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Hypothermic effects on gas exchange performance of membrane oxygenator and blood coagulation during cardiopulmonary bypass in pigs

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Abstract:	Introduction: Whether hypothermic cardiopulmonary bypass (CPB) could attenuate both blood coagulation and platelet activation compared to normothermic CPB remains elusive. Methods: Biocompatibility of a polymer-coated CPB circuit was comparatively assessed by plasma proteomics between juvenile pigs undergoing hypothermic (23℃) CPB and those undergoing normothermic (37℃) CPB (n = 6, respectively). Plasma samples were taken 3 times: 5 min after initiation of CPB (T5, before cooling), just before declamping and rewarming (Tc) and just before termination of CPB (Trw, 120 min). Proteomic analysis was quantitively performed by isobaric tags for relative and absolute quantification labeling. Thrombin-antithrombin complexes (TAT Ⅲ) were measured by enzyme immunoassay, and vitamin K-dependent protein C (PROC), β-thromboglobulin (TG) and p-selectin were measured by enzyme-linked immunosorbent assay. Blood gas analyses evaluated oxygenator performance.

	Results: Hypothermic CPB had a significantly higher PaO2 at Tc and lower PaCO2 at Trw than normothermic CPB. 224 proteins were identified with statistical criteria of both protein confidence (>95%) and false discovery rate (FDR<5%). Six of these proteins significantly decreased at Tc than at T5 in hypothermic CPB (p = 0.02-0.04), with three related to platelet degranulation. Protein C decreased at Trw compared with T5 in normothermic CPB (p = 0.04). TAT had a slightly larger increase with normothermic CPB at Trw than with hypothermic CPB. β-TG and P-selectin levels were significantly lower at Trw with hypothermic CPB than with normothermic CPB (p =0.04). Conclusion : Hypothermic CPB attenuated platelet degranulation/blood coagulation and maintained better oxygenator performance compared to normothermic CPB in juvenile pigs.

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Keywords

Biocompatibility, blood coagulation, platelet activation, hypothermia, cardiopulmonary bypass, proteomics

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Introduction

It remains uncertain whether hypothermia could reduce blood component activation by contact with the cardiopulmonary bypass (CPB) circuit. On the other hand, many studies have shown that hypothermia could reduce inflammation caused by ischemic/traumatic injuries[1]. Evidence has been convincing that there is crosstalk between inflammation and blood coagulation[2,3]. Hypothermia was also shown to decrease complement, coagulation, neutrophil and platelet activities during/after surgery with CPB or therapeutic hypothermia[4-10]. However, there have been several evidences that moderate hypothermic (24℃, 25℃) CPB could not impact on cytokine levels compared to mild (32℃, 34℃) one in pediatric cardiac surgery[11,12]. Furthermore, the CPB-induced inflammation has been shown to be stronger in children compared with adults because of simply small body size relative to CPB circuit size[11]. The objective of this study was to ascertain whether deep (23℃) hypothermic CPB reduces blood component activation including blood coagulation compared with normothermic CPB in juvenile pigs; with both procedures the circuits were fully polymer-coated. The deep hypothermic CPB has be widely used in complex congenital surgeries such as Norwood procedure or aortic arch reconstruction in order to safely extend hypothermic circulatory arrest time. We previously reported that a polymer-coated CPB circuit attenuated up-regulation of proteins in both proteases/protease inhibitors and platelet degranulation[13]. The aim of this study was to verify whether deep hypothermic CPB could reduce upregulation of blood proteins activated by contact with a polymer-coated CPB circuit in juvenile pigs.

Materials and Methods

Animal experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals prepared by Shimane University Faculty of Medicine and the experimental protocol was approved by the Ethics Committee of the Ethics of Shimane University. The anesthetic and experimental procedures were performed as described previously[13]. In brief, 12 domestic juvenile pigs (body weight = 16 kg) were randomized into two groups: the normothermic (37℃) CPB group and hypothermic (23℃) CPB group (n = 6, respectively). After an overnight fast with free access to water, animals were premedicated by intramuscular injection of ketamine (20 mg/kg), xylazine (3 mg/kg) and 0.5 mg of atropine. Anesthesia was induced by bolus infusion of propofol (2 mg/kg), fentanyl (12.4 μg/kg) and rocuronium bromide (1 mg/kg). After tracheal intubation, animals were mechanically ventilated with 50% oxygen in air, and anesthesia was maintained by inhalation of 1.3% of isoflurane. During full CPB support, anesthetic maintenance was switched to continuous infusion of fentanyl (1 μg/kg/h～20 μg/kg/h), propofol (6－8 mg/kg/h) and rocuronium bromide (1.5 mg/kg/h). Throughout the experiment,

acetated Ringer's solution with 40 ml of 20% glucose solution was administered (6 ml/kg/h) in both groups. Heart rate was monitored together with rectal temperature and arterial blood pressure obtained from the right femoral artery by Power Lab (ML846, ADInstruments, Nagoya, Japan)[13].

