Serum Paraoxonase and Arylesterase Activities Throughout Normal Pregnancy

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ABSTRACT

Objective: Pregnancy is characterized with hyperlipidemia and mild oxidative stress. Paraoxonase1 is an antioxidant enzyme which is also suggested to be involved in lipoprotein metabolism. Our aim was to investigate serum paraoxonase and arylesterase activities and their relation with the lipid profile and oxidative-antioxidative system parameters throughout normal pregnancy.

Material and Method: Seventeen healthy pregnant women and 17 age-matched non-pregnant controls were included in the study. Serum paraoxonase and arylesterase activities were determined spectrophotometrically. Plasma malondialdehyde (MDA) level was measured by high-performance liquid chromatography. Serum total antioxidant capacity (TAOC), red blood cell superoxide dismutase and whole blood glutathione peroxidase activities were measured by commercial kits. Lipid profile was studied by routine biochemical methods.

Results: There were not any significant differences in either serum paraoxonase or arylesterase activities between the three trimester values and between each trimester level and non-pregnant group. Plasma MDA levels obtained in each trimester were significantly higher than those of the non-pregnant control group, however there were not any significant differences among the three trimesters. TAOC value of the pregnant groups increased significantly throughout pregnancy. However, TAOC levels were significantly lower in each trimester compared with the non-pregnant group. Triglyceride and total cholesterol levels increased throughout pregnancy.

Triglyceride and total cholesterol levels obtained at each trimester were significantly higher than those of the non-pregnant group.

Conclusion: The results of the present study demonstrated hyperlipidemia and oxidative stress in normal pregnancy, however, failed to show any effects of paraoxonase 1 on lipoprotein metabolism or oxidative-antioxidative system parameters in pregnancy.

Key Words: Pregnancy, paraoxonase, oxidative stress, lipid, hyperlipidemia. **Nobel Med 2011; 7(1): 49-55**

NORMAL GEBELİK SÜRESİNCE SERUM PARAOKSONAZ VE ARİLESTERAZ AKTİVİTELERİ

ÖZET

Amaç: Gebelik hiperlipidemi ve hafif oksidatif stres ile seyretmektedir. Paraoksonaz1 lipoprotein metabolizmasında rol oynadığı öne sürülen antioksidan bir enzimdir. Amacımız gebelik boyunca paraoksonaz ve arilesteraz aktivitelerini ve onların lipid profili ve oksidan-antioksidan sistem parametreleri ile ilişkisini incelemektir.

Materyal ve Metod: Çalışmaya 17 sağlıklı gebe ve yaşa göre eşleştirilmiş 17 kontrol alındı. Serum paraoksonaz ve arilesteraz aktiviteleri spektrofotometrik yöntemlerle tayin edildi. Plazma malondialdehid (MDA) düzeyi yüksek basınçlı sıvı kromatografisi ile ölçüldü. Serum total antioksidan kapasitesi (TAOK), kırmızı küre süperoksid dismutaz ve tam kan glutatyon peroksidaz aktiviteleri ticari kitlerle çalışıldı. Lipid profili rutin biyokimya metodları ile ölçüldü.

Bulgular: Serum paraoksonaz ve arilesteraz aktivitesi gebelik boyunca değişim göstermedi, gebelik dönemleri ile gebe olmayanlar arasında anlamlı fark gözlenmedi. Her gebelik döneminde plazma MDA düzeyi gebe olmayan gruptan yüksekti, gebelik dönemleri arasında MDA düzeylerinde fark yoktu. Serum TAOK değerleri gebelik boyunca anlamlı olarak yükseldi, fakat her dönemdeki değerler gebe olmayan gruptan daha düşük bulundu. Trigliserid ve total kolesterol düzeyleri de gebelik boyunca anlamlı olarak yükseldi ve her dönemde elde edilen değerlerin gebe olmayan gruba göre anlamlı olarak yüksek olduğu gözlendi.

Sonuç: Bu çalışmada gebeliğin hiperlipidemi ve hafif oksidatif stresle seyrettiği gözlendi, fakat paraoksonaz 1 enziminin gebelikte lipoprotein metabolizması veya oksidan-antioksidan sistemler üzerine etkisi gösterilemedi.

