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# Increased Production of Bioactive Lysophosphatidic Acid by Serum Lysophospholipase D in Human Pregnancy[1]

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## ABSTRACT

**Lysophosphatidic acid (LPA) is a prototype of the lysophospholipid mediator family and has multiple effects in the female reproductive system. Although several metabolic routes have been reported for intracellular formation of LPA, a unique route involving lysophospholipase D, an extracellular enzyme that produces LPA in blood and body fluids, is particularly intriguing for its agonistic role. In this study, using an assay with radioactive palmitoyl-lysophosphatidylcholine, we found that lysophospholipase D activity producing palmitoyl-LPA in human serum gradually increased during pregnancy. Elevated activity of lysophospholipase D was not caused by changes in levels of their precursors, lysophosphatidylcholines, in nonpregnant women or in pregnant women at different gestational periods. With increasing length of gestation, the elevated activity in pregnant women was found to produce increasing proportions of LPA with a palmitoyl group versus other LPAs. These results suggest that LPA formed by increased activity of lysophospholipase D in blood might participate in maintenance of pregnancy.**

*growth factors, parturition, placenta, pregnancy, uterus*

## INTRODUCTION

Much attention has been paid to lysophosphatidic acid (LPA) because of its diverse biological actions via its specific receptors. LPA was first identified as a smooth muscle-contracting substance [1] or a vasoactive principal [2] more than 20 yr ago. Later, the bioactive lysophospholipid was reassessed as a novel type of growth factor after it was found to induce proliferation of fibroblasts [3]. Today, it is known to exert growth factor- and hormone-like biological activities in a wide range of animal cells [4–8].

In animals, acyl, alkyl, and alkenyl LPAs consist of several molecular species having long methylene chains with distinct chain lengths and degrees of unsaturation [5–7]. Three receptors specific for LPA, which are coded by the endothelial differentiation gene (*Edg*), appear to distinguish minute structural differences in the molecular species of LPA [8–11]. Consistent with the multiplicity of LPA receptors, several distinct metabolic pathways seem to exist and to be relevant to the wide distribution of LPAs throughout the animal body [4–8].

It is noteworthy that LPA is produced both extracellularly and intracellularly [5, 7]. From the viewpoint of the functional role of LPA as an intercellular mediator, an extracellular enzyme that acts on phospholipids in biological fluids or on the cell surface would be important for the generation of bioactive LPA. Lysophospholipase D in mammalian plasma and serum was found to be a novel metalloenzyme [12–15] that exists in various body fluids. We detected a significant amount of LPA produced by lysophospholipase D in human follicular fluid [16]. Previously, we found that LPA stimulated egg maturation [17], suggesting a physiological significance of LPA in the follicular fluid. We also found that LPA accelerated the transport of embryos through the oviducts [18] and promoted embryo development [19]. We speculated that the possible existence of LPA in oviductal fluid might be involved in the development and transport of embryos in the oviduct, because we demonstrated that hen egg white, originating from hen oviductal fluid, contained lysophospholipase D activity that produced unsaturated LPAs [20]. Other groups have provided evidence for the participation of LPA in egg implantation [21] and in growth and regression of the corpus luteum [22].

Taken together, these findings suggest that extracellular production of this interesting lysophospholipid may play important roles in multiple processes during reproduction, especially the success of pregnancy. On the other hand, the physiological significance of LPA production by plasma lysophospholipase D remains unclear. We assume that alteration in plasma or serum lysophspholipase D, if detected under certain physiological situations, will throw some light on its physiological role. This expectation allowed us to examine, in the present study, whether production of LPA by serum lysophospholipase D changes significantly in human pregnancy, a very important physiological event. Some of the preliminary results have already been reported elsewhere [17].

## MATERIALS AND METHODS

### Materials

Phospholipase D from cabbage, 1,2-diheptadecanoyl (17:0/17:0)-*sn*-glycero-3-phosphocholine (17:0/17:0-phosphatidylcholine; 17:0/17:0-PC), 1-palmitoyl (16:0)-2-lyso-*sn*-glycero-3-phosphocholines (16:0-lysophosphatidylcholine; 16:0-LPC), 1-heptadecanoyl (17:0)-2-lyso-*sn*-glycero-3-phosphocholine (17:0-LPC), and 1-oleoyl (18:1)-2-lyso-*sn*-glycerol-3-phosphate (18:1-LPA) were obtained from Sigma Chemical Co. (St. Louis, MO). The 1-[$^{14}$C]palmitoyl-2-lyso-*sn*-glycero-3-phosphocholine (57.0 mCi/mmol; [$^{14}$]16:0-LPC) was purchased from Amersham International (Buckinghamshire, U.K.). Sintisol EX-H was from Wako Pure Chemical (Osaka, Japan). Choline oxidase, horseradish peroxidase, and 3-(4-hydroxyphenyl)propionic

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acid were products of Toyobo Co. (Osaka, Japan), Funakoshi Co. (Tokyo, Japan), and Dojindo Laboratories (Kumamoto, Japan), respectively. 1-Heptadecanoyl-2-lyso-*sn*-glycerol-3-phosphate (17:0-LPA) was prepared by hydrolysis of 17:0-LPC with cabbage phospholipase D as described elsewhere [13].

