Free Radical Research, Volume 38 Number 9 (September 2004), pp. 913–918

Taylor & Francis
healthsciences

Isoprostanes, Prostaglandins and Tocopherols in Pre-eclampsia, Normal Pregnancy and Non-pregnancy

OSAMU ISHIHARA[a], MASATOSHI HAYASHI[a], HIROYUKI OSAWA[a], KOICHI KOBAYASHI[a], SATORU TAKEDA[a], BENGT VESSBY[b] and SAMAR BASU[b,*]

[a]*Department of Obstetrics and Gynaecology, Saitama Medical School, Moroyama, Japan;* [b]*Sections of Geriatrics and Clinical Nutrition Research, Faculty of Medicine, Uppsala University, Uppsala, Sweden*

Accepted by Professor B. Halliwell

(Received 4 May 2004; In revised form 15 June 2004)

This study is designed to evaluate whether oxidative stress and inflammation are involved in severe pre-eclampsia compared to normal pregnancy and non-pregnancy. We have measured plasma and urinary levels of 8-iso-$PGF_{2\alpha}$, a major isoprostane as an indicator of oxidative stress; plasma and urinary 15-keto-dihydro-$PGF_{2\alpha}$, a major metabolite of cyclooxygenase-catalysed $PGF_{2\alpha}$ as an indicator of inflammatory response, and plasma -α-and -γ-tocopherol in 18 pre-eclamptic, 19 normal pregnancy and 20 non-pregnant women. Pregnant women had significantly higher levels of 8-iso-$PGF_{2\alpha}$ and $PGF_{2\alpha}$ metabolite as compared to the non-pregnancy. Levels of 8-iso-$PGF_{2\alpha}$ in the pre-eclamptic women did not differ from the normal pregnancy but $PGF_{2\alpha}$ metabolite levels were significantly higher in normal pregnancy. On the other hand, γ-tocopherol levels were significantly lower in pre-eclampsia than normal pregnancy. In contrast, the concentration of α-tocopherol was very similar between the groups. α-and γ-tocopherol levels were significantly lower in pregnancy compared to non-pregnancy. Although no direct evidence of oxidative stress and inflammatory response was observed in severe pre-eclampsia, a reduction of γ-tocopherol suggests the possible precedence of oxidative stress in this condition. Higher levels of isoprostanes and prostaglandin metabolite in late pregnancy suggest the importance of both free radicals and cyclooxygenase-catalysed oxidation products in normal biological processes of pregnancy.

Keywords: Pre-eclampsia; Oxidative stress; Isoprostanes; Inflammation; Prostaglandins; Antioxidants; Human

INTRODUCTION

The etiology of pre-eclampsia is a complex process and is poorly understood. Oxidative stress has been implicated in the pathophysiology of pre-eclampsia that are characterized by hypertension and proteinuria in pregnant women. Recent reports suggest that several biomarkers might be used as predictors of pre-eclamptic manifestations or as an indicator of endothelial dysfunction in severe pre-eclampsia.[1,2] Further, it was also shown that polymorphoneuclear leukocytes (PMN), an important inflammatory cell in the circulatory system might generate reactive oxygen species inducing oxidative stress, inflammation and endothelial damage.[3] However, several of the biomarkers that are commonly used have proved to be unreliable for the assessment of free radical generation due to the limitations of these methods.[4]

Recently, 8-iso-$PGF_{2\alpha}$, a major isoprostane generated through the non-enzymatic peroxidation of arachidonic acid, has shown to be a reliable indicator of oxidative stress.[5,6] Several studies in pre-eclampsia and normal pregnancy have been performed by assessing circulatory isoprostanes but the results are conflicting.[7–9] However, a recent study questioned the importance of oxidative stress in pre-eclampsia, since urinary isoprostane levels did not differ significantly between the pre-eclamptic cases and control.[10] Further, the role of cyclooxygenase-catalysed prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) as an intravascular inflammatory reponser due to endothelial cell dysfunction in pre-eclampsia is relatively unknown. Intracellular reactive oxygen species may increase in various cell types in pre-eclampsia, which may release inflammatory stimuli to the maternal

