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Augmented PLA_2 Activity in Pre-eclamptic Decidual Tissue—A Key Player in the Pathophysiology of 'Acute Atherosis' in Pre-eclampsia?

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Decidual acute atherosis is associated with pre-eclampsia, but the underlying mechanism is still unclear. We have previously demonstrated elevated level of the oxidative stress marker 8-isoprostaglandin $F_{2\alpha}$ (8-isoprostane) and lipids in pre-eclamptic decidual tissue. Arachidonic acid (AA) in tissue phospholipids is a source for 8-isoprostane generation, and 8-isoprostane is liberated from tissue phospholipids by phospholipase A_2 (PLA_2). The aims of this study were to explore whether AA content or PLA_2 expression in pre-eclamptic decidual tissue differed from controls. Decidua basalis tissues were obtained by vacuum aspiration at Caesarean delivery in pre-eclamptic and control pregnancies. We demonstrated a statistically significantly higher total PLA_2 activity in pre-eclamptic decidua compared to control tissue. On the other hand, no differences in AA content of tissue phospholipids or protein expression of secretory and cytosolic PLA_2 between pre-eclamptic and control decidual tissue were found. In conclusion, the elevated level of free 8-isoprostane in pre-eclamptic decidual tissue could be caused by augmented PLA_2 activity. We speculate that an elevated PLA_2 enzyme activity in pre-eclamptic decidual tissue could be of importance in the pathogenesis of 'acute atherosis', comparable to the atherogenesis in cardiovascular diseases.
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INTRODUCTION

Pre-eclampsia complicates 3–7 per cent of all human pregnancies, and is diagnosed by hypertension and proteinuria in the latter half of the pregnancy. The etiology of the disease is still unknown, but dysfunction of maternal systemic endothelial cells is a key feature of the syndrome (Roberts and Redman, 1993). Shallow placentation associated with dysfunctional trophoblast invasion (Zhou et al., 1993) of the maternal spiral arteries in the gestational endometrium (decidua) has been proposed to cause inadequate uteroplacental circulation and local ischaemia. Among uteroplacental compounds that are proposed to mediate disturbance of the maternal endothelium are lipid products, in particular lipid peroxides (Hubel et al., 1989). Patients with pre-eclampsia have many predisposing factors associated with the risk of developing atherosclerosis and cardiovascular diseases: hyperlipidemia, hypertension, obesity, insulin resistance, elevated serum homocysteine concentrations, inflammation and oxidative stress (Ross, 1999; Roberts and Hubel, 1999). In pre-eclampsia there are often areas of lipid deposition in the maternal spiral arterial walls, resembling early stages of atherosclerotic lesions, named 'acute atherosis' (Roberts and Redman, 1993). The 'acute atherosis' is characterized by fibrinoid necrosis of the vessel wall, an accumulation of lipid-laden macrophages, an intimal thickening in addition to different immune components (i.e. mononuclear cells and immunoglobulins, macrophages) (Labarrere, 1988). The mechanisms that contribute to the development of 'acute atherosis' in pre-eclampsia are largely unknown.

Oxidative stress has been proposed to be involved in the pathophysiology of pre-eclampsia (Roberts and Redman, 1993; Roberts and Hubel, 1999). Isoprostanes are stable end products of lipid peroxidation, and are used as reliable markers of oxidative stress. They are prostaglandin-isomers formed by free radical peroxidation of arachidonic acid (AA, *20:4w6*) present in phospholipids. The 8-isoprostanes are liberated by the action of phospholipase A_2 (PLA_2) (Morrow et al., 1992), and are abundantly expressed in atherosclerotic plaques (Pratico et al., 1997). In women with pre-eclampsia, plasma level of free 8-isoprostane is elevated compared to control pregnancies (Barden et al., 1996). We have in previous studies demonstrated elevated levels of lipid peroxides (Staff et al., 1999a) and free 8-isoprostane (Staff et al., 1999b) in gestational endometrium (decidua) at delivery in pre-eclamptic pregnancies compared to controls.

Hyperlipidemia in the maternal systemic circulation is a general feature of pregnancy, and is excessive in pre-eclampsia (Lorentzen and Henriksen, 1998). We have previously demonstrated an elevated level of lipid contents (i.e. cholesterol and

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phospholipids) in gestational endometrium (decidual tissue) in pre-eclamptic pregnancies as compared to controls (Staff et al., 1999a). Low-density lipoprotein (LDL) and oxidized LDL (oxLDL) are among several factors well established as important components in the general development of atherosclerosis (Lusis, 2000). Interestingly, circulating autoantibodies against oxLDL are found in pre-eclampsia (Branch et al., 1994; Uotila et al., 1998), and such circulating antibodies are recently reported to correlate positively with endothelial dysfunction (Fang et al., 2002), carotid atherosclerosis, inflammatory parameters and phospholipase A_2 (PLA_2) activity (Hulthe et al., 2001). Secretory PLA_2 ($sPLA_2$) has the ability to transform LDL into smaller atherogenic particles in vitro (Sartipy et al., 1999) through fragmentation of apoB-100, a major low-density lipoprotein (LDL)-derived apolipoprotein, resulting in a pro-inflammatory and atherogenic lipoprotein particle (Hurt-Camejo et al., 2001). The blood level of type II PLA_2 has been shown to be elevated in pre-eclampsia in several studies (Pulkkinen, Kivikoski and Nevalainen, 1993; Pulkkinen et al., 1996), and especially in severe pre-eclampsia (Lim et al., 1995). However, there is no information available on the PLA_2 activity in decidual tissue, the area with acute atherosis often seen in pre-eclamptic pregnancies.

The primary aim of this study was to investigate various possible pathways leading to the previously demonstrated elevated level of free 8-isoprostane in decidual tissue in pre-eclampsia. We explored whether pre-eclamptic decidual tissue had an altered level of AA in tissue phospholipids and/or changes in the expression and activity of PLA_2 as compared to control decidual tissue. We investigated two types of expressed PLA_2 in gestational tissue, namely secretory ($sPLA_2$) and cytosolic PLA_2 ($cPLA_2$) (Freed et al., 1997). We also measured apoB levels and degree of apoB fragmentation as indicators of LDL-derived lipid deposition and product of endogenous PLA_2 activity in decidua basalis, respectively.