### *Human Serum and Plasma*

Blood was withdrawn through an antecubital vein of healthy, nonpregnant volunteers who had not taken any medicine for at least 2 wk and of pregnant women at different stages of pregnancy. The ages of the pregnant women ranged from 21 to 36 yr. Informed consent was obtained from the all volunteers and patients according to the guidelines established by the Tokushima University Hospital. Sera from nonpregnant and pregnant women were prepared by centrifugation of the blood at 1000 × *g* for 30 min at 4°C after standing for 1.5–2 h at room temperature. Plasma preparations were obtained from venous blood of nonpregnant women anticoagulated with citrate-phosphate-dextrose (CPD) solution (102 mM sodium citrate, 17 mM citrate, 129 mM dextrose, and 16 mM $NaH_2PO_4$).

### *Extraction and Separation of Lipids*

Lipids were extracted according to the method of Bligh and Dyer [23], with some modifications as described previously [13]. Briefly, samples of serum and plasma were diluted with an equal volume of 2% (v/v) KCl and acidified with 1 N HCl to pH 2.5. Known amounts of 17:0/17:0-PC, 17:0-LPC, and 17:0-LPA were added to the acidified sample as internal standards. For quantifications of PC, LPC, and LPA by gas-liquid chromatography (GLC), the lipid extracts were separated by thin-layer chromatography (TLC) on Whatman Silica gel K6 plates (Whatman International, Kent, U.K.), and PC, LPC and LPA were recovered from the silica gels and converted to fatty acid methyl esters by transmethylation reaction as described below. In experiments with radioactive 16:0-LPC for assaying lysophospholipase D activity, the acidified plasma was mixed with 18:1-LPA as a carrier just before lipid extraction, and the lipid extract was subjected to TLC on Merck Silica gel 60 plates (Merck, Darmstadt, Germany).

Lipids separated by TLC on Merck Silica gel 60 plates with a solvent system of chloroform:methanol:20% $NH_4OH$ (60:35:8, v/v/v) were visualized under an ultraviolet lamp after spraying a 6-*p*-(toluidino)-2-naphthalene sulfonic acid solution (1 mM in 50 mM Tris-HCl buffer). The PC and LPC bands were scrapped off the plate and extracted from the suspension of silica gel in 2 ml of distilled water by the method of Bligh and Dyer [23]. The LPA was recovered from the acidified suspension of the silica gel by the same method.

### *Fatty Acid Composition of PC, LPC, and LPA*

Purified PC, LPC, and LPA from various samples were converted to fatty acid methyl esters by heating them in 0.5 ml of 5% HCl in methanol at 100°C for 3 h. The fatty acid methyl esters were extracted with *n*-hexane and analyzed by GLC with a capillary column (DB-225, 30 m × 0.24 mm, 0.25 μm thickness; J & W Scientific, Foster City, CA). The column was kept at 120°C for 1 min and then increased in temperature to 220°C at 10°C/min.

### *Measurement of Lysophospholipase D Activity*

Human sera were mixed with 0.05 volume of a solution of [$^{14}$C]16:0-LPC (final concentration, 3.3 nmol/ml; 0.2 μCi/ml) in saline containing 0.25% BSA. Immediately after the addition of [$^{14}$C]16:0-LPC, 0.2-ml aliquots of serum were withdrawn as 0-h samples. The remaining portion of the serum was incubated at 37°C for 6 h. Samples of serum (0.2 ml each) were withdrawn after 2-, 4-, and 6-h incubation and diluted to 2 ml with 2% KCl solution, and lipids were then extracted from the diluted samples by a reported method [23] and separated by TLC as described above. Bands of individual phospholipids were scraped off the gel, mixed with 4 ml of Sintisol EX-H, and counted in a liquid scintillation counter. Lysophospholipase D activity was calculated from the radioactivities in the product LPA against the total radioactivity (PC + LPC + LPA + fatty acid) and expressed as % conversion/h.

Serum lysophospholipase D activity was also measured by determining the choline from LPC. In brief, 0.01-ml aliquots of human sera were diluted with saline to 0.1 ml and incubated with 0.05 ml of 0.45 mM 16:0-LPC solution in saline containing 0.25% BSA at 37°C for 24 h. Then, 0.1-ml aliquots of the first-assay solutions were mixed with 2.6 ml of 0.1 M Tris-HCl buffer (pH 8.5), 0.2 ml of 7.5 mM 3-(4-hydroxyphenyl)propionic acid, 0.1 ml of horse radish peroxidase (concentration, 2 U/ml), and 0.01 ml of choline oxidase (concentration, 300 U/ml). After incubation at 37°C for 15 min, fluorescent intensity from the second-assay solutions was measured at 404 nm with an excitation wavelength of 320 nm essentially as described elsewhere [24, 25].