*Corresponding author. Tel.: +46-18-6117958. Fax: +46-18-6117976. E-mail: samar.basu@pubcare.uu.se

ISSN 1071-5762 print/ISSN 1029-2470 online © 2004 Taylor & Francis Ltd
DOI: 10.1080/10715760412331273421

circulation.[11] We, therefore, investigated the extent of oxidative stress and successive involvement of inflammation in normal pregnancy, pre-eclamptic patients and non-pregnant cases by measuring both plasma and urinary lipid peroxidation products, i.e. 8-iso-$PGF_{2\alpha}$ and 15-keto-dihydro-$PGF_{2\alpha}$, a major metabolite of $PGF_{2\alpha}$ as well as plasma antioxidants, i.e. α-tocopherol and γ-tocopherol.

MATERIALS AND METHODS

Patients and Sample Collection

Plasma and urinary samples were collected from 23 pre-eclamptic patients (31.2 ± 5.2; mean ± SD) who had been transferred to Saitama Medical School from other local clinics and hospitals between November 2000 and November 2001. All the patients were referred as cases for emergent control of hypertension or for an emergency cesarean section at the time of 27–40 weeks of gestation. Pre-eclampsia was defined as hypertension (BP > 140/90, Proteinuria > 300 mg/24 h or >1+ dipstick). Five cases were diagnosed as hemolysis elevated liver enzymes and low platelet counts (HELLP) syndrome from their individual laboratory data, and remaining cases were in the criteria of pre-eclampsia. In this paper, we describe the non-HELLP pre-eclamptic patients since we had a technical difficulty at the assays because of the hemolysis of the samples from HELLP patients. All these pre-eclamptic cases had hypertension and proteinuria and a part of the patients had already been treated with various medications at the time of admission as shown in Table I. One woman took 81 mg of aspirin within 24 h of sampling, however no patient was supplemented with ascorbic acid, vitamin E or multi-vitamins. All the patients enrolled in this study gave life to healthy babies within 48 h after the admission to our hospital.

We also collected blood and urinary samples from 21 apparently normal pregnant women (33.3 ± 3.9) without a sign of pre-eclampsia and they had visited regularly to our outpatient clinic. We deleted two samples out of them since the patients resulted in pre-term labour. Therefore, we used the samples from 19 cases whose pregnancy were uneventful and resulted in term pregnancy. The sample collection from the patients and the normal pregnant women were undertaken after obtaining written informed consent. Blood and urine samples were taken as a part of regular clinical practice. In addition, 20 non-pregnant healthy volunteers (28.1 ± 4.2) with regular menstruation were recruited. None of the subjects was using contraceptive pills, any vitamin supplementation or NSAIDs during the cycles of sample collection. All plasma and urine samples were stored frozen at −70°C. Samples were then transported to Sweden in dry ice and kept frozen at −70°C until analysis.

Measurement of Plasma and Urinary 8-iso-$PGF_{2\alpha}$ (Oxidative Stress Indicator)

The plasma and urinary samples were analysed for 8-iso-$PGF_{2\alpha}$ by a radioimmunoassay as described by Basu.[12] The cross-reactivity of the 8-iso-$PGF_{2\alpha}$ antibody with 15-keto-13, 14-dihydro-8-iso-$PGF_{2\alpha}$, 8-iso-$PGF_{2\beta}$, $PGF_{2\alpha}$, 15-keto-$PGF_{2\alpha}$, 15-keto-13, 14-dihydro-$PGF_{2\alpha}$, TXB_2, 11β-$PGF_{2\alpha}$, 9 β-$PGF_{2\alpha}$ and 8-iso-$PGF_{3\alpha}$, respectively, was 1.7, 9.8, 1.1, 0.01, 0.01,

TABLE I Clinical status and the medications at the time of sampling from pre-eclamptic patients and the outcome of the pregnancy

