bar represents the mean values and the box represents ± S.D.

(D-F) Correlations of plasma concentrations of LPA species with serum ATX levels. D, 16:0 LPA; E, 18:1 LPA; and F, 22:5 LPA.
Supplemental Figure II. Altered concentrations of each LPA species during activation of platelets in the presence of platelet-poor plasma.
Washed platelet concentrates at $5 \times 10^8$/mL were stimulated with 1 U/mL of thrombin and incubated for 10 min and 30 min with vehicle or 50 µM HA130 in the presence of corresponding platelet-poor plasma (n = 3). The data shows the mean ± S.D. † $P < 0.05$. 
Supplemental Figure III. Correlations between LPA and other lysophospholipids.

(A, B) Correlation between 18:2 LPA and 18:2 LPS or 18:2 LPG.
(C, D) Correlation between 20:4 LPA and 20:4 LPS or 20:4 LPG.
(E, F) Correlation between 18:2 LPA and 22:6 LPS or 22:6 LPG.
Stratified analysis for total LPA levels in ACS, SAP, and ACS groups. Data are expressed as the mean ± SD, (n). *P < 0.01, vs. NCA; † P < 0.05, vs. NCA; ‡ P < 0.01, vs. SAP; § P < 0.05, vs. SAP; *P < 0.01, vs. yes (male); and bP < 0.05, vs. yes (male).

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<th>SAP (71)</th>
<th>ACS (38)</th>
</tr>
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<td>525 ± 386, (29)*, ‡</td>
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### Supplemental Table II. Stratified analysis for plasma 18:2 LPA

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Stratified analysis for 18:2 LPA levels in ACS, SAP, and ACS groups. Data are expressed as the mean ± SD, (n). *P < 0.01, vs. NCA; †P < 0.05, vs. NCA; § P < 0.01, vs. SAP; § P < 0.05, vs. SAP; aP < 0.01, vs. yes (male); and bP < 0.05, vs. yes (male).
### Supplemental Table III. Stratified analysis for plasma 20:4 LPA

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Stratified analysis for 20:4 LPA levels in ACS, SAP, and ACS groups. Data are expressed as the mean ± SD. *P < 0.01, vs. NCA; † P < 0.05, vs. NCA; ‡ P < 0.01, vs. SAP; § P < 0.05, vs. SAP; aP < 0.01, vs. yes (male); and bP < 0.05, vs. yes (male).
Supplemental Table IV. Stratified analysis for plasma 22:6 LPA

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<td>42 ± 51, (6)</td>
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<td>33 ± 37, (25)</td>
<td>44 ± 69, (38)</td>
<td>151 ± 160, (32)*, ‡</td>
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Stratified analysis for 22:6 LPA levels in ACS, SAP, and ACS groups. Data are expressed as the mean ± SD, (n). *P < 0.01, vs. NCA; † P < 0.05, vs. NCA; ‡ P < 0.01, vs. SAP; § P < 0.05, vs. SAP; aP < 0.01, vs. yes (male); and bP < 0.05, vs. yes (male).
### Supplemental Table V. Plasma concentrations of total LPC and each LPC species