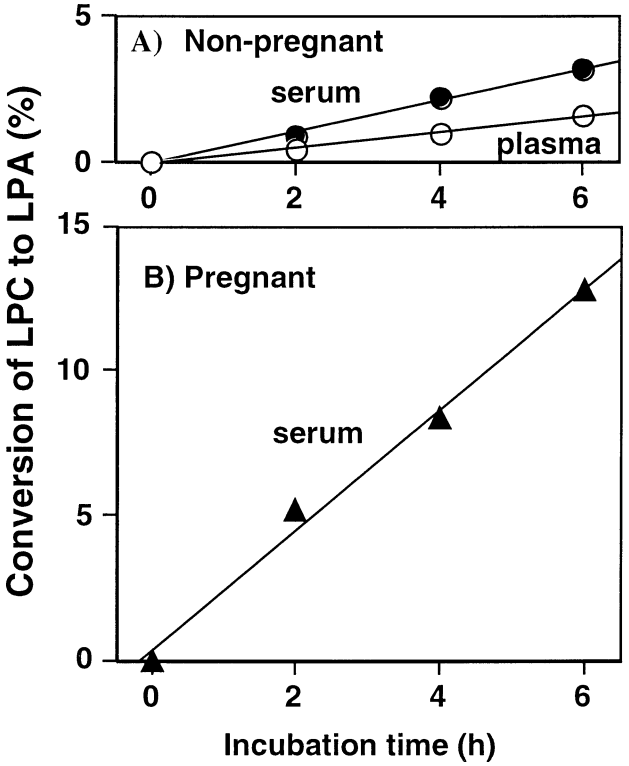


FIG. 1. Time course of metabolic conversion of [$^{14}$C]16:0-LPC to LPA during incubation of human plasma or serum. Serum (**A**, closed circles) and CPD-treated plasma (**A**, open circles) from nonpregnant women and serum from a pregnant woman at 39 wk of gestation (**B**, closed triangles) were incubated for 6 h at 37°C with [$^{14}$C]16:0-LPC.

### *Statistical Analysis*

Values are expressed as the mean ± SEM. Differences between means were compared by the unpaired Student *t*-test. A $P$ value of less than 0.05 was considered to be significant.

## RESULTS

### *Lysophospholipase D Activity Measured with a Radioactive Substrate*

Because we previously found that lysophospholipase D activity in rat plasma varied depending on the anticoagulant used [13], we first examined the influence of the CPD solution on human lysophospholipase D activity. Figure 1A shows typical results concerning metabolic conversion of [$^{14}$C]16:0-LPC into LPA in serum and CPD-treated plasma from nonpregnant women. In both cases, the conversions of LPC to LPA proceeded linearly for 6 h at a rate of 0.54%/h ($r$ = 0.998) and 0.26%/h ($r$ = 0.998) for the plasma and serum, respectively, although 10.6% and 8.7% of the radioactive LPC were also converted to PC and palmitic acid by lecithin:cholesterol acyltransferase and lysophospholipase, respectively, for 6 h.

Consistent with results on lysophospholipase D activity in rat plasma and serum [13], no significant difference was observed between lysophospholipase D activity in human serum and in heparinized human plasma (data not shown). Thus, we decided to compare serum lysophospholipase D activities of nonpregnant and pregnant women, because

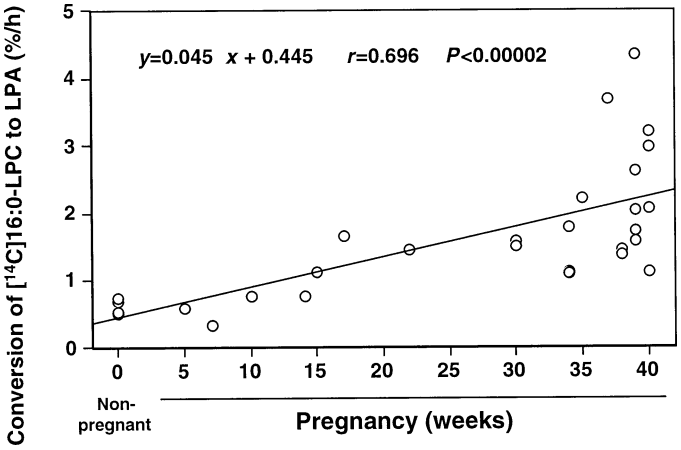


FIG. 2. Apparent activity of serum lysophospholipase D of nonpregnant women and pregnant women at various gestational times. Activity is expressed as the fractional change of [14C]LPC to LPA during incubation of serum.

mixing of human blood with the CPD solution resulted in significant reduction in the lysophospholipase D activity. Figure 1B shows typical results with serum from pregnant women in the third trimester of pregnancy. The LPC was converted to LPA in these women at a constant rate (2.07%/h, $r = 0.996$) that was significantly higher than that of the nonpregnant woman.