MATERIAL AND METHODS

Patient selection

The women selected were previously healthy with uncomplicated pregnancies, except for pre-eclampsia in the study group. In particular, there were no women with chronic hypertension, renal disease, or diabetes included. The women, both in the pre-eclamptic group and the control group, were delivered by the Caesarean route. Caesarean section was performed in the pre-eclamptic patients (n=47) due to disease progression and/or unfavourable cervical ripening. Controls (n=44) were healthy, normotensive women undergoing Caesarean section on one of the following indications: breech presentation, cephalopelvic disproportion or psychological reasons. All pregnancies were singletons, and none of the women were in active labour at the time of Caesarean section.

Pre-eclampsia was defined as rise in blood pressure after 20 weeks' gestation to >140/90 on $\geq$2 occasions 6 h apart in a previously normotensive woman, combined with proteinuria. Proteinuria was defined as protein dip stix $\geq$1+ on $\geq$2 midstream urine samples 6 h apart, in the absence of urinary infection. Korotkoff's phase V was used to determine diastolic blood pressure. Pregnancy duration was based on routine ultrasonographic screening between gestational weeks 17 and 20.

Tissue samples

The decidua basalis samples were collected during Caesarean sections with a vacuum suction technique developed and previously described by our group (Staff et al., 1999a,b). In brief, decidua basalis tissue was obtained by careful vacuum aspiration of the uterine wall after the delivery of the baby and placenta. Decidual tissue from the area underlying the placenta was collected by the suction force directly on nylon net, which was immediately flushed with 500 ml sterile saline in order to remove blood. A random tissue portion of each sample was taken for histopathological confirmation of the decidual nature of the tissues. The remaining tissue was snap frozen without delay in liquid nitrogen and stored at $-80°$C, and was homogenized into powder with a pestle and mortar in liquid nitrogen until analyses. All organic solutions used in the analyses were of reagent grade. The study protocol was approved by the Regional Committee of Medical Ethics in Norway, and informed written consent was obtained from each patient.

Fatty acid pattern of phospholipids measured by gas liquid chromatography (GC)

Fatty acid pattern of decidual tissue (0.1 g) phospholipids from 18 pre-eclamptic women and 12 controls were analyzed by gas liquid chromatography as described elsewhere (Ranheim et al., 1994).

Cytosolic PLA_2 protein expression (Western blot analysis)

Proteins were extracted from decidual tissue from 17 pre-eclamptic women and 15 controls. The tissue powders were homogenized in ice-cold lysis buffer (PBS containing protease inhibitor cocktail (Gibco, Paisley, UK) with 1 per cent Triton X-100 and 0.1 per cent Tween 20) at a ratio of 0.1 ml per 10 mg wet weight tissue by a metal blade homogenizer. Extracts were incubated on ice for 15 min and then centrifuged at 12 000 g for 15 min at 4°C. The supernatants were retained and protein concentrations in the samples were measured with the BCA method (Pierce, Cheshire, UK). The protein extracts were separated on 15 per cent SDS–PAGE gels (Bio-Rad, Hercules, CA, USA) and electrophoretically transferred on to nitro-cellulose. After blocking overnight in TBS with 10 per

cent skimmed dried milk, the membranes were incubated with mouse anti-human secretory PLA_2 (Europa Bioproducts Ltd, Cambridge, UK) or mouse anti-human cytosolic PLA_2 (Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA). Each membrane was stripped and reprobed with mouse-anti-human β-actin (Sigma, St Louis, MO, USA) to ensure equal loading. Proteins were detected by enhanced chemiluminescence (ECL) with horseradish peroxidase-labelled anti-mouse IgG (Vector Laboratories, Burlingame, CA, USA) visualized after exposure to Hyperfilm ECL (Amersham). The values for $cPLA_2$ were obtained after standardizing the relative intensity using β-actin as a standard.

Secretory PLA_2 protein expression (ELISA)

Pulverized decidual tissue samples from 17 pre-eclamptic women and 15 controls were resuspended in ice-cold lysis, as described above. The supernatants were retained and protein concentrations in the samples were measured with the BCA method (Pierce). The supernatants were analyzed for $sPLA_2$ type II protein levels by an ELISA kit (Cayman). The values for $sPLA_2$ were calculated as pg $sPLA_2$ per mg extracted tissue protein.

Secretory PLA_2 mRNA (Northern blot analysis)

The mRNA was isolated from the decidual tissue samples from 23 pre-eclamptic and 19 control pregnancies as described by the manufacturer, using Dynabeads Oligo $(dT)_{25}$ (Dynal A.S., Oslo, Norway) and the Northern blot was performed as described earlier (Halvorsen et al., 1998). The $sPLA_2$ cDNA probe (which was kindly provided from Dr Jean-Luc Olivier) was labelled with [^{32}P]dCTP, using a Megaprime DNA labelling kit (Amersham, Buckinghamshire, UK). The size of the mRNA was determined with reference to a RNA standard. Finally, the signals were analyzed by a Phosphor Imager (Molecular Dynamics, Sunnyvale, CA, USA). A probe for human β-actin (ATCC, Rockville, MD, USA) was used to calibrate the mRNA signal levels.

PLA_2 enzyme activity

Total PLA_2 activity in decidual tissue extracts from 13 patients with pre-eclampsia and 10 control patients were examined. The samples were homogenized in the same lysis buffer as described above, but without protease inhibitors. The PLA_2 activity decidual extract was assessed by a method described by Petrovic et al., 2001, using 2 mM 1,2-bis (heptanoylthio) glycerol phosphocholine (Cayman, Ann Arbor, MI, USA) as substrate, which is a substrate for all PLAs with exception of $cPLA_2$ and PAF-acetyl hydrolase. The absorbance at 412 and 600 nm (the latter to correct for any turbidity in the sample) were detected on a microtiter reader. Specific activity for PLA_2 enzyme activity was calculated using $\varepsilon_{412\ nm}$ for DTNB [5,5′-dithiobis(2-nitrobenzoic acid)] of $1.36 \times 10^4\ M^{-1}\ cm^{-1}$ (Winterbourn, 1985).

Apolipoprotein B protein levels (immunoassay)

Proteins were extracted from decidual tissue from 17 pre-eclamptic women and 13 controls as described above, and the apoB levels in the samples were assessed by an immunoturbimetric assay (Bio Merieux, Marcy-l'Etoile, France).