Surgical procedures and hypothermic/normothermic CPB

All animals underwent median sternotomy in the supine position under aseptic conditions[13]. After a bolus infusion of heparin (4 mg/kg), a 14 Fr aortic cannula was inserted into the ascending aorta and an 18 Fr angled single venous cannula was inserted into the superior vena cava. After initiation of CPB, an 18 Fr straight venous single cannula was inserted into the right atrium and advanced into the inferior vena cava. Pump flow was set about 1 l/min, which was equal to the cardiac output determined just before CPB initiation by a conductance catheter and its system (Unique Medical, Tokyo, Japan). When necessary, acetated Ringer's solution was added to maintain pump flow. In the hypothermic group animals, 5 min after initiation of ECC and the first blood sampling (T5), hypothermia was introduced by rapid core cooling to a rectal temperature of 22℃ in 25 min with surface cooling. In contrast, animals in the normothermic CPB group were kept under a normothermic condition of 37℃ during 120 min of the CPB procedure. Thirty min later, the ascending aorta was cross-clamped, and cardioplegic arrest of the heart was achieved by delivering blood cardioplegic solution. The same dose of cardioplegic solution was administered 30 min later. At 60 min of aortic cross clamp, the second blood sample (Tc) was taken, and then the cross clamping was released after infusion of 20 mg xylocaine. After 30 min of assisted circulation in the normothermic group or rapid rewarming up to 37℃ in the hypothermic CPB group and the third blood sampling (Trw), all animals were successfully weaned from the CPB and received all blood that remained in the reservoir and the tubing.

The CPB circuit consisted of a hollow-fiber membrane oxygenator combined with a hard-shell reservoir and a roller pump (Terumo Sarns 8000, Tokyo, Japan). In both groups, all areas of the CPB circuit in contact with blood were coated with ternalpolymer (SEC-coating, HPO-06RHF-C, Senko Medical Instrument Manufacturing Co., Ltd., Tokyo, Japan)[13]. The CPB priming fluid consisted of 440 ml of acetated Ringer's solution (including 1500 U of heparin and 50 ml of 20% mannitol) in order to achieve the minimum filling level (55 ml) of the reservoir.

Data collection and measurement

Pump flow, gas flow, percentage of oxygen, arterial pressure, heart rate and rectal temperature were manually recorded every 5 min. Arterial blood gases were analyzed every 30 min by i-STAT (Abbott Point Of Care, Princeton, NJ, USA), $PaCO_2$ was measured at 37℃ (α-stat strategy), and gas flow was adjusted to keep PaO_2 >100 mmHg. Hypoglycemia was treated by bolus infusion of 20% glucose

solution. Rectal temperature was controlled by a heat exchanger, cooling/warming blanket and room temperature control. Blood samples were taken 5 min after initiation of CPB and just before cooling (T5), just before declamping and rewarming (90 min after the initiation of CPB, Tc) and just before termination of CPB (Trw, after rewarming). Blood samples were centrifuged at 1,400 x g for 5 min, and the plasma layers were stored at -80°C.

Immunodepletion of abundant proteins

The two most abundant plasma proteins, albumin and immunoglobulin G, were removed using an immunodepletion column (Albumin & IgG Depletion SpinTrap, GE Healthcare, Buckinghamshire, UK) in accordance with the manufacturer's instructions and as reported previously[14].

iTRAQ labeling and strong cation exchange (SCX) chromatography

Samples were prepared according to instructions published by SCIEX (Concord, Canada) and as published previously[14]. Briefly, equal amounts of immunodepleted T5, Tc and Trw samples from each animal were denatured and reduced and the cysteines were alkylated and digested with trypsin (SCIEX). Each digest was labeled with a different iTRAQ tag using the iTRAQ reagent 4-plex kit (SCIEX). iTRAQ label 114 was chosen for the T5 sample, and iTRAQ labels 115, 116 or 117 were randomly selected for the Tc and Trw samples**;** the three samples from each animal were then combined. The combined samples were then fractionated into six fractions by SCX chromatography according to the manufacturer's instructions (SCIEX), and each fraction was desalted according to the manufacturer's manuals (Waters, Milford, MA, USA).