We confirmed the linearity of the production of LPA from LPC by lysophospholipase D activity during 6-h incubation for all serum preparations from pregnant and nonpregnant women tested in this study, allowing us to calculate its activities from the slopes of the straight lines. Figure 2 shows apparent activities of lysophospholipase D expressed as percentages of conversion of the radioactive LPC to LPA in sera of pregnant women after various gestational periods. For comparison, values of sera from three nonpregnant women are also included in the figure. The activity of lysophospholipase D gradually increased with increasing length of gestation ($r = 0.696$, $P < 0.00002$), reaching approximately fivefold that of nonpregnant women during late stages of pregnancy.

### *Corrected Activity of Lysophospholipase D Based on the Level of Endogenous LPC*

The apparent activity of lysophospholipase D was measured in human serum incubated with a small amount of exogenous [14C]16:0-LPC and, therefore, may have been affected by both the amount and the molecular-species composition of endogenous LPC during pregnancy. To overcome this criticism, the apparent activity of lysophospholipase D should be corrected for the levels of the endogenous LPC pool that could be utilized by lysophospholipase D. For this purpose, we first examined whether the endogenous levels of individual LPCs were altered during incubation of serum preparations at 37°C. Figure 3 shows typical results with serum from a pregnant woman at 39 wk of gestation. The levels of two saturated LPCs (16:0 and stearoyl [18:0]) greatly increased after 6-h incubation, possibly because of the action of lecithin:cholesterol acyltransferase, and thereafter declined slowly, whereas the levels of polyunsaturated LPCs (linoleoyl [18:2] and arachidonoyl [20:4]) remained nearly constant or decreased slightly. The level of 18:1-LPC also significantly increased after 6-h incubation, but the degree and duration of its increase were less than those observed for saturated LPCs.

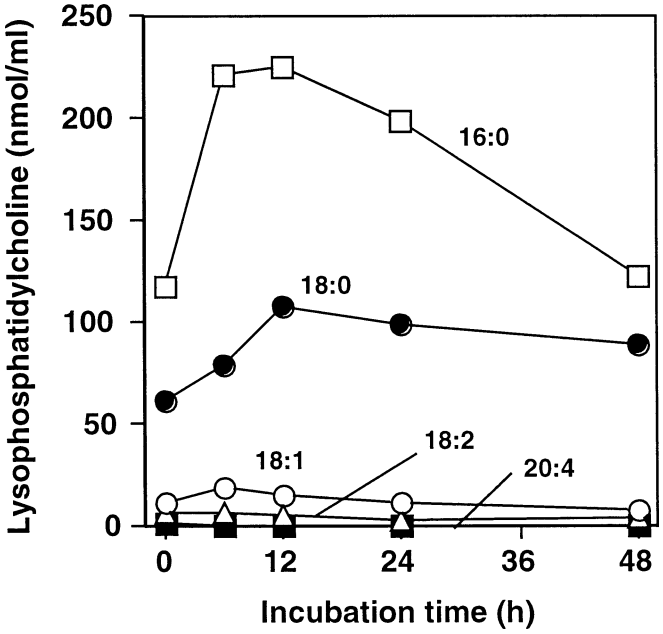


FIG. 3. Changes in levels of individual LPCs during incubation of serum from a pregnant woman at 39 wk of gestation. The symbols used are as follows: 16:0 (□), 18:0 (●), 18:1 (○), 18:2 (△) and 20:4 (■).

From these results, we selected the levels of endogenous 16:0-LPC in 6-h incubated serum (Fig. 4A), but not those in unincubated serum, for correction of apparent activities of serum lysophospholipase D measured with [14C]16:0-LPC. The corrected activities of lysophospholipase D are expressed as nmol 16:0-LPC converted to LPA/ml of serum (Fig. 4B); the activity gradually increased with increasing length of gestation ($r = 0.51$, $P < 0.01$).

### *Quantification of Individual LPAs and LPCs*

Next, we analyzed TLC-purified LPA from lipid extracts of sera incubated for 6 and 24 h by GLC after their conversion to fatty acid methyl esters. Figure 5A shows the time course of LPA production during incubation of sera from a nonpregnant and a pregnant woman at 39 wk of gestation. In both sera, LPA accumulated throughout the incubation. Consistent with results of experiments with [14C]16:0-LPC, the rate of production of total LPA (the sum of five major molecular species) in serum from the pregnant woman was higher than that in serum from the nonpregnant woman. Based on the results of the time course experiments, we selected 6- and 24-h incubated sera as an early and late stage of incubation for comparison of LPA accumulation in sera from pregnant women at three different trimesters and from nonpregnant women. As shown in Figure 5B, the total amounts of LPA accumulated in the 6- and 24-h incubated sera were the highest in the third trimester, followed in decreasing order by the second trimester, the first trimester, and the nonpregnant group. Figure 5C shows results on individual LPAs in 24-h incubated serum of the four groups. The order of five major species of LPA in serum from nonpregnant women was as follows: 16:0 > 18:0 = 18:1 > 18:2 > 20:4. With increasing length of gestation, the levels of individual LPAs increased, with the increase in 16:0 species being higher than those in other LPA species. Thus, in incubated serum, 16:0-LPA became more predominant than other LPAs with progression of pregnancy.