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<th>SAP</th>
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<td>Total LPC (μM)</td>
<td>168.23 ± 50.45</td>
<td>183.56 ± 41.96</td>
<td>192.73 ± 69.36</td>
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<tr>
<td>14:0 LPC (μM)</td>
<td>0.63 ± 0.29</td>
<td>0.78 ± 0.36</td>
<td>0.69 ± 0.31</td>
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<tr>
<td>16:0 LPC (μM)</td>
<td>91.85 ± 27.74</td>
<td>100.59 ± 26.72</td>
<td>92.72 ± 29.99</td>
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<td>16:1 LPC (μM)</td>
<td>1.83 ± 0.82</td>
<td>2.00 ± 0.73</td>
<td>1.90 ± 0.77</td>
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<tr>
<td>18:0 LPC (μM)</td>
<td>32.79 ± 11.02</td>
<td>35.33 ± 8.35</td>
<td>32.79 ± 11.30</td>
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<td>18:1 LPC (μM)</td>
<td>15.96 ± 5.80</td>
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<td>18:2 LPC (μM)</td>
<td>15.52 ± 6.56</td>
<td>16.66 ± 4.82</td>
<td>26.64 ± 16.34*, ‡</td>
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<td>18:3 LPC (μM)</td>
<td>0.24 ± 0.12</td>
<td>0.27 ± 0.13</td>
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<td>20:3 LPC (μM)</td>
<td>1.03 ± 0.59</td>
<td>1.23 ± 0.57</td>
<td>2.22 ± 1.64*, ‡</td>
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<td>20:4 LPC (μM)</td>
<td>3.94 ± 2.00</td>
<td>4.28 ± 1.57</td>
<td>6.25 ± 3.50*, ‡</td>
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<td>20:5 LPC (μM)</td>
<td>1.49 ± 0.82</td>
<td>1.82 ± 1.09</td>
<td>2.46 ± 1.84*, ‡</td>
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<td>22:5 LPC (μM)</td>
<td>0.35 ± 0.14</td>
<td>0.42 ± 0.22</td>
<td>0.72 ± 0.46*, ‡</td>
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<td>22:6 LPC (μM)</td>
<td>2.61 ± 0.89</td>
<td>2.97 ± 1.63</td>
<td>5.80 ± 3.69*, ‡</td>
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Total and molecular species of plasma LPC in NCA, SAP, and ACS groups. Data are expressed as the mean ± SD. *P < 0.01, vs. NCA; † P < 0.05, vs. NCA; ‡ P < 0.01, vs. SAP; and § P < 0.05, vs. SAP.
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<td>18:1 LPE (µM)</td>
<td>0.37 ± 0.35</td>
<td>0.35 ± 0.35</td>
<td>0.97 ± 0.69*, †</td>
</tr>
<tr>
<td>18:2 LPE (µM)</td>
<td>0.93 ± 0.82</td>
<td>0.92 ± 0.97</td>
<td>2.90 ± 1.82*, †</td>
</tr>
<tr>
<td>18:3 LPE (µM)</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td>0.01 ± 0.01*, †</td>
</tr>
<tr>
<td>20:3 LPE (µM)</td>
<td>0.00 ± 0.01</td>
<td>0.01 ± 0.02</td>
<td>0.04 ± 0.08*, †</td>
</tr>
<tr>
<td>20:4 LPE (µM)</td>
<td>0.85 ± 0.82</td>
<td>0.95 ± 1.08</td>
<td>3.06 ± 1.62*, †</td>
</tr>
<tr>
<td>20:5 LPE (µM)</td>
<td>0.09 ± 0.10</td>
<td>0.14 ± 0.24</td>
<td>0.55 ± 0.58*, †</td>
</tr>
<tr>
<td>22:5 LPE (µM)</td>
<td>0.01 ± 0.01</td>
<td>0.01 ± 0.02</td>
<td>0.09 ± 0.14*, †</td>
</tr>
<tr>
<td>22:6 LPE (µM)</td>
<td>2.30 ± 1.70</td>
<td>2.31 ± 2.48</td>
<td>7.16 ± 3.90*, †</td>
</tr>
</tbody>
</table>

Total and molecular species of plasma LPE in NCA, SAP, and ACS groups. Data are expressed as the mean ± SD. *P < 0.01, vs. NCA; † P < 0.01, vs. SAP; and ‡ P < 0.05, vs. SAP.
### Supplemental Table VII. Plasma concentrations of total LPG and each LPG species

<table>
<thead>
<tr>
<th></th>
<th>NCA</th>
<th>SAP</th>
<th>ACS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total LPG (nM)</td>
<td>254 ± 180</td>
<td>244 ± 153</td>
<td>546 ± 409* †</td>
</tr>
<tr>
<td>14:0 LPG (nM)</td>
<td>1 ± 1</td>
<td>1 ± 2</td>
<td>0 ± 1</td>
</tr>
<tr>
<td>16:0 LPG (nM)</td>
<td>23 ± 18</td>
<td>23 ± 20</td>
<td>36 ± 28‡</td>
</tr>
<tr>
<td>16:1 LPG (nM)</td>
<td>1 ± 3</td>
<td>1 ± 3</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>18:0 LPG (nM)</td>
<td>26 ± 33</td>
<td>21 ± 17</td>
<td>31 ± 27</td>
</tr>
<tr>
<td>18:1 LPG (nM)</td>
<td>172 ± 118</td>
<td>165 ± 107</td>
<td>378 ± 280* †</td>
</tr>
<tr>
<td>18:2 LPG (nM)</td>
<td>28 ± 24</td>
<td>30 ± 29</td>
<td>94 ± 94* †</td>
</tr>
<tr>
<td>18:3 LPG (nM)</td>
<td>0 ± 1</td>
<td>0 ± 1</td>
<td>0 ± 1</td>
</tr>
<tr>
<td>20:3 LPG (nM)</td>
<td>0 ± 1</td>
<td>1 ± 1</td>
<td>1 ± 1</td>
</tr>
<tr>
<td>20:4 LPG (nM)</td>
<td>1 ± 1</td>
<td>1 ± 1</td>
<td>2 ± 3* †</td>
</tr>
<tr>
<td>20:5 LPG (nM)</td>
<td>1 ± 3</td>
<td>0 ± 0</td>
<td>0 ± 1</td>
</tr>
<tr>
<td>22:5 LPG (nM)</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>22:6 LPG (nM)</td>
<td>1 ± 5</td>
<td>1 ± 1</td>
<td>3 ± 7</td>
</tr>
</tbody>
</table>