NanoLC and MALDI-TOF/TOF MS/MS analysis

One fraction from SCX chromatography was fractionated to 171 spots using a DiNa nanoLC system (KYA Tech, Tokyo, Japan) and collected onto an Opti-TOF LC/MALDI 384 target plate (SCIEX) according to the manufacturer's instructions and as reported previously[14]. Spotted peptide samples were analyzed by a 5800 MALDI-TOF/TOF MS/MS Analyzer with TOF/TOF Series software (version 4.0, SCIEX). MS/MS data were analyzed using ProteinPilot™ software (version 3.0) and the Paragon™ protein database (SCIEX) after adding a sus scrofa FASTA file downloaded from NCBI for database searching. Quantitative changes in protein abundance at Tc or Trw were calculated using the iTRAQ ratios Tc:T5 or Trw:T5, respectively.

ITRAQ data analysis

Proteins identified were assessed to determine if they met the following two conditions: 1) a false discovery rate (FDR) <5% (FDR was estimated by "decoy database searching" using ProteinPilot Software); and 2) protein confidence >99% ("unused ProtScore" >2). Proteins meeting those criteria are defined here as having "statistical significance"[14,15]. The annotations of identified proteins were acquired from the Uniprot database (http://www.uniprot.org/). For an unclassified protein whose protein name or gene name for sus scrofa was not attainable by the ProteinPilot software, we searched for its orthologous protein by a database for the orthologous group, EggNOG4.5 (http://eggnogdb.embl.de/#/app/home)[16] and Inparanoid8 (http://inparanoid.sbc.su.se/cgi-bin/index.cgi)[17].

Enzyme-linked immunosorbent assay (ELISA)

Levels of vitamin K-dependent protein C (PROC), β-thromboglobulin (TG) and P-selectin were measured using each sandwich ELISA kit according to its respective manual (Pig PROC kit, LifeSpan BioSciences, Inc., Seattle, WA, USA; pig β-TG ELISA kit, MyBioSource, San Diego, CA, USA; Pig soluble P-selectin ELISA kit, CUSABIO, Houston, TX, USA). The levels of thrombin-antithrombin complexes (TAT Ⅲ) were measured by enzyme immunoassay.

Statistical analysis

iTRAQ ratios (Tc/T5 ratio, Trw/T5 ratio) were expressed as mean ± standard deviation (SD) and statistically analyzed regarding differences within groups by using 1-sample t-test of the average protein ratio against 1 to evaluate the validity of protein expression changes[14,15]. Continuous variables were expressed as mean ± standard deviation (SD) or expressed as median and quartiles [Q1, Q3] or box-and -whisker plot. Statistical significance was assessed between two groups by the Mann-Whitney U test, and comparisons among three sampling/measurement points in each group were analyzed by the Wilcoxon signed-ranked test with Bonferroni corrections (SPSS Statistics 24.0 for Windows, IBM Corp. Chicago, IL, USA). $P<0.05$ was defined as statistically significant.

Results

There were no significant differences between the two groups in pump parameters and results of blood gas analyses at T5 before cooling (Table 1)**, although there were relatively large individual differences in PO2, PCO2, and glucose values at T5.**

. After cooling, PaO_2 increased more markedly at Tc than at T5 in the hypothermic group ($P = 0.188$), while it decreased in the normothermic group; thereby the difference in PaO_2 between groups reached statistical

significance (P = 0.019) despite a significant difference in FiO_2 between groups (P = 0.045). Therefore, the P/F ratio, i.e., the ratio of PaO_2 divided by FiO_2, an index of pulmonary oxygenation[18], increased at Tc in the hypothermic group, but decreased in the normothermic group at Tc (P = 0.094); the difference at Tc between groups was statistically significant (P = 0.019). Level of $PaCO_2$ was significantly higher at Trw in the normothermic group than in the hypothermic group (P = 0.01), despite the significantly larger gas flow in the normothermic group than in the hypothermic group (P = 0.048). In animals having hypothermic CPB, the hemoglobin level had a greater decrease at Tc than at T5 (P = 0.375) and thereafter increased at Trw, although the difference between groups did not reach statistical significance (P = 0.062). Infusion volume during CPB was 785 [450, 880] ml in animals undergoing hypothermic CPB and 275 [60, 355] ml in those undergoing normothermic CPB, for a statistically significant difference (P =0.0017).