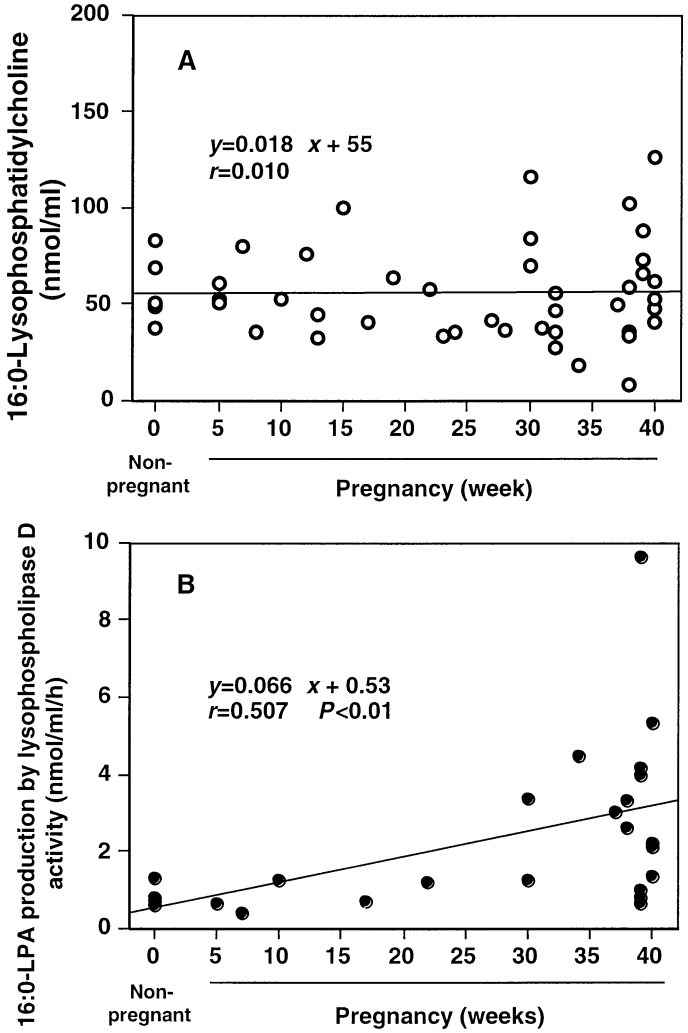


FIG. 4. Correction of lysophospholipase D based on absolute level of endogenous 16:0-LPC in sera. **A**) Levels of 16:0-LPC in 6-h incubated sera from nonpregnant and pregnant women. **B**) Corrected activity of lysophospholipase D was calculated by multiplying the fractional conversion of [$^{14}$C]16:0-LPC to LPA (see Fig. 2) by the absolute level of 16:0-LPC in 6-h incubated serum (**A**) and expressed as nmol 16:0-LPA expected to be produced by lysophospholipase D/ml/h.