Total and molecular species of plasma LPG in NCA, SAP, and ACS groups. Data are expressed as the mean ± SD. *P < 0.01, vs. NCA; † P < 0.01, vs. SAP; and ‡ P < 0.05, vs. SAP.
Supplemental Table VIII. Plasma concentrations of total LPI and each LPI species

<table>
<thead>
<tr>
<th></th>
<th>NCA</th>
<th>SAP</th>
<th>ACS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total LPI (µM)</td>
<td>1.539 ± 0.991</td>
<td>1.505 ± 1.229</td>
<td>2.945 ± 1.884*, †</td>
</tr>
<tr>
<td>14:0 LPI (µM)</td>
<td>0.000 ± 0.000</td>
<td>0.000 ± 0.000</td>
<td>0.000 ± 0.000</td>
</tr>
<tr>
<td>16:0 LPI (µM)</td>
<td>0.069 ± 0.055</td>
<td>0.062 ± 0.037</td>
<td>0.103 ± 0.123‡</td>
</tr>
<tr>
<td>16:1 LPI (µM)</td>
<td>0.001 ± 0.002</td>
<td>0.002 ± 0.006</td>
<td>0.020 ± 0.046*, †</td>
</tr>
<tr>
<td>18:0 LPI (µM)</td>
<td>1.157 ± 0.782</td>
<td>0.963 ± 0.348</td>
<td>1.186 ± 0.626</td>
</tr>
<tr>
<td>18:1 LPI (µM)</td>
<td>0.111 ± 0.109</td>
<td>0.147 ± 0.269</td>
<td>0.458 ± 0.459*, †</td>
</tr>
<tr>
<td>18:2 LPI (µM)</td>
<td>0.102 ± 0.079</td>
<td>0.168 ± 0.334</td>
<td>0.644 ± 0.585*, †</td>
</tr>
<tr>
<td>18:3 LPI (µM)</td>
<td>0.000 ± 0.000</td>
<td>0.000 ± 0.000</td>
<td>0.000 ± 0.000†</td>
</tr>
<tr>
<td>20:3 LPI (µM)</td>
<td>0.019 ± 0.023</td>
<td>0.040 ± 0.109</td>
<td>0.142 ± 0.136*, †</td>
</tr>
<tr>
<td>20:4 LPI (µM)</td>
<td>0.079 ± 0.061</td>
<td>0.122 ± 0.230</td>
<td>0.384 ± 0.298*, †</td>
</tr>
<tr>
<td>20:5 LPI (µM)</td>
<td>0.000 ± 0.000</td>
<td>0.000 ± 0.001</td>
<td>0.000 ± 0.000</td>
</tr>
<tr>
<td>22:5 LPI (µM)</td>
<td>0.000 ± 0.000</td>
<td>0.001 ± 0.001</td>
<td>0.003 ± 0.005*, †</td>
</tr>
<tr>
<td>22:6 LPI (µM)</td>
<td>0.000 ± 0.000</td>
<td>0.000 ± 0.002</td>
<td>0.002 ± 0.003*, †</td>
</tr>
</tbody>
</table>

Total and molecular species of plasma LPI in NCA, SAP, and ACS groups. Data are expressed as the mean ± SD. *P < 0.01, vs. NCA; † P < 0.01, vs. SAP; and ‡ P < 0.05, vs. SAP.
### Supplemental Table IX. Plasma concentrations of total LPS and each LPS species

<table>
<thead>
<tr>
<th></th>
<th>NCA</th>
<th>SAP</th>
<th>ACS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total LPS (nM)</td>
<td>66 ± 71</td>
<td>104 ± 110</td>
<td>213 ± 317* ‡</td>
</tr>
<tr>
<td>14:0 LPS (nM)</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>16:0 LPS (nM)</td>
<td>0 ± 0</td>
<td>0 ± 1</td>
<td>0 ± 1</td>
</tr>
<tr>
<td>16:1 LPS (nM)</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>18:0 LPS (nM)</td>
<td>59 ± 68</td>
<td>89 ± 91</td>
<td>73 ± 103</td>
</tr>
<tr>
<td>18:1 LPS (nM)</td>
<td>3 ± 6</td>
<td>7 ± 12</td>
<td>55 ± 99* †</td>
</tr>
<tr>
<td>18:2 LPS (nM)</td>
<td>0 ± 1</td>
<td>0 ± 1</td>
<td>2 ± 3* †</td>
</tr>
<tr>
<td>18:3 LPS (nM)</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0 ± 1</td>
</tr>
<tr>
<td>20:3 LPS (nM)</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0 ± 1</td>
</tr>
<tr>
<td>20:4 LPS (nM)</td>
<td>2 ± 3</td>
<td>4 ± 13</td>
<td>56 ± 125* †</td>
</tr>
<tr>
<td>20:5 LPS (nM)</td>
<td>0 ± 0</td>
<td>0 ± 1</td>
<td>1 ± 2</td>
</tr>
<tr>
<td>22:5 LPS (nM)</td>
<td>0 ± 1</td>
<td>0 ± 1</td>
<td>1 ± 2</td>
</tr>
<tr>
<td>22:6 LPS (nM)</td>
<td>1 ± 2</td>
<td>3 ± 13</td>
<td>25 ± 38* †</td>
</tr>
</tbody>
</table>

