



Correlation between the thermodynamic features of blood plasma and polymorphisms in thrombophilia genes of women with pre-eclampsia and early pregnancy loss



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Background Normal pregnancy is accompanied by significant changes in the hemostasis – complex protective mechanism associated with the fine balance between pro- and anticoagulation systems and fibrinolysis. These processes are regulated by the endothelium, the platelets and fibrinolytic plasma proteins. A failure of appropriate hemostatic function due to a number of mutations and other factors results in different pathological states – either in increased cardiovascular and thrombotic incidents or in hemorrhagic risk. All changes in blood coagulation, anticoagulation and fibrinolysis during pregnancy, lead to increased tendency of blood clotting. The changed balance between the pro- and anticoagulation system and the fibrinolytic system leads to maintenance of stable placental function during pregnancy and prevents per partum blood loss.

Thrombophilia, genetic or immunological, is a condition in which there is a systemic disorder in the process of haemostasis¹. Hereditary forms of thrombophilia due to gene mutations, including mutation of Factor V clotting (FV Leiden) and Prothrombin 20210A, and the gene encoding the enzyme methylenetetrahydrofolatereductase (MTHFR) may induce placental hypercoagulation and microthrombosis in uterine-placental blood vessels with subsequent ischemia, vasoconstriction and endothelial dysfunction². The thrombogenic mechanism has an inhibitory effect on the process of implantation and hormone production and can lead to recurrent miscarriage in the first trimester. Another serious problem that may arise at a later stage of pregnancy is due to disturbance in the normal trophoblastic invasion, leading to a reduction of the blood flow in the uteroplacental system and a risk of developing preeclampsia.

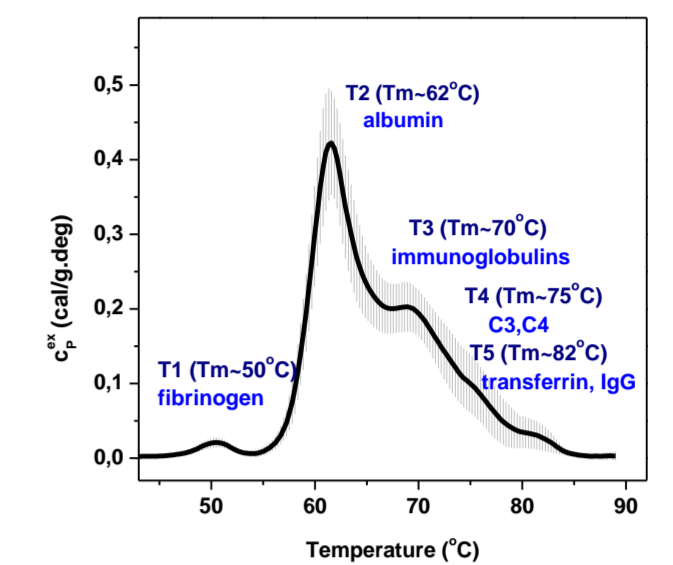
In recent years, differential scanning calorimetry (DSC) has emerged as a new tool for characterization of the thermodynamic properties of body fluids like blood plasma/serum with the aim to detect disease related changes in the thermodynamic behavior of the major plasma proteins (human serum albumin (HSA), immunoglobulins, transferrin, fibrinogen) and their binding state.

Aim The aim of this study is to identify specific calorimetric features of blood plasma proteome of women with preeclampsia and early pregnancy loss.

Methods The protein thermal stability was probed by means of differential scanning calorimetry (DSC) using DASM-4 instrument (Biopribor, Pushchino).

Blood plasma was linearly heated in the range 30–100°C with a scan rate of 1°C/min. The thermodynamic parameters: excess heat capacity (c_p^{ex}) and transition temperature (T_m) of the successive thermal transitions. The polymorphism in the genes of thrombophilia factors was determined as in Ridker et al. (1997)³.