All proteins identified with high confidence are shown in a scatter plot graph in which each protein is expressed by the log-transformed iTRAQ ratio and p-values (Figure 1). At the midpoint of CPB, the trend of protein downregulation was prominent in the hypothermic group although there was a wide range of variation of protein fold changes in the normothermic group. Several proteins related to coagulation/fibrinolysis were upregulated in the normothermic group, although without statistical significance (Figure 1A, B). Eight proteins decreased with statistical significance during both hypothermic and normothermic CPB (Table 2). Among these, proteins belonging to platelet degranulation, APOH, APOA1, SERPINA3-1, were slightly but significantly downregulated during cooling (Tc). Furthermore, the level of vitamin K-dependent protein (PROC), a physiologic anticoagulant, was significantly downregulated in animals with normothermic CPB at Trw (P= 0.001) although the level of PROC increased in animals undergoing hypothermic CPB (P = 0.069).

ELISA analysis demonstrated that levels of β-TG and P-selectin were significantly higher in normothermic animals than in hypothermic animals at Trw (P =0.041, Figure 2A, B). TAT Ⅲ was more upregulated at Trw in the normothermic group than in the hypothermic group, although the difference did not reach statistical significance (P = 0.258, Figure 3A). There was no difference in administered heparin doses between groups (61 [48, 68] mg in normothermic animals, 56 [48, 64] mg in hypothermic animals). In ELISA analysis, protein C was upregulated at Tc (during cooling), with a small difference between hypothermic and normothermic animals (P = 0.180, Figure 3B).

Discussion

A wide range of measures have been studied so far to improve biocompatibility in CPB[19]. However, to our knowledge, this is the first study to assess the effects of hypothermia on the biocompatibility of CPB circuits by comparing both oxygenator performance and plasma proteomes between hypothermic CPB and normothermic CPB in which all experiments in both groups were performed under the same conditions

except for temperature management. Hypothermic CPB improved oxygenation by oxygenators during blood cooling at Tc. This improvement was simply thought to reflect the physiologic changes whereby lowering the temperature increases blood solubility of oxygen by 1.2%/℃ and hemoglobin affinity of oxygen by 7.4%/℃ (at SO_2<80%)[20,21], resulting in increased oxygen transfer by oxygenators[22]**.** However, reduced oxygen consumption by hypothermia leads to increased venous oxygen tension. This consequence necessarily decreases oxygen transfer in oxygenators by Fick's law because oxygen diffusion decreases due to the decreased oxygen partial pressure gradient between gas flowing inside capillary membranes and venous blood flowing outside membranes in the oxygenator[23]. However, hemodilution by the pump priming solution can reduce oxygen delivery (oxygen in ml/100ml of blood) and thereby increase the oxygen extraction rate resulting in a lower venous PO_2. Taken together, hypothermic CPB could augment the oxygen transfer ability of oxygenators, but the actual oxygen transfer varies with body temperature, oxygen consumption, pump flow and hemoglobin levels.

After two hours of CPB, elimination of CO_2 gas by oxygenators decreased in normothermic CPB compared to hypothermic CPB. This phenomenon could reflect some sort of deterioration of membrane oxygenator performance in normothermic CPB. However, further study is needed to elucidate the true mechanism.

Hypothermia is generally thought to impair or suppress blood coagulation which was evaluated by bleeding time, APTT, PT, thrombelastography, and enzyme activity[4,5,7,9,24]. Our study also demonstrated that hypothermic CPB suppressed upregulation of proteins belonging to coagulation/fibrinolysis and TAT Ⅲ. Proteomic and ELISA analyses demonstrated that levels of protein C were slightly increased during cooling (at Tc) in hypothermic CPB, while proteomic analysis showed a significant decreased level of protein C at Trw in normothermic CPB. A recent study showed that protein C, a physiologic anticoagulant, was upregulated in animals having mild hypothermia (33℃) after cardiac arrest[9].

Whether platelet degranulation could be suppressed or activated by hypothermia remains controversial. In this study, levels of two platelet activation markers, β-TG and P-selection, were lower in the hypothermic CPB group than in the normothermic CPB group at Trw, indicating that hypothermia could attenuate platelet degranulation induced by exposure to the CPB circuit. This was consistent with previous studies[7,9]. However, Straub et al. reported that hypothermia or lowering the temperature activated platelet function and thereby upregulated p-selection expression by increased levels of ADP[25,26]. On the other hand, Swoboda et al. reported that hypothermia inhibited expressions of CD11b and CD162 (p-selectin) on monocytes in an in vitro circulation model, which may lead to the inhibition of monocyte-endothelial and monocyte-platelet interaction[27].