Total and molecular species of plasma LPS in NCA, SAP, and ACS groups. Data are expressed as the mean ± SD. *P < 0.01, vs. NCA; † P < 0.01, vs. SAP; and ‡ P < 0.05, vs. SAP.
<table>
<thead>
<tr>
<th></th>
<th>LPC</th>
<th>LPE</th>
<th>LPI</th>
<th>LPS</th>
<th>LPG</th>
</tr>
</thead>
<tbody>
<tr>
<td>14:0 LPA</td>
<td>-0.195†</td>
<td>-0.136</td>
<td>0.005</td>
<td>-0.038</td>
<td>-0.059</td>
</tr>
<tr>
<td>16:0 LPA</td>
<td>-0.063</td>
<td>-0.018</td>
<td>0.262*</td>
<td>0.009</td>
<td>-0.114</td>
</tr>
<tr>
<td>16:1 LPA</td>
<td>-0.006</td>
<td>-0.018</td>
<td>0.014</td>
<td>-0.069</td>
<td>-0.116</td>
</tr>
<tr>
<td>18:0 LPA</td>
<td>0.088</td>
<td>0.427*</td>
<td>0.287*</td>
<td>-0.099</td>
<td>0.121</td>
</tr>
<tr>
<td>18:1 LPA</td>
<td>0.300*</td>
<td>0.520*</td>
<td>0.622*</td>
<td>0.233*</td>
<td>0.256*</td>
</tr>
<tr>
<td>18:3 LPA</td>
<td>-0.007</td>
<td>0.094</td>
<td>0.169†</td>
<td>0.049</td>
<td>-0.101</td>
</tr>
<tr>
<td>20:3 LPA</td>
<td>0.352*</td>
<td>0.027</td>
<td>0.547*</td>
<td>0.040</td>
<td>0.009</td>
</tr>
<tr>
<td>20:5 LPA</td>
<td>0.528*</td>
<td>0.576*</td>
<td>0.327*</td>
<td>0.181†</td>
<td>0.193†</td>
</tr>
<tr>
<td>22:5 LPA</td>
<td>0.266*</td>
<td>0.095</td>
<td>0.288*</td>
<td>0.062</td>
<td>0.110</td>
</tr>
</tbody>
</table>

Correlation between LPA and other lysophospholipids. The Spearman $r$ value is expressed. *$P < 0.01$; †$P < 0.05$. 

**Supplemental Table X. Correlations between LPA and other lysophospholipids**
Supplemental Table XI. Multiple regression analysis for plasma LPA species

A.

<table>
<thead>
<tr>
<th></th>
<th>B</th>
<th>95% CI</th>
<th>Standardized β</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>18:1 LPI</td>
<td>0.038</td>
<td>(0.030 - 0.047)</td>
<td>0.598</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ATX</td>
<td>0.019</td>
<td>(0.010 - 0.028)</td>
<td>0.208</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>18:1 LPE</td>
<td>0.009</td>
<td>(0.004 - 0.015)</td>
<td>0.226</td>
<td>0.001</td>
</tr>
</tbody>
</table>

B.

<table>
<thead>
<tr>
<th></th>
<th>B</th>
<th>95% CI</th>
<th>Standardized β</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>22:5 LPI</td>
<td>0.357</td>
<td>(0.271 - 0.443)</td>
<td>0.573</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

C.

<table>
<thead>
<tr>
<th></th>
<th>B</th>
<th>95% CI</th>
<th>Standardized β</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>16:0 LPI</td>
<td>0.093</td>
<td>(0.058 - 0.128)</td>
<td>0.383</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>16:0 LPC</td>
<td>-0.00016</td>
<td>(-0.000 - -0.001)</td>
<td>-0.250</td>
<td>0.001</td>
</tr>
<tr>
<td>ATX</td>
<td>0.017</td>
<td>(0.006 - 0.028)</td>
<td>0.221</td>
<td>0.003</td>
</tr>
</tbody>
</table>

Multiple regression analysis for plasma 18:1 LPA (A), 22:5 LPA (B), and 16:0 LPA (C). The LPLs of the corresponding molecular species and ATX were utilized as the possible explanatory factors.