Apolipoprotein B fragmentation (Western blots)

To assess the degree of apoB fragmentation in decidual tissue, proteins were extracted from decidual tissue in 17 pre-eclamptic women and 17 controls as explained above. The membranes were incubated with mouse anti-human apoB (Santa Cruz Biotechnology, Santa Cruz, CA, USA). Each membrane was stripped and reprobed with mouse-anti-human β-actin (Sigma) to ensure equal loading. Proteins were detected by enhanced chemiluminescence (ECL). The primary antibody was also examined for the ability to demonstrate Cu^{2+}-oxidized LDL (Halvorsen et al., 1998), and tested positive, verifying that the antibody used in the Western blot binds oxLDL, thus measuring apoB fragmentation qualitatively.

Statistical analysis

The results are presented as the mean values as well as 95 per cent confidence intervals (CI) for the means for the main results. Statistical analyses were performed with the Statistical Package for the Social Sciences (SPSS-PC), version 10.0. Differences between groups were tested by non-parametric Mann–Whitney *U* tests. Pearson correlation was used to calculate a correlation coefficient between apoB and PLA_2 enzyme activity measurements. Probability of less than 0.05 was considered statistically significant.

RESULTS

Clinical characteristics

Clinical characteristics for all the patients included in the study (47 pre-eclamptic patients and 44 controls) are shown in Table 1. The patient groups were similar regarding age at delivery. The mean body mass index (BMI) of the pre-eclamptic group was higher than in the control group both before pregnancy and at delivery. The mean parity and gravidity number was slightly higher in the control group compared to the pre-eclampsia group. The systolic and diastolic blood pressures differed significantly between the pre-eclamptic and control

Table 1. Clinical characteristics of all the 91 women included in the study

	Pre-eclampsia (n=47)	Controls (n=44)	Statistical significance
Patient age at delivery (y)	30.5	32.4	P=0.09
Body mass index before pregnancy (kg/m^2)	24.6	21.6	P=0.003*
Body mass index at delivery (kg/m^2)	30.3	26.3	P<0.001*
Gravidity	1.8	2.3	P=0.02*
Parity	0.4	0.8	P=0.01*
Gestational age at delivery (wk)	33.8	38.4	P<0.001*
Neonatal weight (g)	2178	3370	P<0.001*
Neonatal weight percentile	39	50	P=0.01*
Systolic blood pressure at delivery (mmHg)	169	117	P<0.001*
Diastolic blood pressure at delivery (mmHg)	106	75	P<0.001*

Values are presented as means.

* $P \leq 0.05$.

groups, due to the inclusion blood pressure criteria in the study. The mean pregnancy duration at delivery was shorter in the pre-eclampsia group compared to the control group. The mean neonatal weight was therefore not surprisingly lower in the pre-eclampsia group compared to the control group. When corrected for gestational length, the mean neonatal weight percentile was also lower in the pre-eclampsia compared to the control group. For the various experiments of this study, subgroups from this pool of 91 patients were investigated, as described in the 'material and methods' section. The clinical descriptions for the two patient groups in the diverse experiments did not differ significantly from the characteristics of the total pool of patients (results not shown).

Fatty acid composition in decidua basalis

Due to our previously reported elevated 8-isoprostane level in pre-eclamptic decidual tissue (Staff et al., 1999b), we examined the AA substrate availability in the decidual tissue. As Figure 1 shows there was no difference in the mean content of phospholipid-bound AA (*20:4ω6*) in the decidual tissue (shown as μg AA per gram wet weight tissue) between the pre-eclamptic and control pregnancies. For the pre-eclamptic group, the mean value was 907 μg AA in the phospholipid fraction per gram decidual tissue (95 per cent CI: 584–1231), whereas the mean value for the control group was 903 (95 per cent CI: 532–1275) (P=0.8). For the pre-eclamptic group, the mean percentage of AA as percentage of total FFAs was 17.6 per cent (95 per cent CI: 16.2–18.9), whereas the mean value for the control group was 17.3 per cent (95 per cent CI 16.4–18.1) (P=0.5).

Table 2 lists the relative and absolute content of the quantitatively most important fatty acids (which we defined as more than 1 per cent of total fatty acids) in the phospholipids of the decidual tissue from the two patient groups. We did not demonstrate any major differences between the pre-eclampsia and control groups in fatty acid content for the decidual tissues analyzed in this study.

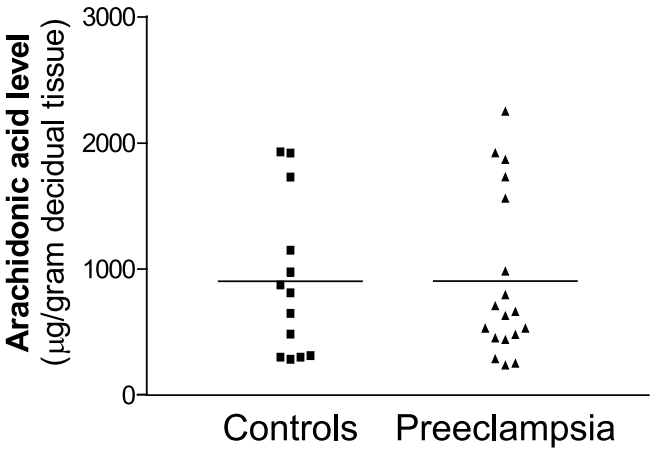

Figure 1. Scatterplot of content of arachidonic acid (AA) in phospholipids extracted from decidual tissue measured by GC as described in 'Methods'. The horizontal lines represent the mean values for the patient groups (13 pre-eclamptic and 18 control cases). AA content in phospholipids is expressed as μg AA/gram wet weight tissue.