In hypothermic CPB, the hemoglobin level decreased at Tc and then increased at Trw; however, such changes did not occur in normothermic CPB. This could result from the significantly larger infusion volume in hypothermic CPB than in normothermic CPB (785 [450, 880]ml vs. 275 [60, 355]ml). Unlike surface

cooling that increases venous return, perfusion hypothermia, i.e., core cooling by CPB, decreases venous return because of an increased vascular capacity[28]. Therefore, in order to maintain the pump reservoir level, a larger volume of infusion is mandatory in hypothermic CPB compared with normothermic CPB. **This large infusion volume might affect protein abundance in hypothermic CPB group, but the equal amounts proteins (100 μg) of immunodepleted plasma samples were prepared to analyze and thereby such dilution cannot affect iTRAQ ratios in both groups.**

The limitations of the present study are: 1) inadequate investigation into p-selection degranulation from platelets and 2) no macro- and microscopic investigations of membrane oxygenators after experiments in order to search for any thrombus outside hollow fibers or the water content stored in hollow fibers due to condensation. Therefore, further studies are needed to elucidate these issues in order better to understand hypothermic effects on CPB biocompatibility. **In order to obtain more wide range of findings, additional studies using adult animals at different temperatures, i.e., moderate hypothermia (25-30℃) or mild hypothermia (31-34℃) are required.**

In conclusion, **the present study of juvenile pig CPB experiments revealed that** hypothermic (23℃) CPB attenuated both blood coagulation and platelet degranulation activated by contact with the CPB circuit compared with those in normothermic CPB. Furthermore, hypothermic CPB was accompanied by a higher performance of the oxygenator compared with normothermic CPB.

Declaration of Conflicts of Interest

The authors declare that there are no conflicts of interest.

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Figure Legend

Figure 1. Scatter plot graphs of identified proteins demonstrated by log-transformed iTRAQ ratio and one-sample t-test p-values. Fold changes >1.2 or <0.833 were defined as significant up- or downregulation (indicated by two vertical lines). P<0.05 was defined as statistically significant (shown by the horizontal line). Upper graphs (A) show proteins belonging to the defense system in both the hypothermic and normothermic CPB groups at midpoint (Tc/T5) of CPB. Lower graphs (B) show the above proteins in both groups at termination (Trw/T5) of CPB. iTRAQ: isobaric tags for relative and absolute quantification; CPB: cardiopulmonary bypass. a: PLG (Plasminogen, P06867); b: FGB (fibrinogen beta, F1RX37); c: FGG(uncharacterized protein, F1RX35); d: FGA (fibrinogen alpha chain, F1RX36); e: fibrinogen A-alpha-chain (N/A, Q28936); f: fibrinogen beta chain (FGB, I3L651); g: vitamin K-dependent protein C (PROC, I3LRJ4); h: SERPINC1 (uncharacterized protein, F2Z5E2).

Figure 2. Platelet degranulation activities that were evaluated by the levels of both β-TG and P-selectin in the hypothermic (HYPO) CPB group and normothermic (NORMO) CPB group. Both were measured using a sandwich ELISA kit. A: β-TG, B: P-selectin. T0, just before cardiopulmonary bypass (CPB) initiation; T5, at 5 min after CPB initiation; Tc, just before declamping (and rewarming); Trw, just before termination of CPB.

Figure 3. Blood coagulation activities that were evaluated by levels of both TAT Ⅲ and protein C in the hypothermic (HYPO) group and normothermic (NORMO) CPB group. A: TAT Ⅲ, B: Protein C. T0, just before cardiopulmonary bypass (CPB) initiation; Tc, just before declamping (and rewarming); Trw, just before termination of CPB.

Table 1. Arterial blood gas analysis and physiological variables at three sampling points during polymer-coating cardioplumonary bypass