Case no.	Age	G	P	BP	Proteinuria	Medication	Mode of delivery	Outcome of the neonates	
								NBW (g)	Apgar (1 min/5 min)
1	40	2	2	158/92	0.25 g/day	None	C.S.	2002	5/7
2	29	0	0	170/100	11.92 g/day	Nafamostat mesilate	C.S.	1048	Intubated
3	35	1	1	147/91	3.0 g/day	Amosulalol hydrochloride	C.S.	2660	4/8
4	30	0	0	176/80	(+) dipstick	Ritodrine hydrochloride	C.S.	2400	8/9
5	32	1	1	190/120	3.0 g/day	None	C.S.	1442	5/9
6	33	0	0	154/90	3.74 g/day	Propylthiouracil	N.V.D	2890	8/9
7	32	1	1	158/102	2.20 g/day	None	C.S.	642	8/9
8	23	0	0	154/96	4.28 g/day	None	N.V.D	1842	8/8
9	35	2	2	166/110	1.29 g/day	None	C.S.	1510	8/9
10	34	1	1	158/94	2.16 g/day	None	C.S.	2128	8/9
11	30	0	0	148/68	1.44 g/day	None	C.S.	2868	6/8
12	37	1	1	180/104	2.73 g/day	None	C.S.	3360	8/9
13	35	2	1	184/110	0.69 g/day	Aspirin	C.S.	1502	Intubated
14	38	5	5	170/100	3.86 g/day	Nicardipine hydrochloride	C.S.	1322	9/10
15	31	0	0	198/108	0.45 g/day	Hydralazine hydrochloride	Forceps	2432	8/9
16	33	0	0	174/112	2.96 g/day	Amosulalol hydrochloride, hydralazine hydrochloride, nicardipine	C.S.	1676	7/8
17	37	0	0	182/102	7.07 g/day	Hydrochloride	N.V.D	2804	9/9
18	35	0	0	142/108	3.0 g/day	None	C.S.	1756	8/9

G: Gravida, P: Para, GW: Gestational weeks, BP: Blood pressure, NBW: Neonatal body weight, C.S: Caesarean section, NVD: Normal vaginal delivery

0.1, 0.03, 1.8 and 0.6%. The detection limit of the assay was 8 pg/ml. The urinary 8-iso-$PGF_{2\alpha}$ levels were corrected for creatinine values, which was measured by a commercial kit (IL™ Test by Monarch Instrument).

Measurement of Plasma and Urinary 15-keto-dihydro-$PGF_{2\alpha}$ (inflammatory Response Indicator)

The plasma and urinary samples were analysed for 15-keto-dihydro-$PGF_{2\alpha}$ as an indicator of inflammatory response by a radioimmunoassay as described by Basu.[13] The cross-reactivity of the antibody with $PGF_{2\alpha}$, 15-keto-$PGF_{2\alpha}$, PGE_2, 15-keto-13, 14-dihydro-PGE_2, 8-iso-15-keto-13, 14-dihydro-$PGF_{2\alpha}$ 11β-$PGF_{2\alpha}$, 9β-$PGF_{2\alpha}$, TXB_2 and 8-iso-$PGF_{2\alpha}$ was 0.02, 0.43, <0.001, 0.5, 1.7, <0.001, <0.001, <0.001 and 0.01%, respectively. The detection limit was 16 pg/ml. The urinary 15-keto-dihydro-$PGF_{2\alpha}$ levels were corrected for creatinine values as described above.

Measurement of α-tocopherol and γ-tocopherol

Since there is a shortage of samples, we assayed 9 cases (those who have plasma samples left for analysis) of pre-eclampsia for α-tocopherol and γ-tocopherol. Plasma α- and γ-tocopherol levels were assayed by using HPLC with fluorescence detection.[14] In brief, 500-μl plasma was extracted with 500-μl ethanol containing 0.005% butylated hydroxytoluene and 2 ml of hexane. A volume of 20 μl of the supernatant was injected to a HPLC column (LiChrospher 100 NH2 250 × 4 mm). The fluorescence detector had an excitation wave-length of 295 nm and an emission wave-length of 327 nm. Plasma tocopherol levels were adjusted for serum lipid concentrations.