Table 2. Content of fatty acids in phospholipids extracted from decidua basalis in pre-eclamptic and control pregnancies

Fatty acid	PE (n=18)	Controls (n=12)	Statistical significance
15:0	219 (4.4)	231 (4.3)	P=0.9 (P=0.5)
16:0	1203 (25.2)	1182 (23.1)	P=1.0 (P=0.06)
18:0	843 (16.0)	1021 (18.3)	P=0.6 (P=0.1)
18:1ω9	539 (11.6)	619 (11.9)	P=0.9 (P=0.7)
18:1ω7	94 (2.2)	79 (1.7)	P=0.4 (P=0.1)
18:2ω6	451 (9.3)	514 (10.1)	P=0.5 (P=0.2)
20:3ω6	142 (2.7)	163 (2.7)	P=0.8 (P=0.7)
20:4ω6 (*AA*)	907 (17.6)	953 (17.4)	P=0.6 (P=0.7)

Data are presented as mean μg fatty acid/gram tissue (and mean percentage of fatty acid relative to total fatty acids in the tissue phospholipids).

Decidual protein content of cytosolic and secretory PLA_2

Next, we examined the protein level of $cPLA_2$ and $sPLA_2$. Table 3 shows that the protein expression of $cPLA_2$ in decidual tissue (measured by Western blots and corrected for β-actin protein expression) did not differ between the pre-eclampsia and control group of patients. The mean $cPLA_2$ protein expression was 2.7 (95 per cent CI 1.2–4.2) for the pre-eclamptic group and 1.7 (95 per cent CI 1.1–2.2) for the control group (P=0.6). The immunoreactive band of $cPLA_2$ was observed at the expected molecular weight about 100 kDa. Protein expression of $sPLA_2$ (measured with an ELISA kit) was not statistically different between the two patient groups

Table 3. Cytosolic and secretory PLA_2 protein expression in decidual tissue

	PLA2 protein level		
	Controls	Pre-eclampsia	Statistical significance
$cPLA_2$	1.7 (1.1–2.2) (n=15)	2.7 (1.2–4.2) (n=17)	P=0.6
$sPLA_2$	14.9 (7.1–22.7) (n=15)	11.4 (4.0–18.8) (n=17)	P=0.6

Data are presented as means (and 95 per cent confidence intervals). For cPLA2, Western blots were used to determine protein level relative to an internal control decidua standard on each blot; all results are relative to β-actin protein expression. For sPLA2, ELISA kits were used to determine protein content, and results are given in pg/mg decidual tissue protein.

(P=0.6). The mean protein value of $sPLA_2$ in the pre-eclampsia group was 11.4 (95 per cent CI: 4.0–18.8) pg/mg decidual tissue protein, as compared to 14.9 (95 per cent CI: 7.1–22.7) in the control group (Table 3).

Secretory PLA_2 mRNA expression

The relative expression of $sPLA_2$ mRNA was 123 (95 per cent CI: 96–149) for the pre-eclamptic group (n=23) compared to 100 (95 per cent CI: 86–114) for the control group (n=19) (blots not shown). This difference between the patient groups was not statistically significant (P=0.3).

Total PLA_2 enzyme activity

Even though no significant differences were observed on the protein expression of $cPLA_2$ and $sPLA_2$ in decidual tissue, we investigated the PLA_2 enzyme activity. And notably, we demonstrated an augmented enzyme activity of total PLA_2 in decidual tissues from pre-eclamptic pregnancies compared to the control group (Figure 2). The mean PLA_2 activity in the pre-eclampsia group was 571 (95 per cent CI: 346–795) nmol/min/g tissue protein, as compared to 257 in the control group (95 per cent CI: 173–341), and this difference was statistically significant (P=0.047).

ApoB protein content and degree of apoB fragmentation

We have previously demonstrated an augmented cholesterol content in pre-eclamptic decidual tissue compared to controls (Staff et al., 1999a). Another parameter associated with LDL-derived lipid deposition in tissues is the apoB content, which we therefore analyzed in decidual tissue. Figure 3A shows a scatterplot of apoB protein level in the decidual tissue of 30 patients (17 with pre-eclampsia and 13 controls), measured by immunoturbidity assay. The mean content of apoB protein (mg per mg tissue protein) in the pre-eclampsia group (1.35, 95 per cent CI: 0.97–1.73) was not statistically significantly different from the slightly higher mean apoB protein content of the control group (1.71, 95 per cent CI: 0.99–2.43) (P=0.1).

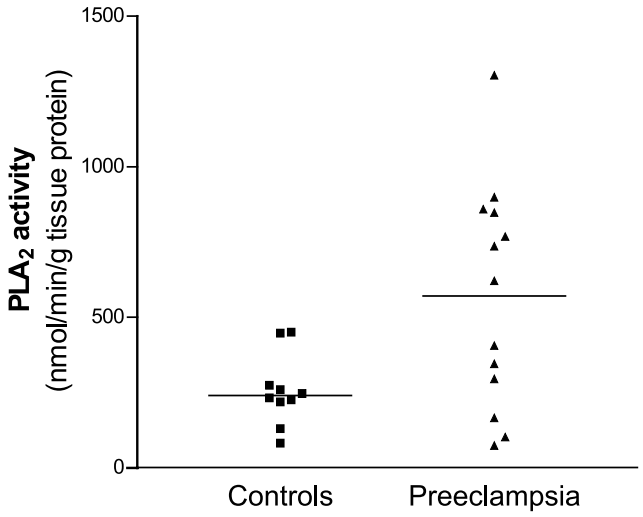

Figure 2. Enzyme activity level of PLA_2 in decidua basalis. The horizontal lines represent the mean values for the patient groups (13 pre-eclamptic and 12 control cases). The PLA2 activity in the tissue extract sample is expressed as nmol/min/g tissue protein.

ApoB-100 is a major LDL-derived apolipoprotein with a molecular weight of ∼220 kDa. ApoB could proceed into smaller molecular weight bands caused by i.e. oxidative stress and PLA_2 activity (Nishi et al., 2002) that qualitatively could be observed as fragmentation on a Western blot. Figure 3B demonstrates the qualitative measurement of fragmented apoB related to protein expression of β-actin (42 kDa) in decidual tissue. There seems, however, to be a wide variability in apoB fragmentation in both the two patient groups, and no evident qualitative difference in apoB fragmentation in decidual tissue between the pre-eclampsia and the control group could be demonstrated. Also, pregnancy duration does not seem to be associated with degree of apoB fragmentation in the pre-eclampsia group (data not shown).