Anahtar Kelimeler: Gebelik, paraoksonaz, oksidatif stres, lipid, hiperlipidemi. **Nobel Med 2011; 7(1): 49-55**

INTRODUCTION

Paraoxonase1 (PON1), which is a member of PON enzyme family (PON1-3) was identified in 1953 and its physiological role is still uncertain. PON1 exerts paraoxonase, arylesterase and lactonase activities.[1,2] Paraoxonase activity varies widely among individuals, partly related to polymorphisms. The PON1 gene has two common coding region polymorphisms (M/L 55 and R/Q 192). Arylesterase activity is not affected by either polymorphisms and can be considered as an index of actual protein concentration.[3] In the circulation, PON1 is bound to high density lipoprotein (HDL) and some of the antioxidative and antiinflammatory actions of HDL are attributed to PON1[4]. PON1 hydrolyzes oxidized lipids in lipoproteins and thus prevents or inhibits low density lipoprotein (LDL) and HDL oxidation[5,6]. LDL oxidation plays a pivotal role in atherogenesis and therefore PON1 is an antiatherogenic enzyme. By preventing or inhibiting HDL oxidation PON1 preserves the integrity and function of HDL and thus contributes to lipoprotein metabolism.[7] Besides its antioxidant activities, the enzyme is also belived to be directly involved in the lipoprotein metabolism and it was suggested that PON1 may also be of importance in the expression of lipid disorders,[8] so the exact mechanisms underlying these associations of PON1 with the lipids and lipoproteins remain to be elucidated. Pregnancy is a physiological state in which, over a relatively short period, several metabolic alterations occure. Pregnancy is characterized with marked hypertriglyceridemia and moderate hypercholesterolemia[9,10] and can be accepted as a hyperlipidemia model. Pregnancy is also associated with changes in the oxidative-antioxidative systems and it is generally believed that there is an increment in free radical production with an accompanying increase in the antioxidative defence forces.[11,12] However, after a critical level or status hyperlipidemia or disturbed oxidative-antioxidative system balance (oxidative stress), is associated with pregnancy related complications such as preeclampsia and diabetes[13,14]. Furthermore, these states have also been related to future risk of coronary events for the mother and the child.[15,16]

There are several studies that investigated lipid profile and various oxidative-antioxidative system parameters in normal pregnancy however, the number of studies that investigated PON1 is limited and yielded conflicting results.[17-19] Therefore, the aim of the present study was to investigate serum paraoxonase and arylesterase activities and their relation with the lipid profile and oxidative-antioxidative system parameters throughout normal pregnancy. For this purpose, we measured plasma malondialdehyde (MDA) levels, one of the most frequently investigated indices of lipid peroxidation and, in order to evaluate antioxidative system, we determined serum total antioxidant capacity (TAOC), serum paraoxonase, arylesterase, red blood cell (RBC) superoxide dismutase (SOD), whole blood glutathione peroxidase (GPx) activities, uric acid, albumin and total bilirubine levels.

MATERIAL and METHOD

Healthy pregnant women (n:17, age: 26±5 years) were recruited at the first prenatal visit in the outpatient clinic of Gynecology and Obstetrics Department of Uludag University Medical Faculty, Bursa, Turkey. Normal pregnancy was diagnosed on the basis of a clinical and ultrasound perspective. A longitudinal study was performed during the three trimesters of pregnancy and healthy pregnant women had normal course of pregnancies without any complications (such as preeclampsia, in utero growth retardation or abortion). For the control group, age-matched healthy non-pregnant women (n:17, age: 26±5 years) were recruited from the hospital and university staff. None of the participants smoked, used alcohol, had history of thyroid disease, diabetes mellitus or hypertension. None of the subjects were taking medications, including lipid lowering drugs, hormonal therapies and non-steroidal anti-inflammatory drugs. This study was approved by the Ethics Committee of Uludag University. All women gave informed consent to participate in the study.

Blood was withdrawn from the antecubital vein in the fasting state in heparin-coated, EDTA-containing and non-additive tubes and were processed in the laboratory immediately after collection. Sera and plasma were separated by centrifugation at 1,500 g for 10 minutes. Serum triglyceride, total cholesterol, HDL-cholesterol, apolipoprotein AI and B, and lipoprotein (a) levels were measured the same day that the blood was collected.