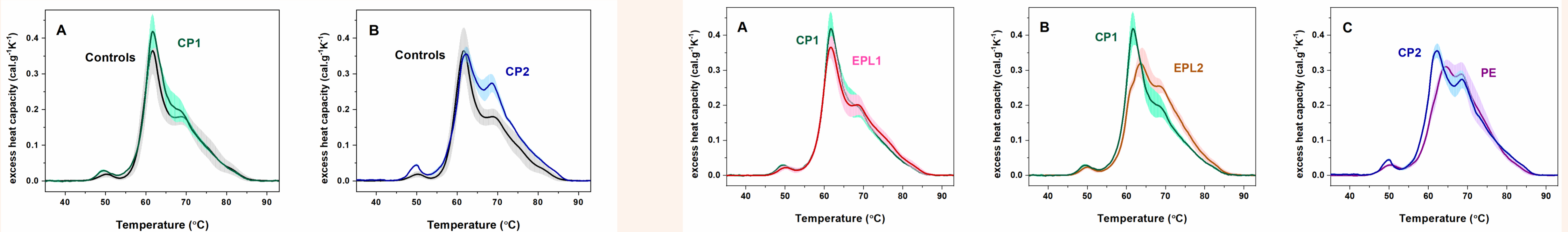
Differential scanning calorimetry



Typical DSC profile (solid line) and standard deviation of blood plasma derived from healthy non-pregnant women (controls). The contribution of the major plasma proteins to the calorimetric transitions is indicated.

Results

Calorimetric profiles of blood plasma from non-pregnant and pregnant controls, women with spontaneous abortions and preeclampsia



Average DSC profiles (solid line) and standard deviation (shadow) of blood plasma proteome recorded for non-pregnant (Controls) and pregnant (CP) women for the first (A) and second (B) trimester of pregnancy. For clarity the plasma profile of healthy non-pregnant women is plotted in black (grey shadow).

Average DSC profiles and standard deviations (shadows) of blood plasma proteome registered for women with early pregnancy loss (EPL1 and EPL2) and preeclampsia (PE). For clarity the plasma profile of healthy pregnant women at respective gestational trimester is plotted in green (cyan shadow) for women in first trimester of pregnancy (CP1) and in blue line (blue shadow) for women in third trimester of pregnancy (CP2).

Calorimetric parameters (mean values \pm SD), excess heat capacity (c_p^{ex}), transition temperature (T_m) of the successive transitions and similarity parameters (r , P , ρ), estimated from the DSC profiles of blood plasma for healthy non-pregnant and pregnant women (CP), women with early pregnancy loss (EPL) and preeclampsia (PE).

Groups	c_{p}^{fib} [cal/g.K]	T_m^{HSA} [°C]	c_{p}^{Alb} [cal/g.K]	c_{p}^{Alb}/c_{p}^{Igs}	ΔH [cal/g]	T_{FM} [°C]	r	P	ρ
Controls	0.018 \pm 0.007	61.5 \pm 0.10	0.37 \pm 0.05	2.10 \pm 0.2	4.2 \pm 0.3	64.7 \pm 0.7	-	-	-
CP 1	0.029* \pm 0.002	61.6 \pm 0.08	0.41 \pm 0.04	2.05 \pm 0.3	4.4 \pm 0.3	64.3 \pm 0.2	0.97	0.85	0.87
CP 2	0.042* \pm 0.001	62.0 \pm 0.06	0.36 \pm 0.03	1.30* \pm 0.2	5.2* \pm 0.3	66.0* \pm 0.4	0.88	0.76	0.79
EPL1	0.022 \pm 0.005	61.6 \pm 0.10	0.37 \pm 0.02	1.85 \pm 0.2	4.5 \pm 0.3	68.8 \pm 0.5	0.94	0.82	0.81
EPL2	0.023 \pm 0.004	63.8* \pm 0.15	0.31 \pm 0.04	1.20* \pm 0.3	4.7* \pm 0.1	67.0* \pm 0.2	0.86	0.67	0.70
PE	0.003 \pm 0.002	64.38 \pm 0.05	0.33 \pm 0.005	1.06 \pm 0.1	4.99 \pm 0.32	67.3* \pm 0.3	0.95	0.85	0.88

*indicates statistically significant difference from the Controls, $p < 0.05$

Polymorphism in the genes of thrombophilia factor (FVL, MTHFR C677T, 4G/5G PAI-1, and PLA1/A2 ITGB3) in the studied individuals