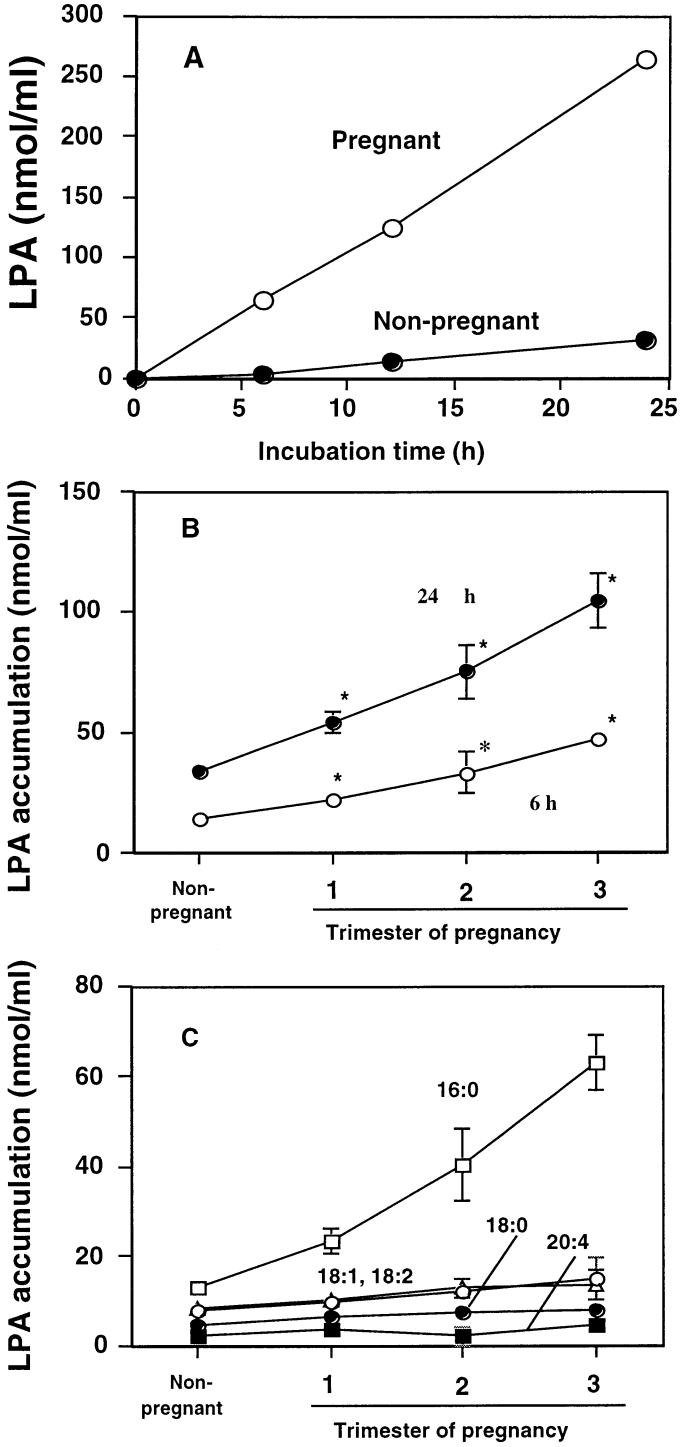


FIG. 5. Production of LPA from endogenous substrates during incubation of sera from pregnant and nonpregnant women. **A**) Time-dependent increases in levels of total LPA during incubation of serum from a nonpregnant woman (closed circles) and from a pregnant woman at 39 wk of gestation (open circles). **B**) Levels of total LPA in 6-h (open circles) and 24-h (closed circles) incubated sera from nonpregnant women and pregnant women in the first, second, or third trimesters of pregnancy. *Significant versus nonpregnant controls ($P < 0.05$). **C**) Levels of individual LPA in 24-h incubated sera from nonpregnant and pregnant women in the first, second, and third trimesters of pregnancy. Symbols used are as follows: 16:0 (□), 18:0 (●), 18:1 (○), 18:2 (△), and 20:4 (■). Values are the mean ± SEM of five or more persons in each group. Some symbols mask error bars.

As shown in Figure 6A, total amounts of LPC, the precursor of LPA, in both unincubated and 24-h incubated sera from pregnant women were significantly less than those in sera from nonpregnant women. On the other hand, the levels of total PC increased during pregnancy. Figure 6B shows changes in levels of individual LPCs. Although the levels of LPCs varied in the four groups tested (Fig. 6B), this alone does not seem to account for the preferable production of 16:0-LPA compared to other LPAs during incubation of sera from pregnant women, especially in the second and third trimesters, as shown in Figure 5C. To strengthen the above assumption, we devised a convenient assay for lysophospholipase D activity with use of exogenous, nonradioactive 16:0-LPC and compared the activity in sera from pregnant women with that in sera from nonpregnant women. In this assay system, sera were diluted 10-fold to minimize the interference by endogenous LPCs for the measurement of the enzymatic activity. The lysophospholipase D in sera from 12 pregnant women (37.5 ± 5.3 wk of gestation) was 280 ± 33 nmol choline/ml per 24 h, being approximately fourfold higher than the 66.7 ± 7.3