Correlation between apoB and PLA_2 activity

LDL has been reported to perform PLA_2 activity itself (Parthasarathy et al., 1985). Therefore, we examined the correlation between apoB content and PLA_2 activity in decidual tissue. As Figure 4 shows there was positive correlation (Pearson's correlation=0.45) between apoB values and PLA_2 activity in decidua for the pre-eclampsia group, but not reaching statistical significance (P=0.1).

DISCUSSION

In the present study, we showed augmented PLA_2 activity in decidual tissue from pre-eclamptic patients compared to controls. On the other hand, we did not find any altered content of

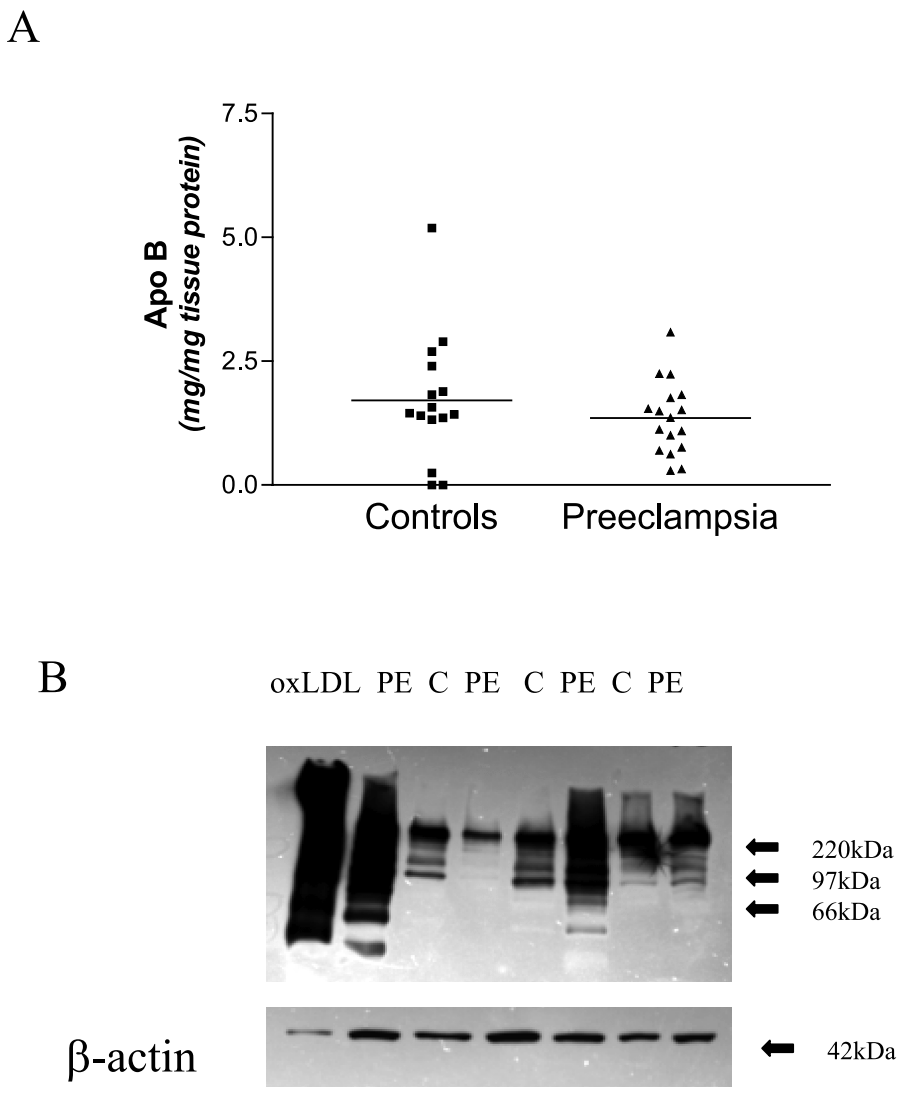

Figure 3. Apolipoprotein B (apoB) protein content in decidua basalis measured by immunoturbidity assay (A). The horizontal lines represent the mean values for the patient groups (17 pre-eclamptic and 15 control cases). ApoB fragmentation (upper panel) and β-actin (lower panel), analyzed by Western blots (17 pre-eclamptic and 15 control cases) (B). The apoB content (A) is expressed as mg apoB/mg tissue protein.

AA or altered protein expression of secretory or cytoplasmatic PLA_2 in pre-eclamptic decidual tissue compared to control pregnancies.

We have previously reported an elevated content of total phospholipids (including all types of phospholipids, also lysophosphatidylcholine) in decidual tissue in pre-eclampsia compared to control pregnancies (Staff et al., 1999a). In the present study we did not find a difference between the pre-eclamptic and control group regarding the content of AA bound to phospholipids per gram wet weight decidua. Adding these two studies, it is possible that the total amount of AA available for 8-isoprostane formation on the tissue phospholipids is in fact lower in the pre-eclampsia group than in the control group. This latter situation could possibly result from an augmented oxidative stress situation in pre-eclamptic decidua, where peroxidation has already transformed some of the available AA on the phospholipids to 8-isoprostane, which subsequently is released in free form to the decidual tissue and the maternal circulation by the augmented activity of decidual PLA_2, the latter being demonstrated in the present study. Notably, oxLDL is indirectly shown to be elevated in pre-eclamptic circulation (Branch et al., 1994) and is also reported as a potent activator of PLA_2 (Ozaki et al., 1999). In fact, an augmented decidual PLA_2 activity in pre-eclampsia as shown in the present paper could explain the findings in several of our previous reports.