	Deep hypothermic cardiopulmonary bypass			Normothermic cardiopulmonary bypass		
	T5, 5min after ECC start, just before cooling	Tc, just before declamping , just before rewarming	Trw, just before termintion of ECC	T5, 5min after ECC start	Tc, just before declamping	Trw, just before termintion of ECC
pH	7.51 [7.40, 7.60]	7.42 [7.33, 7.49]	7.57 [7.53, 7.64][a]	7.43 [7.38, 7.59]	7.32 [7.29, 7.47]	7.44 [7.35, 7.52][a]
PCO2 (mmHg)	33.4 [26.3, 44.3]	44.7 [39.2, 57.1]	30.9 [28.9, 31.6][b]	41.4 [32.6, 55.6]	48.4 [41.1, 54.0]	37.1 [33.0, 41.3][b]
PO2 (mmHg)	82.0 [66.3, 286.8]	369.0[297.3, 416.8][c]	257.0[220.0, 291.8]	228.0[173.8, 323.0]	115.5 [97.8, 177.5][c]	207.0 [198.5, 236.5]
BE (mmol/L)	3.5 [0, 7.0]	5.0 [2.5, 6.8]	6.5 [3.3, 10.5]	7.0 [4.0, 8.3]	1.0 [-2.0, 4.0]	2.0 [-4.0, 4.0]
Na (mmol/L)	134.5 [130.3, 138.0]	134.0 [129.5, 135.5]	135.0 [132.5, 139.0]	134.0 [133.0, 136.5]	136.5 [132.3, 137.3]	137.5 [135.0, 138.5]
K (mmol/L)	4.4 [4.0, 4.9]	3.4 [3.3, 5.9]	3.6 [3.4, 3.8]	4.1 [3.8, 4.3]	4.1 [3.5, 4.5]	3.4 [3.1, 3.7]
Glucose (mg/dL)	205.5 [65.5, 268.3]	165.5 [66.0, 238.8]	142.0 [54.0, 169.3]	146.5 [85.5, 210.0]	165.5 [66.0, 238.8]	76.5 [40.5, 142.3]
Hb (g/dL)	6.2 [5.5, 6.5]	4.8 [4.4, 5.3]	7.0 [5.8, 7.9]	5.8 [5.3, 6.4]	5.8 [4.8, 7.4]	5.3 [4.3, 6.3]
Rectal temperatue (℃)	36.0 [35.7, 36.9]	23.7 [22.4, 24.3][d]	37.0 [36.4, 37.4]	37.4 [35.6, 37.9]	35.3 [34.9, 37.4][d]	37.4 [36.4, 38.0]
Arterial blood temperature (℃)	35.8 [35.4, 36.4]	22.7 [22.0, 23.5][e]	39.3 [38.7, 39.6][f]	34.7 [32.0, 36.9]	34.3 [34.1, 37.5][e]	37.4 [37.1, 37.9][f]
Venous blood temperature (℃)	34.6 [34.3, 36.6]	22.9 [21.9, 23.6][g]	36.7 [36.4, 37.0]	35.6 [32.8, 36.4]	34.4 [33.9, 37.1][g]	36.5 [36.4, 37.0]
Pump flow (L/min)	1.1 [0.7, 1.2]	0.9 [0.8, 1.2]	1.2 [0.9, 1.3]	1.1 [0.9, 1.2]	1.2 [1.0, 1.2]	1.1 [0.8, 1.3]
Gas flow (L/min)	1.0 [1.0, 1.0]	1.1 [0.8, 1.4]	1.0 [0.9, 1.4][h]	1.0 [1.0, 1.3]	1.1 [1.0, 2.7]	1.6 [1.2, 2.6][h]
Oxygen (FiO2)	0.65 [0.60, 1.00]	0.70 [0.65, 0.70][i]	0.80 [0.70, 0.93]	0.80 [0.60, 1.00]	0.88 [0.70, 1.00][i]	0.90 [0.68, 1.00]
P/F ratio[l]	125 [110, 297]	527 [425, 595][j]	292 [285, 399]	233 [193, 538]	134 [98, 254][j]	255 [208, 310]
Mean perfusion pressre (mmHg)	54 [39, 57]	56 [37, 62]	61 [58, 66][k]	47 [36, 60]	61 [56, 68]	51 [42, 57][k]

Valuables are expressed as median and quatiles [Q1, Q3]. Statistical significances between groups were assed by Mann-Whitney U test. The statistical significances ($p<0.05$) were as follows:

a, p =0.010; b, p = 0.010; c, p = 0.019, d, p = 0.005; e, p = 0.004; f, p = 0.017; g, p = 0.004; h, p = 0.048; I, p = 0.045; j, p = 0.019.;k, p = 0.015.

l = the ratio of arterial oxygen tension (mmHg) to fraction of inspired oxygen gas (%) (PaO_2/FiO_2).