EDTA-containing plasma aliquots for MDA and serum aliquots for TAOC, paraoxonase / arylesterase measurements were kept at -80° C until the analyses were performed. A part of whole blood was frozen for GPx determination. RBC for SOD determination were washed by saline and frozen after hemolysis. Paraoxonase activity was determined as described by Eckerson et al.[20]. The rate of hydrolysis of paraoxon was measured by monitoring the increase in absorbance at 412 nm at 25°C. The amount of p-nitrophenol generated was calculated from the molar absorptivity coefficient at pH: 10.5, which was 18 290 M^{-1} cm^{-1}. Phenylacetate was used as a substrate to measure the arylesterase activity. Enzymatic activity was calculated by using its molar absorptivity coefficient, 1310 M^{-1} cm^{-1}. [21]

Plasma MDA concentrations were determined with the high-performance liquid chromatography (HPLC) procedure of Young and Trimble.[22] A calibration curve was prepared for each day by using 1,1',3,3' tetramethoxypropane as the standard. The mobile phase contained 50% methanol and 50% 25 mM phosphate

Table 1: Oxidative and antioxidative system parameters of the non-pregnant and pregnant groups

Group	Non-pregnant	Pregnant		
		First trimester	Second trimester	Third trimester
Paraoxonase (U/L)	218 ± 105	229 ± 106	216 ± 85	207 ± 89
Arylesterase (kU/L)	86 ± 33	87 ± 27	89 ± 27	103 ± 36
RBC-SOD (U/g Hb)	88 ± 51	67 ± 30	66 ± 28	69 ± 37
Whole blood GPx (U/g Hb)	57 ± 38	51 ± 33	63 ± 55	60 ± 55
Uric acid (mg/dL)	3.56 ± 1.03	2.36 ± 0.72 [a*]	2.49 ± 0.65 [a*]	3.14 ± 0.97 [a*,b*,c*]
Albumin (g/dL)	4.69 ± 0.17	4.31 ± 0.42 [a*]	4.02 ± 0.44 [a*]	3.92 ± 0.37 [a*,b**]
Total bilirubin (mg/dL)	0.67 ± 0.26	0.51 ± 0.32 [a*]	0.37 ± 0.15 [a*]	0.42 ± 0.17 [a*]

[a]: Compared with the non-pregnant group [b]: Compared with the first trimester [c]: Compared with the second trimester * p< 0.05, ** p< 0.01

Table 2: Lipid profiles of the non-pregnant and pregnant groups

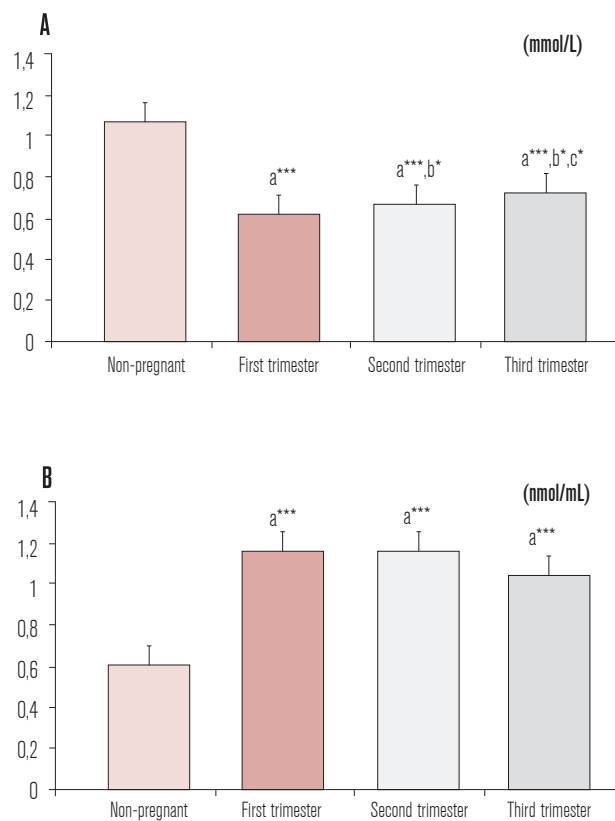| Group	Non-pregnant	Pregnant		
(mg/dL)		First trimester	Second trimester	Third trimester
Triglyceride	83 ± 52	119 ± 54 [a*]	151 ± 43 [a***,b**]	180 ± 55 [a***,b**]
Total cholesterol	168 ± 31	199 ± 45 [a*]	234 ± 40 [a**,b**]	252 ± 47 [a**,b**]
HDL-cholesterol	50 ± 10	61 ± 11 [a*]	69 ± 16 [a*]	70 ± 11 [a*]
Apolipoprotein AI	163 ± 22	173 ± 29	178 ± 38	187 ± 21 [a*]
Apolipoprotein B	76 ± 19	91 ± 24 [a*]	106 ± 20 [a**]	128 ± 13 [a**,b**,c***]
Lipoprotein (a)	22 ± 27	21 ± 17	26 ± 18	26 ± 17