Pre-eclampsia is a syndrome associated with augmented inflammatory response compared to control pregnancies (Redman, Sacks and Sargent, 1999), including augmented TNFα (Vince et al., 1995; Yoneyama et al., 2002) and NF- κB activities in plasma (Takacs et al., 2001). Moreover, $sPLA_2$ is involved in AA release during inflammatory conditions, and it is produced in vascular cells after stimulation of pro-inflammatory cytokines (Andreani et al., 2000). Several

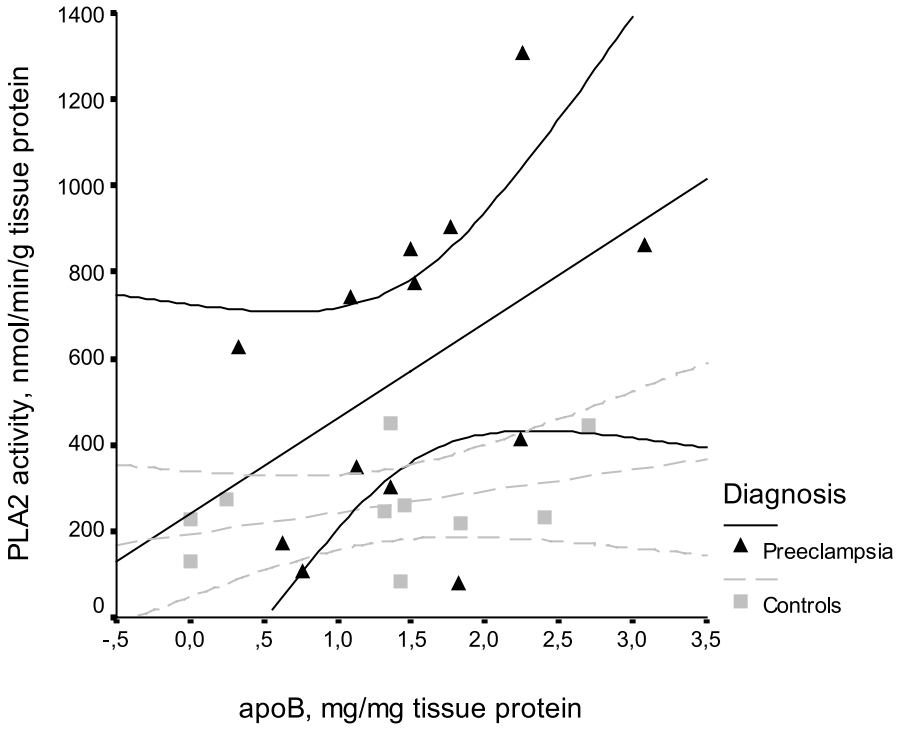

Figure 4. Scatterplot of apoB protein levels and PLA_2 activity in decidua basalis. The lines demonstrate the regression lines for each patient group (13 pre-eclamptic and 12 control cases).

pro-inflammatory cytokines are stimulated through an activation of NF-κB (Tak and Firestein, 2001), and we have shown in another study that 8-isoprostane increases NF-κB activity (Halvorsen et al., 2001). In addition to this, 8-isoprostane is found to increase the expression of pro-inflammatory IL-8 in human macrophages (Schulz et al., 2002), suggesting an important link between lipids and inflammation in atherosclerosis, which could also be relevant for the formation of acute atherosis in pre-eclampsia.

We were not able to detect any changes in either apoB content or apoB fragmentation in pre-eclamptic decidual tissue in the present study, which we expected due to our previous published report of increased lipids (i.e. cholesterol and phospholipids) as well as the increased PLA_2 activity demonstrated in the present study. An important consideration should be taken into account regarding the commercial kit used in this paper for quantitative measurements of apoB. In test experiments the kit proved to underestimate Cu2+-modified LDL about 30 per cent, as compared to native LDL. This could at least partly explain the unaltered content of apoB in decidual tissue between pre-eclamptic and normal pregnancies found in our present study even though we earlier reported elevated content of cholesterol and phospholipids in the decidual tissue from pre-eclamptic women (Staff et al., 1999a). We showed in this study a great variation in biochemical markers (i.e. apoB fragmentation, PLA_2 expression) that could be associated with oxidative stress in decidual tissue, both among normal pregnant women and pre-eclamptic patients at Caesarean delivery. Although apoB fragmentation has been demonstrated to be a reliable index of atherosclerosis (Hashimoto et al., 2002), we were not able to detect any differences between the patient groups, presumably due to the fact that pre-eclampsia is a heterogeneous disease with various clinical degrees of severity and large variety in pregnancy length at delivery. And importantly, acute atherosis is not always present in spiral arteries of decidua basalis in pre-eclampsia. Meekins et al., 1994, reported that 62 per cent of decidual spiral arteries demonstrated acute atherosis.

We have in a previous study demonstrated an increased oxLDL-mediated foam cell formation of trophoblastic cells incubated with 8-isoprostane compared with control cells (Halvorsen et al., 2001). It has been suggested that type II-$sPLA_2$ could play an important role in early atherosclerosis because it is present in the pre-atherosclerotic arterial wall, where it may induce LDL-modification and foam cell formation (Schiering et al., 1999). In addition to an augmented PLA_2 activity in decidual tissue, an upregulated uptake of oxLDL by the LOX receptor (Halvorsen et al., 2001) could be a mechanism contributing to the development of decidual tissue 'acute atherosis' in pre-eclampsia.

We have previously suggested that decidua basalis might contribute to the endothelial dysfunction and the pathophysiology of pre-eclampsia by the release of 8-isoprostane into the maternal circulation (Staff et al., 1999b). There are several possible mechanisms for an elevated content of free 8-isoprostane in decidual pre-eclamptic tissue. An increase in AA substrate could not be responsible for the elevated 8-isoprostane level, since there was no difference between the