Table 2. Significantly upregulated or downregulated plasma proteins during either normothermic or hypothermic cardiopulmonary bypass (CPB) in pigs

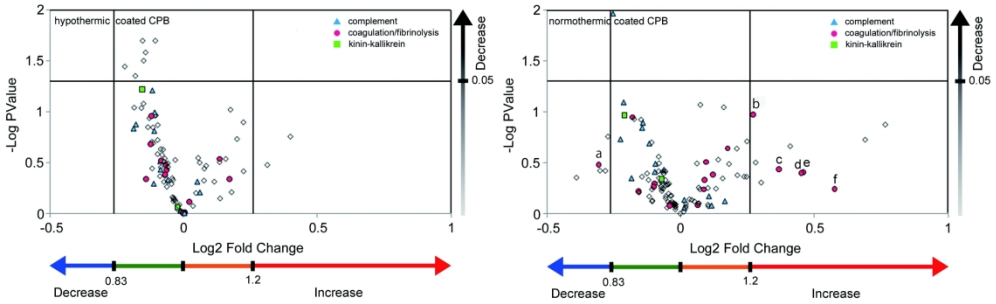
				Just before declamping (and rewarming,Tc/T5)		Just before termination of CPB (Trw/T5)	
Uniprot KB Accession number	**Protein name**	**Gene name**	**Protein function**	**Deep hypothermic CPB**	**Normothermic CPB**	**Deep hypothermic CPB**	**Normothermic CPB**
I3LGN5	Uncharacterized protein (Fragment)	Putative APOH[a]	**Platelet degrnaulation**	0.91±0.07*	0.98±0.14	1.02±0.08	0.99 ± 0.14
F1RN76	Uncharacterized protein	CD5L	Scavenger receptor activity	0.90±0.07*	0.95±0.12	0.92 ± 0.11	1.04 ± 0.19
A0A0C3SG01	Apolipoprotein A-I	APOA1	Lipid transport, **platelet degranulation**, scavenging of heme from plasma	0.88±0.11*	0.97±0.25	1.03 ± 0.24	1.10 ± 0.11
F1SCC7	Uncharacterized protein	Putative SERPINA3-1[b]	**Platelet degrnaulation**, **neutrophil degranulation**, serine-type endopeptidase inhibitor activity	0.90±0.07*	0.97±0.22	1.05 ± 0.14	1.00 ± 0.19
F1S1A9	Uncharacterized protein	APOA2	Chylomicron assembly & remodeling	0.86±0.10*	0.96±0.21	1.08 ± 0.25	1.08 ± 0.10
I3LD86	N-acetylmuramoyl-L-alanine amidase	PGLYRP2	Peptidoglycan receptor activity	0.93±0.04*	0.96±0.38	0.95 ± 0.14	0.98 ± 0.26
A0SEH3	Complement component C8G	C8G	Terminal pathway of complement, regulation of complement cascade	0.88±0.16	0.84±0.03*	1.04 ± 0.17	0.91 ± 0.14
I3LRJ4	Vitamin K-dependent protein C	PROC	Serine-type endopeptidase activitiy, **negative regulation of blood coagulation**	1.10±0.15	0.90±0.20	1.20 ± 0.15	0.88 ± 0.01*

Mean iTRAQ ratio (Tc/T5, Tend/T5) are indicated as mean ± SD. T5: at 5minutes after CPB initiation, Tc: just before declamping (and rewarming), Trw: just before termination of CPB

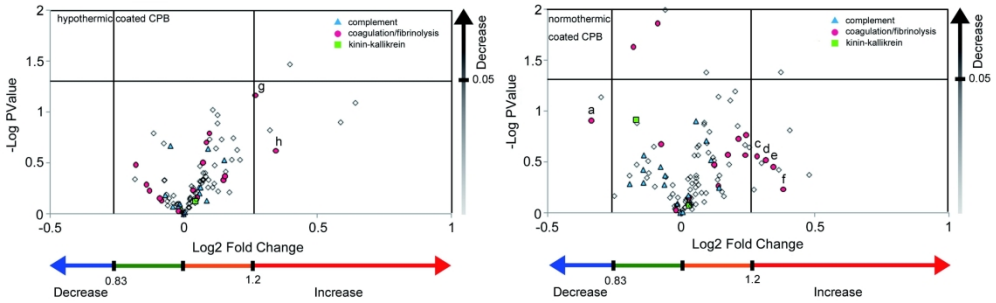
*: shows significant defferences (P<0.05) within groups by 1-sample t-test. a: searched by Inparanoid 8, b: searched by eggNOG4.5.1

Bold face indicates "Platelet/neutrophil degranulation and blood coagulation"