[a]: Compared with the non-pregnant group [b]: Compared with the first trimester [c]: Compared with the second trimester, * p< 0.05, ** p< 0.01, *** p< 0.001

buffer at pH 6.5. A flow rate of 0.8 mL/min was used. The spectrophotometric detector was set at 532 nm.

TAOC was measured in serum by means of a commercial kit (Randox Laboratories, Antrim, UK). The assay is based on the incubation of 2,2'-azino-di-(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) with a peroxidase (metmyoglobin) and H_2O_2 to produce the radical cation ABTS+* which has a relatively stable blue-green colour, measured at 600 nm. When the colored ABTS+* is mixed with antioxidant substance, it is reduced to its original colorless ABTS form. Antioxidants in the added sample cause suppression of this color production to a degree that is proportional to their concentration. The suppression of the color is compared with that of the Trolox, which is widely used as a traditional standard for TAOC measurement assays, and the assay results are expressed as Trolox equivalent (mmol/L).

RBC SOD and whole blood GPx activities were determined using Randox kits (Antrim, UK). Briefly, the determination of SOD activity was based on the production of O_2^- anions by the xanthine/xanthine oxidase system. GPx catalysed by the oxidation of reduced glutathione in the presence of cumene hydroperoxide. The depletion of nicotinamide →

A

B

a: Compared with the non-pregnant group b: Compared with the first trimester
c: Compared with the second trimester * p< 0.05, *** p< 0.01

Figure. Serum total antioxidant capacity (A) and plasma malondialdehyde levels (B) of the non-pregnant and pregnant groups.

adenine dinucleotide phosphate was measured spectrophotometrically at 340 nm. Serum levels of albumin, total bilirubin, uric acid, total cholesterol, HDL-cholesterol and triglyceride were determined using enzymatic assays on an Aeroset autoanalyzer (Abbott Laboratories. Irving, Texas, USA). Apo AI, apo B and lipoprotein (a) were assayed by immunonephelometry (Dade Behring Marburg GmbH, Germany).

All data were expressed as mean ± S.D. The pregnancy values obtained at each trimester were compared with the non-pregnant group by using t-test for independent groups. The pregnancy values were compared with the Friedman test, and the 2 trimesters were compared with the Wilcoxon test. Spearman correlation test was performed to investigate the relation between the serum paraoxonase and arylesterase activities with the lipid profile and oxidative-antioxidative system parameters. A p value <0.05 was considered significant.

RESULTS

There were not significant differences in either serum paraoxonase or arylesterase activities between the

three trimester values and between each trimester level and non-pregnant group (Table 1). TAOC value of the pregnant groups increased significantly throughout pregnancy (Figure A). TAOC level was significantly higher in the third trimester (0.73±0.13 mmol/L) compared to those of the first (0.64±0.12 mmol/L) and second (0.68±0.16 mmol/L) trimesters, and significantly higher in the second tirmester compared with the first trimester. However, TAOC levels were significantly lower in each trimester compared with the non-pregnant group (1.08±0.23 mmol/L). Plasma MDA levels obtained in each trimester (1.13±0.40 nmol/mL, 1.12±0.37 nmol/mL and 1.05±0.33 nmol/mL first, second and third trimester values, respectively) were significantly higher than those of the non-pregnant control group (0.61±0.21 nmol/mL), however there were not any significant differences among the three trimesters (Figure B). RBC-SOD and whole blood-Gpx levels in each trimester were not significantly different from each other and from those of the non-pregnant values. Serum uric acid levels increased throughout pregnancy, while albumin levels decreased. Albumin levels were significantly lower in the third trimester than in the first trimester. Third trimester value of uric acid was significantly higher than those of the first and second trimesters. Uric acid and albumin levels obtained in each trimester were significantly lower than those of the non-pregnant control group. Total bilirubin levels of each trimester were significantly lower than the non-pregnant control group, while there was no differences among the three trimesters (Table 1).