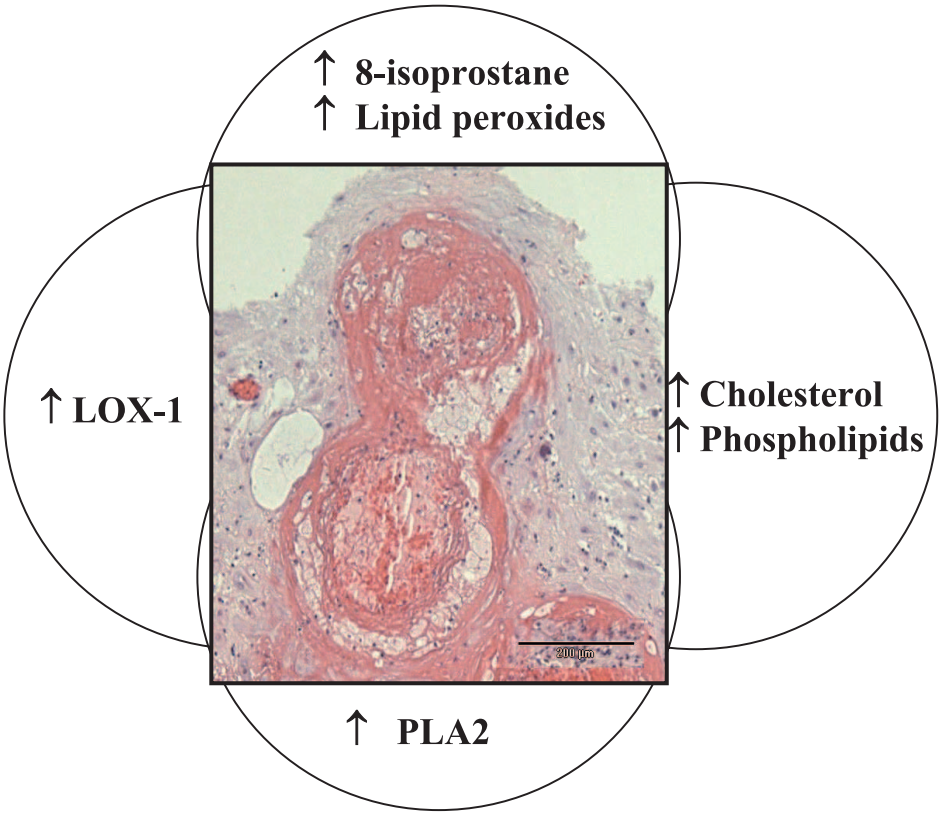

Figure 5. A hypothetical model of a PLA_2 driven process that could promote 'acute atherosis' in spiral arteries in pre-eclamptic pregnancies. The HE (haematoxilin, eosin) stained decidual tissue section shows a decidual spiral artery with areas of 'acute atherosis', including foam cells and vessel lumen filled with a thrombus. The tissue is from a patient with pre-eclampsia included in the present stduy, and the tissue is collected by the decidual suction method. Scale bar: 200 µm.

pre-eclamptic and control group in this regard in the present study. However, one explanation could be that there are more lipids in pre-eclamptic decidual tissue (such as phospholipids, free fatty acids and cholesterol) as previously reported (Staff et al., 1999a). Importantly, we also have reported an increased content of lipid peroxides (Staff et al., 1999a) in decidua basalis in pre-eclampsia, which together with augmented 8-isoprostane suggest that an oxidative stress could be a driving force of the pathogenesis of 'acute atherosis' seen in pre-eclampsia, as outlined in Figure 5. Along with this we speculate that an oxidative stress condition could stimulate the PLA_2 activity in decidual basalis during pre-eclampsia leading to (i) accelerated release of more 8-isoprostane, and (ii) enhanced inflammation and (iii) promoted lipid depositions, thus promoting essential features of pre-eclampsia, namely endothelial dysfunction and 'acute atherosis'. However, further studies are needed to elucidate the mechanisms for development of 'acute atherosis' in gestational tissue.

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REFERENCES

Andreani M, Olivier JL, Berenbaum F, Raymondjean M & Bereziat G (2000) Transcriptional regulation of inflammatory secreted phospholipases A(2). *Biochim Biophys Acta*, **1488**, 149–158.

Barden A, Beilin LJ, Ritchie J, Croft KD, Walters BN & Michael CA (1996) Plasma and urinary 8-iso-prostane as an indicator of lipid peroxidation in pre-eclampsia and normal pregnancy. *Clin Sci*, **91**, 711–718.

Branch DW, Mitchell MD, Miller E, Palinski W & Witztum JL (1994) Pre-eclampsia and serum antibodies to oxidised low-density lipoprotein. *Lancet*, **343**, 645–646.

Fang JC, Kinlay S, Behrendt D, Hikita H, Witztum JL, Selwyn AP & Ganz P (2002) Circulating autoantibodies to oxidized LDL correlate with impaired coronary endothelial function after cardiac transplantation. *Arterioscler Thromb Vasc Biol*, **22**, 2044–2048.

Freed KA, Moses EK, Brennecke SP & Rice GE (1997) Differential expression of type II, IV and cytosolic PLA2 messenger RNA in human intrauterine tissues at term. *Mol Hum Reprod*, **3**, 493–499.

Halvorsen B, Ranheim T, Nenseter MS, Huggett AC & Drevon CA (1998) Effect of a coffee lipid (cafestol) on cholesterol metabolism in human skin fibroblasts. *J Lipid Res*, **39**, 901–912.

Halvorsen B, Staff AC, Henriksen T, Sawamura T & Ranheim T (2001) 8-iso-Prostaglandin $F_{2\alpha}$ increases expression of LOX-1 in JAR cells. *Hypertension*, **37**, 1184–1190.

Hashimoto R, Matsukawa N, Nariyama Y, Ogiri Y, Hamagawa E, Tanaka K, Usui Y, Nakano S, Maruyama T, Kyotani S, Tushima M & Kojo S (2002) Evaluation of apolipoprotein B-100 fragmentation and cross-linkage in serum as an index of atherosclerosis. *Biochim Biophys Acta*, **1584**, 123–128.

Hubel CA, Roberts JM, Taylor RN, Musci TJ, Rogers GM & McLaughlin MK (1989) Lipid peroxidation in pregnancy: new perspectives on preeclampsia. *Am J Obstet Gynecol*, **161**, 1025–1034.

Hulthe J, Wiklund O, Hurt-Camejo E & Bondjers G (2001) Antibodies to oxidized LDL in relation to carotid atherosclerosis, cell adhesion molecules, and phospholipase A(2). *Arterioscler Thromb Vasc Biol*, **21**, 269–274.

Hurt-Camejo E, Camejo G, Peilot H, Oomi K & Kovanen P (2001) Phospholipase A(2) in vascular disease. *Circ Res*, **89**, 298–304.

Labarrere CA (1988) Acute atherosis. A histopathological hallmark of immune aggression?. *Placenta*, **9**, 95–108.

Lim KH, Rice GE, de Groot CJ & Taylor RN (1995) Plasma type II phospholipase A2 levels are elevated in severe preeclampsia. *Am J Obstet Gynecol*, **172**, 998–1002.

Lorentzen B & Henriksen T (1998) Plasma lipids and vascular dysfunction in preeclampsia. *Semin Reprod Endocrinol*, **16**, 33–39.

Lusis AJ (2000) Atherosclerosis. *Nature*, **407**, 233–241.