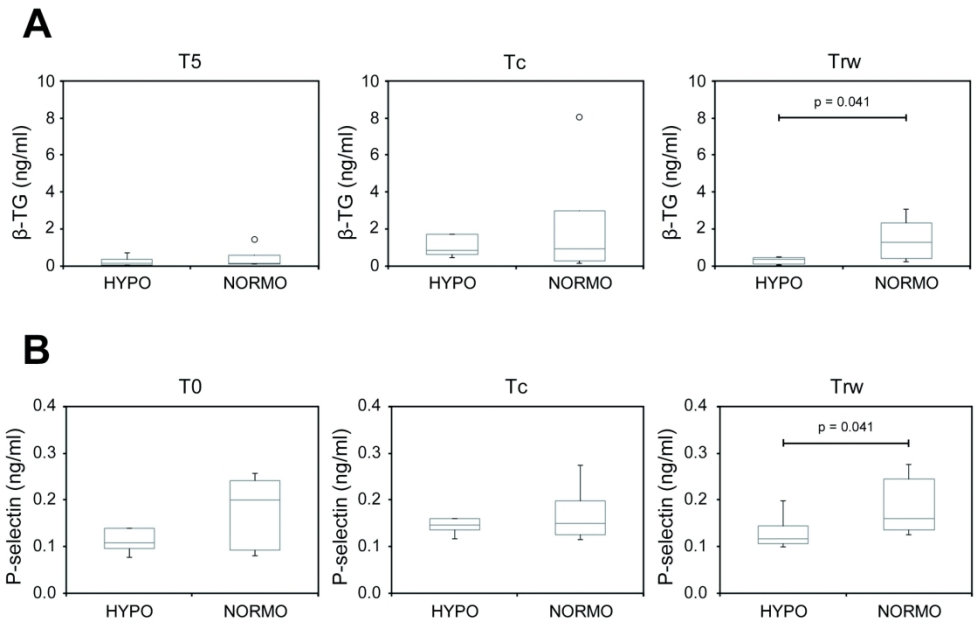
A. Defense system (complement, coagulation/fibrinosis, and kinin-kallikrein) at midpoint of cardiopulmonary bypass (CPB)



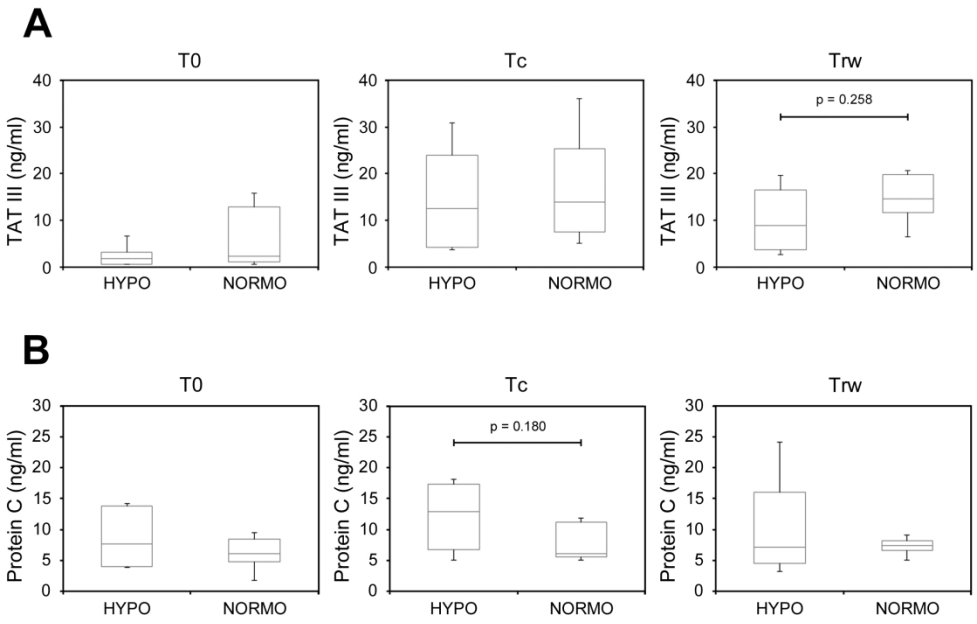
B. Defense system (complement, coagulation/fibrinolysis, and kinin-kallikrein) at termination of cardiopulmonary bypass (CPB)



250x212mm (300 x 300 DPI)



209x143mm (300 x 300 DPI)


A
T0
Tc
Trw
TAT III (ng/ml)
p = 0.258
HYPO
NORMO
B
Protein C (ng/ml)
p = 0.180