Triglyceride and total cholesterol levels increased throughout pregnancy (Table 2). Triglyceride level was significantly higher in the third trimester compared to those of the first and second trimesters, and significantly higher in the second trimester compared with the first trimester value. Second and third trimester values of total cholesterol were significantly higher than those of the first trimester. Triglyceride and total cholesterol levels obtained at each trimester were significantly higher than those of the non-pregnant group. HDL-cholesterol and apolipoprotein B levels were significantly higher in each trimester than those of the non-pregnant group. There were not any differences in HDL-cholesterol levels among the trimesters. Third trimester apolipoprotein B level was significantly higher than those of the first and second trimesters. Apolipoprotein AI level was significantly higher in the third trimester compared with those of the non-pregnant control group. There were not any significant differences in lipoprotein (a) levels between the trimesters and the non-pregnant group and among the three trimesters (Table 2). →

Table 3: Correlations between serum paraoxonase and arylesterase activities with the lipid profile parameters (cofficient of correlation / significance; r /p)								
	Non-pregnant		First trimester		Second trimester		Third trimester	
	Paraoxonase	Arylesterase	Paraoxonase	Arylesterase	Paraoxonase	Arylesterase	Paraoxonase	Arylesterase
Triglyceride	.534 / .090	-.480 / .020	-.220 / .882	.275 / .056	.255 / .252	-.019 / .934	,060 / ,845	-,132 / ,668
Total cholesterol	.01 / .990	.221 / .312	.055 / .705	.448 / .001	-.072 / .751	.307 / .165	-,267 / ,378	,151 / ,622
HDL-cholesterol	-.274 / .205	.816 / .000	.171 / .240	.437 / .002	-.023 / .920	.297 / .179	-,098 / ,751	,117 / ,703
Apolipoprotein AI	-.016 / .947	.634 / .002	.078 / .694	.474 / .011	.077 / .803	.077 / .803	-,055 / ,881	-,073 / ,841
Apolipoprotein B	.120 / .603	-.097 / .675	.147 / .455	.370 / .053	-.339 / .257	-.041 / .893	-,237 / ,510	-,182 / ,614

Statistical analysis

Triglyceride levels were negatively correlated with arylesterase and positively correlated with paraoxonase activities in the non-pregnant control group. Arylesterase activity was positively correlated with HDL-cholesterol and apolipoprotein AI levels in the non-pregnant control group and first trimester pregnant group. There was also a positive correlation between the arylesterase activity and total cholesterol levels in the first trimester pregnant group. There was no significant correlation between the paraoxonase or arylesterase activities and the oxidative-antioxidative system parameters (Table 3).

DISCUSSION

In the present study, we investigated serum paraoxonase and arylesterase activities throughout normal pregnancy and did not observe any significant changes; furthermore there were not any significant differences in these enzyme activities between the pregnant (determined at all time points) and non-pregnant groups. According to our literature research, there are three studies[17-19] that investigated serum PON1 enzyme activities (paraoxonase or arylesterase activity) in normal pregnancy. Roy et al.[17] reported that serum paraoxonase activity was higher at 28 and 32 weeks of normal pregnancy compared with the postnatal values and those of the non-pregnant controls. Carpintero et al.[18] investigated serum arylesterase activity in three groups of healthy pregnant women in their first, second and third trimesters and found that arylesterase activity was higher than the non-pregnant controls in the third trimester of pregnancy. However, Ferre et al.[19] investigated longitudinal changes in serum paraoxonase activity and found that serum paraoxonase activity was reduced at 32 week of pregnancy and at labour. Since paraoxonase activity is strongly influenced by the genotype, Ferre et al. proposed that the discrepancy between their study and the previous two studies could be related to the comparison of pregnant and non-pregnant subjects in the other two. However, in the present study we also determined serum paraoxonase and arylesterase activities throghout pregnancy and there were not any significant changes.