Serum cholesterol and triglyceride levels were measured by enzymatic method using Test Cholesterol Trinard method 181618-80 in a Monark apparatus (Instrument laboratory analysis, MS, USA). An external serum standard is used in all analyses.

Statistical Analysis

Results are expressed as mean ± SD and statistical significances were calculated using unpaired *t*-test.

RESULTS

The concentration of 8-iso-$PGF_{2\alpha}$ in plasma was 62.9 ± 26.1 in pre-eclampsia and 75.5 ± 21.5 pg/ml in normal pregnancy, presenting no significant difference (Table II). However, 15-keto-dihydro-$PGF_{2\alpha}$ concentrations were significantly lower ($p < 0.01$) in pre-eclampsia than in the normal pregnant women (107.8 ± 33.9 in pre-eclampsia and 147.0 ± 48.1 pg/ml in normal pregnancy). There was no apparent change of plasma 8-iso-$PGF_{2\alpha}$ and 15-keto-dihydro-$PGF_{2\alpha}$ between gestational weeks 20 and 40, though the number of cases was limited (data not shown). In addition, both 8-iso-$PGF_{2\alpha}$ and 15-keto-dihydro-$PGF_{2\alpha}$ concentrations increased significantly ($p < 0.001$) during pregnancy in comparison with non-pregnant status regardless of the presence of pre-eclampsia. Similarly, there was no significant difference in pre-eclampsia and normal pregnancy concerning urinary concentration of 8-iso-$PGF_{2\alpha}$, but urinary 15-keto-dihydro-$PGF_{2\alpha}$ levels were lower in the pre-eclamptic than the normal pregnant women ($p < 0.01$). The urinary samples from non-pregnant healthy volunteers showed significantly lower levels of 8-iso-$PGF_{2\alpha}$ and 15-keto-dihydro-$PGF_{2\alpha}$ than pregnant women, as well.

Regarding antioxidant levels in plasma, lipid-adjusted α-tocopherol level was very similar between normal and pre-eclamptic patients (3.31 ± 0.30 vs. 2.65 ± 0.32; Table II) and presented very small fluctuation and individual variability between 20 and 40 weeks (Fig. 1, upper panel). However, the levels were significantly lower from that of non-pregnant healthy volunteers ($p < 0.001$). On the contrary, γ-tocopherol levels were relatively variable between cases during pregnancy (Fig. 1, lower panel), and showed significantly lower levels in pre-eclamptic cases compared to normal pregnancy ($p < 0.01$) and non-pregnancy ($p < 0.001$). The concentration of γ-tocopherol in

TABLE II Levels of 8-iso-$PGF_{2\alpha}$, 5-keto-dihydro-$PGF_{2\alpha}$ and tocopherols in plasma and/or urinary samples

	Normal pregnant	Preeclamptic	Significance (*t*-test)	Non-pregnancy
No of cases	19	18		20
Plasma				
8-iso-$PGF_{2\alpha}$ (pg/ml)	75.5 ± 21.5***	62.9 ± 26.1*	NS	42.5 ± 33.5
15-keto-dihydro-$PGF_{2\alpha}$ (pg/ml)	147.0 ± 48.1***	107.8 ± 33.9*	$p < 0.01$	86.2 ± 29.7
α-tocopherol (μmol/mmol)	3.31 ± 0.30***	2.65 ± 0.32***	NS	4.20 ± 0.49
γ-tocopherol (μmol/mmol)	0.28 ± 0.06***	0.21 ± 0.06***	$p < 0.01$	0.58 ± 0.16
Urine				
8-iso-$PGF_{2\alpha}$ (nmol/mmol creat.)	0.45 ± 0.19***	0.36 ± 0.19*	NS	0.24 ± 0.075
15-keto-dihydro-$PGF_{2\alpha}$ (nmol/mmol creat.)	0.73 ± 0.38***	0.46 ± 0.17**	$p < 0.01$	0.33 ± 0.13