Meekins JW, Pijnenborg R, Hanssens M, McFadyen IR & van Asshe A (1994) A study of placental bed spiral arteries and trophoblast invasion in normal and severe pre-eclamptic pregnancies. *Br J Obstet Gynaecol*, **101**, 669–674.

Morrow JD, Awad JA, Boss HJ, Blair IA & Roberts LJ (1992) Non-cyclooxygenase-derived prostanoids (F2-isoprostanes) are formed in situ on phospholipids. *Proc Natl Acad Sci U S A*, **89**, 10721–10725.

Nishi K, Itabe H, Uno M, Kitazato KT, Horiguchi H, Shinno K & Nagahiro S (2002) Oxidized LDL in carotid plaques and plasma associates with plaque instability. *Arterioscler Thromb Vasc Biol*, **22**, 1649–1654.

Ozaki M, Yamada Y, Matoba K, Otani H, Mune M, Yukawa S & Sakamoto W (1999) Phospholipase A2 activity in ox-LDL-stimulated mesangial cells and modulation by alpha-tocopherol. *Kidney Int Suppl*, **71**, S171.

Parthasarathy S, Steinbrecher UP, Barnett J, Witztum JL & Steinberg D (1985) Essential role of phospholipase A2 activity in endothelial cell-induced modification of low density lipoprotein. *Proc Natl Acad Sci U S A*, **82**, 3000–3004.

Petrovic N, Grove C, Langton P, Misso NL & Thompson P (2001) A simple assay for a human serum phospholipase A2 that is associated with high-density lipoproteins. *J Lipid Res*, **42**, 1706–1713.

Pratico D, Iuliano L, Mauriello A, Spagnoli L, Lawson JA, Rokach J, Maclouf J, Violi F & FitzGerald GA (1997) Localization of distinct F2-isoprostanes in human atherosclerotic lesions. *J Clin Invest*, **100**, 2028–2034.

Pulkkinen MO, Kivikoski AI & Nevalainen TJ (1993) Group I and group II phospholipase A2 in serum during normal and pathological pregnancy. *Gynecol Obstet Invest*, **36**, 96–101.

Pulkkinen MO, Poranen AK, Kivikoski AI & Nevalainen TJ (1996) Elevated serum group II phospholipase A2 levels are associated with decreased blood flow velocity in the umbilical artery. *Gynecol Obstet Invest*, **4**, 93–95.

Ranheim T, Gedde-Dahl A, Rustan AC & Drevon CA (1994) Fatty acid uptake and metabolism in CaCo-2 cells: eicosapentaenoic acid (20:5(n-3)) and oleic acid (18:1(n-9)) presented in association with micelles or albumin. *Biochim Biophys Acta*, **1212**, 295–304.

Redman CW, Sacks GP & Sargent IL (1999) Preeclampsia: An excessive maternal inflammatory response to pregnancy. *Am J Obstet Gynecol*, **180**, 499–506.

Roberts JM & Hubel CA (1999) Is oxidative stress the link in the two-stage model of pre-eclampsia?. *Lancet*, **354**, 788–789.

Roberts JM & Redman CW (1993) Preeclampsia: more than pregnancy-induced hypertension. *Lancet*, **341**, 1447–1451.

Ross R (1999) Atherosclerosis—an inflammatory disease. *N Engl J Med*, **340**, 115–126.

Sartipy P, Camejo G, Svensson L & Hurt-Camejo E (1999) Phospholipase A(2) modification of low density lipoproteins forms small high density particles with increased affinity for proteoglycans and glycosaminoglycans. *J Biol Chem*, **274**, 25913–25920.

Schiering A, Menschikowski M, Mueller E & Jaross W (1999) Analysis of secretory group II phospholipase A2 expression in human aortic tissue in dependence on the degree of atherosclerosis. *Atherosclerosis*, **144**, 73–78.

Schulz H, Yndestad A, Aukrust P, Hall C & Halvorsen B (2002). 8-iso-PGF2a stimulates interleukin-8 expression in human THP-1 derived macrophages. *Abstracts of the Molecular Medicine- XXVIII Nordic Congress in Clinical Medicine*, *Reykjavik*, 2002. p.50.

Staff AC, Ranheim T, Khoury J & Henriksen T (1999a) Increased contents of phospholipids, cholesterol and lipid peroxides in decidua basalis in preeclampsia. *Am J Obstet Gynecol*, **180**, 587–592.

Staff AC, Halvorsen B, Ranheim T & Henriksen T (1999b) Elevated level of free 8-iso-prostaglandin $F_{2\alpha}$ in the decidua basalis of women with preeclampsia. *Am J Obstet Gynecol*, **181**, 1211–1215.

Tak PP & Firestein GS (2001) NF-kappaB: a key role in inflammatory diseases. *J Clin Invest*, **107**, 7–11.

Takacs P, Kauma SW, Sholley MM, Walsh SW, Dinsmoor MJ & Green K (2001) Increased circulating lipid peroxides in severe preeclampsia activate NF-kappaB and upregulate ICAM-1 in vascular endothelial cells. *FASEB J*, **15**, 279–281.

Uotila J, Solakivi T, Jaakkola O, Tuimala R & Lehtimaki T (1998) Antibodies against copper-oxidised and malondialdehyde-modified low density lipoproteins in pre-eclampsia pregnancies. *Br J Obstet Gynaecol*, **105**, 1113–1117.

Vince GS, Starkey PM, Austgulen R, Kwiatkowski D & Redman CW (1995) Interleukin-6, tumour necrosis factor and soluble tumour necrosis factor receptors in women with preeclampsia. *Br J Obstet Gynaecol*, **102**, 842–845.

Winterbourn CC (1985) Comparative reactivities of various biological compounds with myeloperoxidase-hydrogen peroxide-chloride, and similarity of the oxidant to hypochlorite. *Biochem Biophys Acta*, **840**, 204–210.

Yoneyama Y, Suzuki S, Sawa R, Yoneyama K, Power GG & Araki T (2002) Increased plasma adenosine concentrations and the severity of preeclampsia. *Obstet Gynecol*, **100**, 1266–1270.

Zhou Y, Damsky CH, Chiu K, Roberts JM & Fisher SJ (1993) Preeclampsia is associated with abnormal expression of adhesion molecules by invasive cytotrophoblasts. *J Clin Invest*, **91**, 950–960.