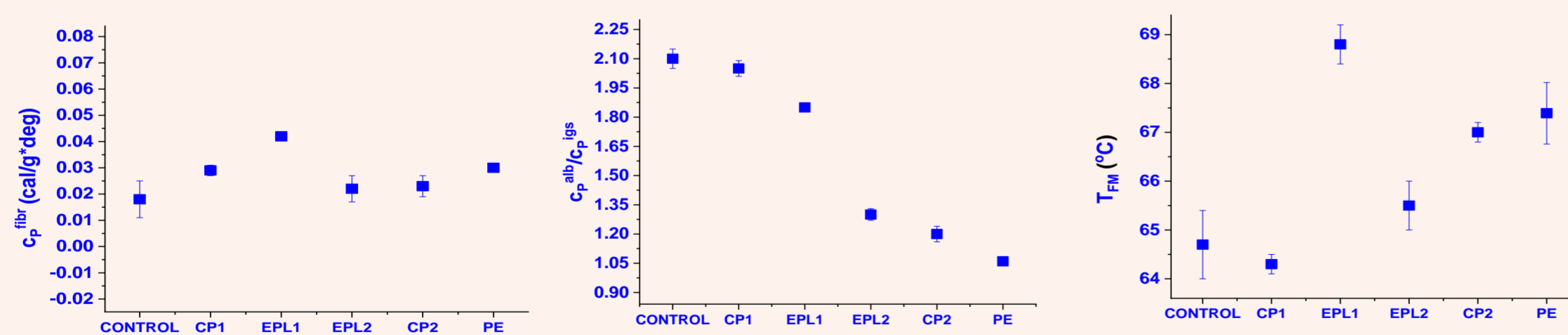
Group	% carrier of polymorphism
no-pregnant controls (n=10)	20
pregnant controls (n=6)	16
EPL1 (n=13)	77
EPL2 (n=4)	100
PE (n=4)	100

Level of plasma proteins determined by capillary electrophoresis

Groups	Albumin	Alpha 1	Alpha 2	Beta 1	Beta 2	Gamma
Reference values	54.7-69.6	2.63-5.03	4.87-10.48	5.35-9.19	2.38-7.11	9.69-18.9
CP 1	54.6 \pm 2	6.2 \pm 0.8	10.2 \pm 0.1	7.6 \pm 0.9	5.4 \pm 0.4	10.1 \pm 0.9
CP 2	54.6 \pm 3	7.4 \pm 1.8	11.6 \pm 0.2*	8.3 \pm 0.8	6.8 \pm 0.5	11.4 \pm 1.1
EPL1	54.3 \pm 2	7.5 \pm 1.1	10.7 \pm 0.7	7.4 \pm 0.3	10.4 \pm 0.8*	9.6 \pm 1.7
EPL2	55.9 \pm 2	7.2 \pm 1.2	9.7 \pm 1.1	7.4 \pm 0.4	9.6 \pm 1.1	10.2 \pm 2
PE	50.7 \pm 2.3*	8.4 \pm 0.7	11.4 \pm 0.8*	9.0 \pm 0.4	7.3 \pm 0.6	13.3 \pm 1.2