***$p < 0.001$ to non-pregnancy, **$p < 0.01$ to non-pregnancy, *$p < 0.05$ to non-pregnancy.

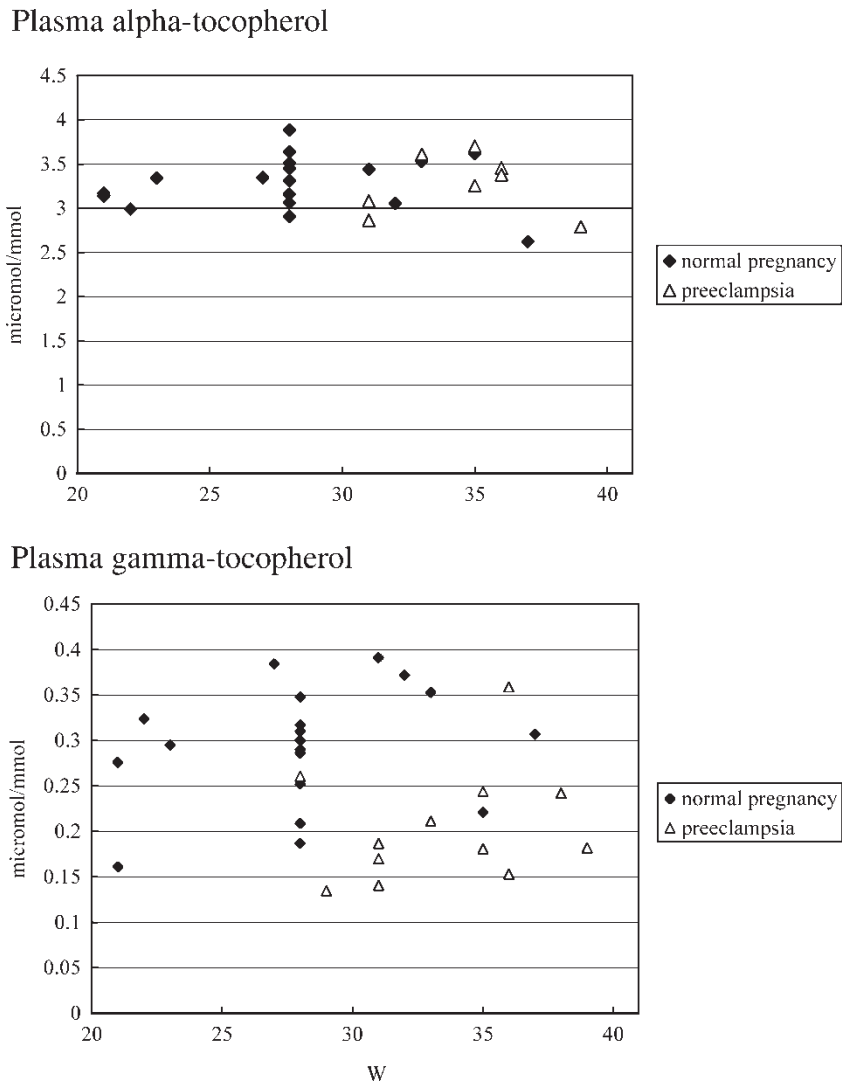

FIGURE 1 The lipid corrected plasma levels of alpha-tocopherol in normal pregnant and pre-eclampsia patients at different gestation weeks (Upper panel). The lipid corrected plasma levels of gamma-tocopherol in normal pregnant and pre-eclampsia patients at different gestation weeks (Lower panel).

non-pregnant healthy volunteers was kept high ($p < 0.001$) compared to pregnant ones.