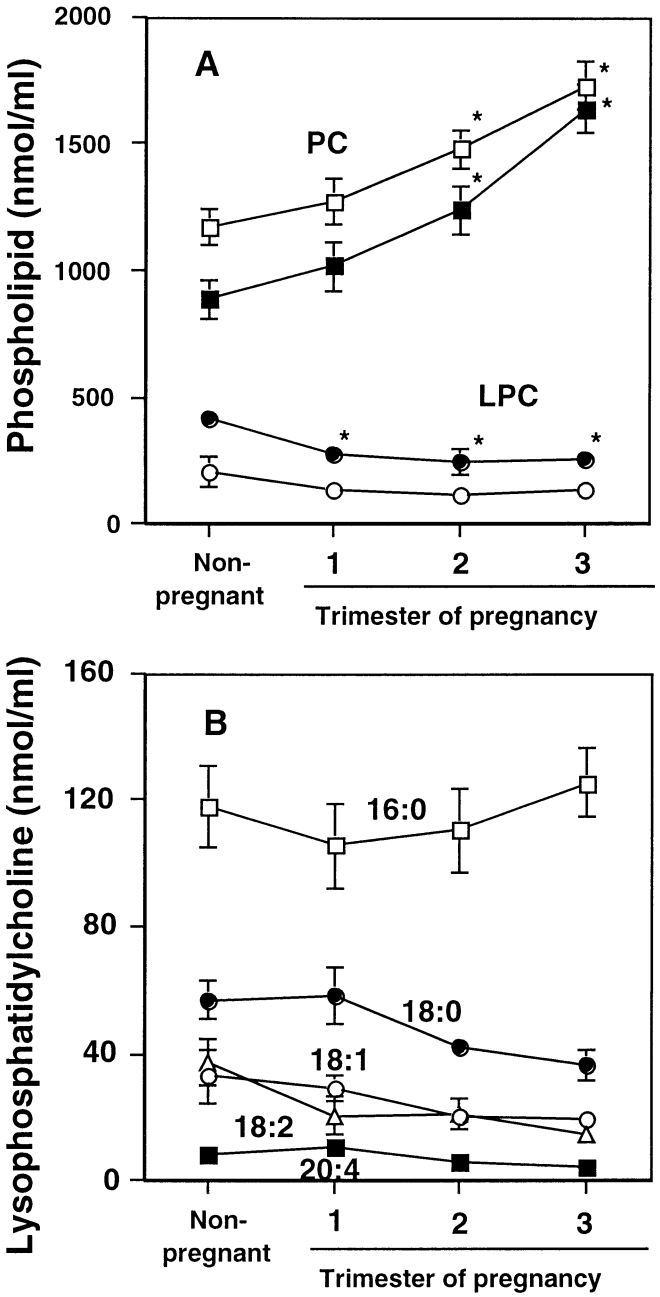


FIG. 6. Levels of LPC in unincubated and incubated sera from nonpregnant and pregnant women. **A**) Levels of PC (□, ■) and LPC (○, ●) in unincubated (■, ●) and 24-h incubated (□, ○) sera from nonpregnant and pregnant women in the first, second, or third trimesters of pregnancy. Values are the mean ± SEM of five or more persons in each group. Some symbols mask error bars. *Significant versus nonpregnant controls ($P < 0.05$). **B**) Levels of individual LPC in unincubated sera from nonpregnant and pregnant women in the first, second, and third trimesters of pregnancy. Symbols used are as follows: 16:0 (□), 18:0 (●), 18:1 (○), 18:2 (△), and 20:4 (■). Values are the mean ± SEM of five or more persons in each group. Some symbols mask error bars.

nmol choline/ml per 24 h obtained for four nonpregnant women.

*Metal Ion Requirement of Lysophospholipase D*

Addition of EDTA to human sera at 3 mM resulted in approximately 60% reduction in lysophospholipase D activity (Fig. 7). Addition of $Zn^{2+}$ at 5 mM restored the lysophospholipase D activity to nearly the control level. Addition of $Co^{2+}$ at 5 mM caused larger recovery than $Zn^{2+}$ when added to EDTA-treated sera. Moreover, it increased the lysophospholipase D activity to approximately twofold that of the control level. These results were consistent with those obtained for lysophospholipase D activity in rat plasma [13].

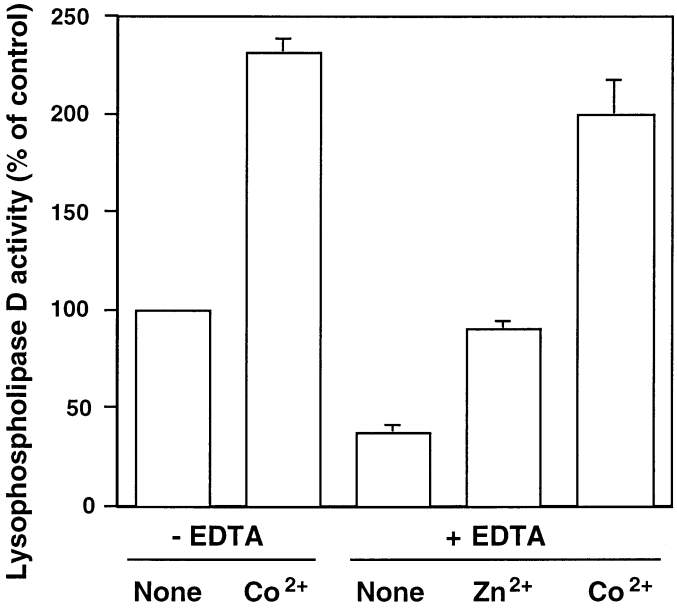


FIG. 7. Metal-ion requirement of lysophospholipase D activity. Human sera were incubated with [$^{14}$C]LPC in the presence or absence of 1 mM $Co^{2+}$ at 37°C for 6 h. The sera treated with 3 mM EDTA were also incubated with [$^{14}$C]LPC in the absence and presence of 5 mM $Co^{2+}$ or $Zn^{2+}$. Relative activities of lysophospholipase D to controls were calculated and expressed as percentages (mean ± SEM for three experiments).