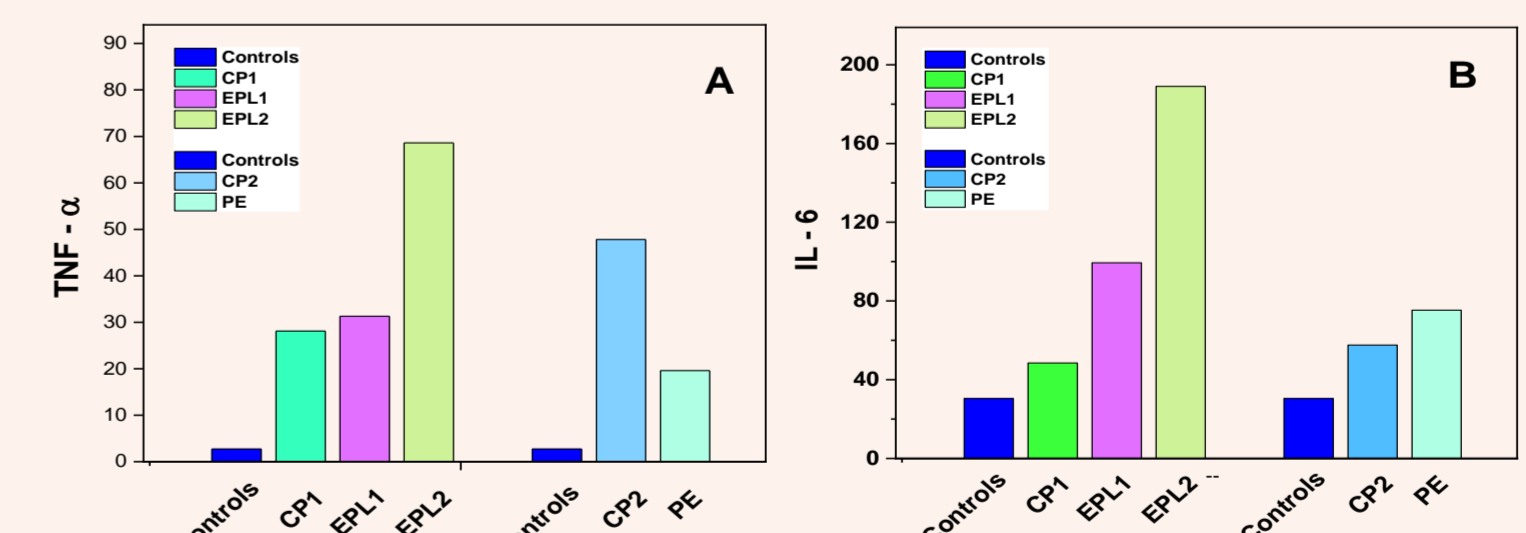
*indicates statistically significant difference from the Controls, $p < 0.05$

Thermodynamic parameters for the different calorimetric groups



Selected thermodynamic parameters determined from the DSC profiles of blood plasma derived from non-pregnant (Controls) and pregnant women in the first (CP1) and second (CP2) trimester of pregnancy. c_p^{fib} – excess heat capacity of the thermal transition assigned to fibrinogen; c_p^{Alb}/c_p^{Igs} – ratio of the excess heat capacities of albumin and immunoglobulins thermal transitions; T_{FM} – temperature weighted average center of the thermograms.

Histogram of tumor necrosis factor- α and interleukin-6 values



Tumor necrosis factor- α (A) and interleukin-6 (B) determined for non-pregnant (Controls) and pregnant women in the first (CP1) and second (CP2) trimester of pregnancy, women with early pregnancy loss (EPL1/EPL2) and preeclampsia (PE)

Conclusions

- The advancement of the pregnancy of healthy pregnant women is associated with increase in the excess heat capacity of the thermal transitions of fibrinogen and immunoglobulins, most probably due to the increase in their level in blood plasma as a consequence of changes in fibrinolytic system, that contributes to maintaining the placental function during pregnancy and prevents excessive bleeding in delivery.
- For high percentage of women with early pregnancy loss the excess heat capacity of fibrinogen is not altered, while the albumin transition is stabilized as compared to healthy pregnant women. These results show altered composition or intermolecular interactions of the plasma proteome for those women.
- Statistically significant shift of the weighted average center (T_{FM}) of the thermograms for nearly half of women with EPL, pregnant women in the second trimester and women with preeclampsia, reveals stabilization of the plasma proteome as compared to healthy non-pregnant controls.
- Increased cytokine levels of TNF- α and IL-6 established in blood plasma of both patient groups, more pronounced for EPL women, indicate a maternal systemic inflammation which might contribute to the pregnancy complication
- The polymorphism in the genes of thrombophilic factors (FVL, MTHFR C677T, 4G/5G PAI-1, and PLA1/A2 ITGB3) in EPL and PE women were significantly more prevalent than in both control groups.
- Strong correlation between the modified thermodynamic features of plasma proteome of women with EPL and PE and the carrier of polymorphism in the genes of thrombophilia is found.

References:

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