DISCUSSION

There has been an on-going argument concerning the contribution of various arachidonic acid metabolites in the pathophysiology of pre-eclampsia. Both free radical- and cyclooxygenase-catalysed products have been used as targets of investigation of this field. Isoprostanes, which are free-radical catalysed prostaglandin isomers, are considered to be reliable indicators of *in vivo* oxygen stress and lipid peroxidation in human.[5,6] Further, the role of cyclooxygenase-catalysed $PGF_{2\alpha}$ as an indicator of inflammation is well known.[13] Since the characteristics of pre-eclamptic patients are general endothelial activation and vascular injury, vasospasm and microthrombosis,[15] isoprostanes and prostaglandins could be strong candidates for investigations in regard to the pathophysiology of pre-eclampsia. In this study, we focused on 8-iso-$PGF_{2\alpha}$, an abundant isoprostane and $PGF_{2\alpha}$, since our previous investigations revealed the significance of these compounds in particular conditions of oxidative stress and inflammation.[16–23]

The lack of significance in isoprostane levels between pre-eclamptic patients and normal pregnancy in the present study was unexpected but may not be surprising, since the patients in this study were already symptomatically completed in terms of the process of pre-eclampsia at the time of sample collection. Though a previous paper demonstrated that plasma F_2-isoprostane levels were higher in the order of severe pre-eclampsia,

mild pre-eclampsia, normotensive pregnancy and non-pregnancy,[9] the present study did not show a sign of overproduction of the isoprostanes at this stage. This was consistent with the report that urinary isoprostane levels did not significantly differ between the pre-eclamptic cases and controls.[10] In addition, our results from non-pregnant patients seem to support the idea that even normal pregnancy may be a state of mild oxidative stress[9] and consequent peroxidation of lipids, namely arachidonic acid. The pregnancy state itself showed higher levels of isoprostanes which is an important observation since the free radical mediated arachidonic acid products might have a significant role in the maintenance of pregnancy. It was earlier hypothesized that free radicals although mainly depicted as potentially damaging factor for various cell components, the role of intracellular oxidant levels might have specific effects on the specific signalling pathways.[24]

In this study, we have also shown that enzymatically produced $PGF_{2\alpha}$ levels were significantly higher during the normal pregnancy period than the non-pregnancy. Perhaps an increased level of $PGF_{2\alpha}$ might be necessary in the normal pregnancy process and parturition. We have earlier shown that the levels of urinary tetranor metabolites of $PGF_{2\alpha}$ had gradually increased in heifer after the establishment of pregnancy and during the whole pregnancy period until parturition, and which subsequently decreased to the basal levels at day 20 after parturition.[25] A significantly lower levels of $PGF_{2\alpha}$ metabolite in the pre-eclamptic women compared to the normal pregnancy further suggest an impairment of the enzymatically derived $PGF_{2\alpha}$ in pre-eclamptic pregnancy.

Earlier studies suggested that maternal level of α-tocopherol progressively increased throughout pregnancy, and significantly decreased in severe pre-eclampsia.[26,27] However, the present study could not demonstrate this change. The lower α-tocopherol levels was seen during pregnancy regardless to the presence of signs of pre-eclampsia though the levels were significantly lower than in non-pregnancy. In addition, γ-tocopherol was further suppressed in pre-eclamptic patients in addition to the lower levels of normal pregnancy. This suggests that the reduction of γ-tocopherol in pre-eclamptic cases might have occurred at an earlier stage. Though α-tocopherol is the predominant form of vitamin E in most human and animal tissues, previous papers suggested relative importance of γ-tocopherol as an indicator for coronary heart disease.[14,28] The supplement of α-tocopherol to the pregnant patients with potential pre-eclampsia was reported to not only improving the plasma biochemical markers of endothelial activation but also decreasing the risk of the disease,[29] though α-tocopherol levels stayed unchanged during pregnancy in the placebo group of the study. In this study, the reduction of γ-tocopherol seems to be more remarkable in pre-eclampsia rather than α-tocopherol. A difference between the α- and γ-tocopherol which accounted for the differences in the diet among the various groups, could not be ruled out. However, the differences of dietary intake of vitamin E in various countries might affect the supplementary effects of this vitamin on the onset of pre-eclampsia.

In conclusion, no direct evidence of oxidative stress and inflammatory response was observed in pre-eclampsia compared to the normal pregnancy. Though the plasma peroxidation products are not significantly changed in pre-eclampsia, a reduction of γ-tocopherol suggests the possible precedence of oxidative stress in this complicated condition. Further, the involvement of isoprostanes and also enzymatically derived $PGF_{2\alpha}$ in normal pregnancy might suggest a biological importance of free radicals- and cyclooxygenases-catalysed products in this state.