## DISCUSSION

In this study, we found that human pregnancy was associated with elevated production of LPA, the phospholipid growth factor, during incubation of serum at 37°C. Our experiments with radioactive 16:0-LPC confirmed that human serum contains lysophospholipase D activity that converts LPC to LPA, and we found that the lysophospholipase D activity gradually increased during the entire period of pregnancy. The mechanism for elevation of lysophospholipase D activity during human pregnancy is still unknown. However, it appears not to result from a change in the level of LPC, the substrate of lysophospholipase D, because the level of LPC declines slightly with the stage of gestation, which is consistent with previous reports [26, 27]. Our results with 10-fold-diluted human serum incubated with exogenous 16:0-LPC showed that the lysophospholipase D activity of pregnant women was much higher than that of nonpregnant women, supporting the assumption mentioned above. Although the reduced serum level of LPC in preeclamptic women has been attributed to increased activity of lysophospholipase [28], our results suggest that increasing activity of lysophospholipase D in pregnancy may also be, in part, related to the early findings showing a decrease in the level of serum LPC in human pregnancy [26, 27].

The most abundant molecular species of LPA in incubated human sera from nonpregnant women was 16:0-LPA. This would mainly be caused by a much higher serum level of 16:0-LPC than of the other LPCs, although we could not exclude the possibility that other lysophospholipids contributed in the production of LPA to minor extents. Our

important finding is more preferable generation of 16:0-LPA than of other LPAs in sera from pregnant women compared to sera from nonpregnant women. Variation in the levels and composition of molecular species of serum LPC in pregnant and nonpregnant women may affect the composition of LPA formed when the sera were incubated at 37°C. However, small changes seen in both the levels and composition of molecular species of human LPC seem unable to account for the unusually higher percentage of 16:0-LPA in incubated sera from pregnant women. A likely explanation is release of additional lysophospholipase D activity that preferentially hydrolyzes 16:0-LPC over other LPCs in blood during pregnancy. It seems likely that this additional lysophospholipase D is of placental origin.

What is the physiological meaning of progressive elevation of LPA production by lysophospholipase D in blood during pregnancy? When LPA was intravenously injected into cats, it induced a sharp drop in the arterial blood pressure because of in vivo platelet aggregation in the pulmonary circulation [29], followed by hypertension [30]. On its intravenous injection into rats, LPA increased both the blood pressure and the intrauterine pressure [31]. Indeed, the level of Rho-activated protein kinase stimulated with LPA was reported to increase during pregnancy, raising the level of phosphorylated myosin light chain and causing increased muscle tone [32]. Apart from the platelet-aggregating and smooth muscle-contracting effects of LPA, attention has recently been directed to its regulatory effects on the integrity and function of vascular endothelial barrier [33–35]. Thus, one can speculate that the increased activity of the LPA-producing enzyme in the serum of pregnant women might relate to the altered fetal-maternal interaction through their circulations, which is essential for the successful maintenance of pregnancy and, thus, to achieve normal parturition at term [36].

As well as lysophospholipase D activity, the capacity of peripheral tissues for cellular uptake of LPA coupled with its metabolic degradation would be an important factor in strict control of the blood level of LPA and seems to be high enough for its rapid clearance from the circulation, because much less LPA was present in fresh human plasma and sera than that expected from its lysophospholipase D activity. The reported ectoenzyme family (lipid phosphate phosphatase) that dephosphorylates phosphatidic acid and LPA may be involved in the clearance of LPA from the circulation [37, 38]. Based on this finding, we speculate that the increased lysophospholipase D activity during pregnancy may provoke only moderate effects on vascular homeostasis through the sustained production of LPA, but we cannot exclude the possibility that the increased enzyme activity simply reflects the increased volume of blood vessels mainly caused by the placental development.

Zinc is known to participate either in the catalytic process or in the stabilization of protein structure of a variety of lipolytic enzymes, such as glycosylphosphatidylinositol-specific phospholipase D [39], sphingomyelinase in fetal bovine serum [40], and phospholipase C from *Bacillus cereus* [41]. Consistent with our previous findings concerning lysophospholipase D in rat plasma [13], the present study showed that treatment of human serum with EDTA resulted in a large reduction in activity of lysophospholipase D, but the enzyme activity was restored by adding a metal ion such as $Zn^{2+}$ and $Co^{2+}$ to the serum, indicating that its catalytic function was more important than its stabilizing effect on the enzyme. The lysophospholipase D in human serum appears to be an uncharacterized metalloenzyme. The $Mg^{2+}$-dependent lysophospholipase D that selectively hydrolyzes alkyl-ether-linked lysophospholipid, but not LPC, has been reported in various tissues [42], enterocytes [43], and epididymal spermatozoa of rats [44], whereas lyso-platelet-activating factor-selective lysophospholipase D in rabbit kidney medulla requires $Ca^{2+}$ [45]. Serum lysophospholipase D differs from intracellular lysophospholipase D in its substrate specificity and metal-ion requirements. We are now purifying the lysophospholipase D in human plasma. Its characterization will shed light on the physiological significance of LPA generated by this circulating lysophospholipase D in the female reproductive system.

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