We observed higher plasma MDA levels in the first, second and third trimesters, compared with those of the non-pregnant group, which is in accordance with several reports and with the notion that there is increased free radical production and a modest oxidative stress in normal pregnancy.[11,12] Although levels of lipid peroxidation indicators were reported as increased in pregnancy compared with the non-pregnant state, there were differences in the results of the studies investigating the duration of these indicators throughout pregnancy. Toescu et al.[15] found a progressive increase in plasma lipid hydroperoxide levels (another indicator of lipid peroxidation) throughout pregnancy whereas Wang et al.[23] reported unchanged values. However, in a very recent study, Guven et al.[24] reported that serum MDA levels decreased throughout pregnancy. We did not find any differences in plasma MDA levels among the three trimesters.

Lipids are substrates for lipid peroxidation and increased lipid levels might be one of the reasons for increased plasma MDA levels. In the present study, in line with the literature, serum triglyceride, total cholesterol, HDL-cholesterol and apolipoprotein B levels were significantly higher in the first, second and third trimesters compared with those of the non-pregnant group. These parameters were significantly higher in the second trimester than those of the first, however, there was no significant difference between the second and third trimester values (except the apolipoprotein B levels). In their studty, Roy et al.[17] found a positive association between serum paraoxonase activity and triglyceride levels in both the pregnant and non-pregnant states, and suggested a possible role of PON1 in energy delivery for fetal development derived from maternal hypertriglyceridemia. However, in the present study, triglyceride levels were positively correlated with paraoxonase activity and negatively associated →

with arylesterase activity in the non-pregnant group. Arylesterase activity was positively associated with total cholesterol, HDL-cholesterol and apolioprotein AI levels in the first trimester of pregnancy. There were not any significant correlations between the lipid parameters and the enzyme activities in the second and third trimesters. The various association of the PON1 with lipid parameters might be related to the hyperlipidemia observed in the pregnant group. In a recent study, van Himbergen et al.[8] investigated PON1 in patients with familial combined hyperlipidemia and their normolipidemic relatives, and proposed that PON1 associates differentially with lipids and lipoproteins in the hypelipidemic and the normolipidemic groups.

In the present study, we measured TAOC which asseses overall effects of many antioxidants working in synergy, and also levels of several antioxidant molecules and antioxidant enzyme activities.   In line with report of Toescu et al.[15] we observed that TAOC was decreased during pregnancy and that the reduced TAOC improved throughout pregnancy (did not reach the level of non-pregnent group). However, Belo et al.[25] who also reported decreased TAOC levels, did not oberve an increment in TAOC throughout pregnancy. Uric acid has been accepted as one of the major contributors of serum TAOC. Since, in their study, TAOC appeared to be unchanged when uric acid was removed, Toescu et al.[15] suggested that the changes in TAOC appears to be related to alterations in uric acid levels. So, the unchanged antioxidative enzyme activities (paraoxonase, arylesterase, RBC-SOD and whole blood Gpx), observed in the present study might be reasonable and increased TAOC might be related to increased uric acid levels. In parallel with our findings, uric acid had been reported as reduced

in early pregnancy and significantly increased at the third trimester of pregnancy.[15,26,27] Uric acid concentrations are reduced in early pregnancy related to increased renal clearence and the end of pregnancy is characterized by an increase in uric acid levels due to an increased rate of catabolism.

SOD dismutases superoxide radicals to form hydrogen peroxide which is in turn decomposed to water and oxygen by GPx and catalase, thereby preventing the formation of hydroxyl radicals. Failure of this antioxidative system results in enhanced lipid peroxidation. Studies concerning the antioxidative enzyme activities of normal pregnancy are limited in number and yielded conflicting results; as decreased[27] or increased[28] GPx activities. Chen et al.[28] suggested that the changes might be related to differences in antioxidant response and dietary fat intake. To compare our results, we could not find any study that investigated SOD activity throughout pregnancy or any comparison with non-pregnant women.

CONCLUSION

Although this study demonstrated hyperlipidemia and oxidative stress (as demonstrated by increased plasma MDA levels and reduced serum TAOC) in normal pregnancy, however, failed to show any effects of PON1 on lipoprotein metabolism or oxidative-antioxidative system parameters in pregnancy. The metabolic changes of normal pregnancy appear to be an area of interest, since understanding the physiological processes would help us to better understand the role of PON1 in lipoprotein metabolism and hyperlipidemia and furthermore physiopathological mechanisms of pregnancy related complications.

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