Acknowledgements

This work was financed by grants from the Geriatrics Research Foundation. Eva Seiby and Barbro Simu are acknowledged for excellent technical assistance.

References

[1] Vanwijk, M.J., Kublickiene, K., Boer, K. and VanBavel, E. (2000) "Vascular function in pre-eclampsia", *Cardiovasc. Res.* **47**, 38–48.
[2] Chappell, L.C., Seed, P.T., Briley, A., Kelly, F.J., Hunt, B.J., Charnock-Jones, D.S., Mallet, A.I. and Poston, L. (2002) "A longitudinal study of biochemical variables in women at risk of pre-eclampsia", *Am. J. Obstet. Gynecol.* **187**, 127–136.
[3] Kristal, B., Shurtz-Swirski, R., Chezar, J., Manaster, J., Levy, R., Shapiro, G., Weisman, I., Shasha, S.M. and Sela, S. (1998) "Participation of pheripheral polymorphoneuclear leukocytes in the oxidative stress and inflammation in patients with essential hypertension", *Am. J. Hypertens.* **11**, 921–928.
[4] Gutteridge, J.M. and Halliwell, B. (1990) "The measurement of free radical reactions in humans", *Trends Biochem. Sci.* **15**, 129–135.
[5] Roberts, L.J. II, and Morrow, J.D. (2000) "Measurement of F_2-isoprostanes as an index of oxidative stress *in vivo*", *Free Radic. Biol. Med.* **28**, 505–513.
[6] Basu, S. (2004) "Isoprostanes: novel bioactive compounds of lipid peroxidation", *Free Radic. Res.* **38**, 105–122.
[7] Barden, A., Beilin, L.J., Ritchie, J., Croft, K.D., Walters, B.N. and Michael, C.A. (1996) "Plasma and urinary 8-iso-prostane as an indicator of lipid peroxidation in pre-eclampsia and normal pregnancy", *Clin. Sci.* **91**, 711–718.
[8] Morris, J.M., Gopaul, N.K., Endresen, M.J.R., Knight, M., Linton, E.A., Dhir, S., Änggård, E.E. and Redman, C.W.G. (1998) "Circulating markers of oxidative stress are raised in normal pregnancy and pre-eclampsia", *Br. J. Obstet. Gynaecol.* **105**, 1195–1199.
[9] McKinney, E.T., Shouri, R., Hunt, R.S., Robert, S., Ahokas, R.A. and Sibai, B.M. (2000) "Plasma urinary and salivary 8-epi-prostaglandin F2α levels in normotensive and

pre-eclamptic pregnancies", *Am. J. Obstet. Gynecol.* **183**, 874–877.
[10] Regan, C.L., Levine, R.J., Baird, D.D., Ewell, M.G., Martz, K.L., Sibai, B.M., Rokach, J., Lawson, J.A. and FitzGerald, G.A. (2001) "No evidence for lipid peroxidation in severe pre-eclampsia", *Am. J. Obstet. Gynecol.* **185**, 572–578.
[11] Redman, C., Sacks, G. and Sargent, I. (1999) "Pre-eclampsia: an excessive maternal inflammatory response to pregnancy", *Am. J. Obstet. Gynaecol.* **1890**, 499–506.
[12] Basu, S. (1998) "Radioimmunoassay of 8-iso-prostaglandin F2alpha: an index for oxidative injury via free radical catalysed lipid peroxidation", *Prostaglandins Leukot. Essent. Fatty Acids* **58**, 319–325.
[13] Basu, S. (1998) "Radioimmunoassay of 15-keto-13, 14-dihydro-prostaglandin F2alpha: an index for inflammation via cyclooxygenase catalysed lipid peroxidation", *Prostaglandins Leukot. Essent. Fatty Acids* **58**, 347–352.
[14] Öhrvall, M., Sundlöf, G. and Vessby, B. (1998) "Gamma, but not alpha, tocopherol levels in serum are reduced in coronary heart disease patients", *J. Int. Med.* **239**, 111–117.
[15] Roberts, J.M., Taylor, R.N., Musci, T.J., Rodgers, G.M., Hubel, C.A. and McLaughlin, M.K. (1989) "Pre-eclampsia: an endothelial cell disorder", *Am. J. Obstet. Gynecol.* **161**, 1200–1204.
[16] Helmersson, J., Vessby, B., Larsson, A. and Basu, S. (2004) "Association of type 2 diabetes with cyclooxygenase-mediated inflammation and oxidative injury in an elderly population", *Circulation* **109**, 1729–1734.
[17] Cederberg, J., Basu, S. and Eriksson, U.J. (2001) "Increased rate of lipid peroxidation and protein carbonylation in experimental diabetic pregnancy", *Diabetologia* **44**, 766–774.
[18] Risérus, U., Basu, S., Jovinge, S., Ärnlöv, J. and Vessby, B. (2002) "Dietary supplementation with conjugated linoleic acid causes isomer-dependent oxidative stress and elevated C-reactive protein-a potential link to fatty acids-induced insulin resistance", *Circulation* **106**, 1925–1929.
[19] Basu, S., Nozari, A., Liu, X.L., Rubertsson, S. and Wiklund, L. (2000) "Development of a novel biomarker of free radical damage in reperfusion injury after cardiac arrest", *FEBS Lett.* **470**, 1–6.
[20] Basu, S., Hellberg, A., Ulus, T., Westman, J. and Karacagil, S. (2001) "Biomarkers of oxidative injury during spinal cord ischemia", *FEBS Lett.* **508**, 36–38.
[21] Basu, S. and Eriksson, M. (1998) "Oxidative injury and survival during endotoxemia", *FEBS Lett.* **438**, 159–160.
[22] Basu, S. (1999) "Oxidative injury induced cyclooxygenase activation in experimental hepatotoxicity", *Biochem. Biophys. Res. Commun.* **254**, 764–767.
[23] Ulus, A.T., Aksovek, A., Ozkan, M., Katircioglu, S.F. and Basu, S. (2003) "Cardiopulmonary bypass as a cause of free radical induced-oxidative stress and enhanced circulatory isoprostanes in humans", *Free Radic. Biol. Med.* **34**, 911–917.
[24] Finkel, T. and Hoolbrook, N.J. (2000) "Oxidants, oxidative stress and the biology of ageing", *Nature* **408**, 239–247.
[25] Basu, S., Kindahl, H., Harvey, D. and Betteridge, K.J. (1987) "Metabolites of $PGF_{2\alpha}$ in blood plasma and urine as parameters of $PGF_{2\alpha}$ release in cattle", *Acta Vet. Scand.* **28**, 409–420.
[26] Wang, Y., Walsh, S.W., Guo, J. and Zhang, J. (1991) "Maternal levels of prostacyclin, thromboxane, vitamin E, and lipid peroxides throughout normal pregnancy", *Am. J. Obstet. Gynecol.* **165**, 1690–1694.
[27] Wang, Y., Walsh, S.W., Guo, J. and Zhang, J. (1991) "The imbalance between thromboxane and prostacyclin in pre-eclampsia is associated with an imbalance between lipid peroxides and vitamin E in maternal blood", *Am. J. Obstet. Gynecol.* **165**, 1695–1700.
[28] Jiang, Q., Christen, S., Shigenaga, M.K. and Ames, B.N. (2001) "γ-tocopherol, the major form of vitamin E in the US diet, deserves more attention", *Am. J. Clin. Nutr.* **74**, 714–722.
[29] Chappell, L.C., Seed, P.T., Briley, A.L., Kelly, F.J., Lee, R., Hunt, B.J., Parmar, K., Bewley, S.J., Shennan, A., Steer, P.J. and Poston, L. (1998) "Effect of antioxidants on the occurrence of pre-eclampsia in women at increased risk: a randomized trial", *Lancet* **354**, 810–816.