Hypothermia in cardiogenic shock reduces systemic t-PA release.

vanderPals, Jesper; Götberg, Michael I; Götberg, Matthias; Hultén, Lillemor Mattsson; Magnusson, Mia; Jern, Sverker; Erlinge, David

Published in:
Journal of Thrombosis and Thrombolysis

DOI:
10.1007/s11239-010-0541-x

2011

Hypothermia in cardiogenic shock reduces systemic t-PA release

Jesper van der Pals[1], Michael I Götberg[1], Matthias Götberg[1], Lillemor Mattsson Hultén[2], Mia Magnusson[3], Sverker Jern[3] and David Erlinge[1]*

[1]Department of Cardiology, Skåne University Hospital, Lund University, Sweden

[2]Wallenberg Laboratory, Sahlgrenska Center for Cardiovascular and Metabolic Research, University of Gothenburg, Sweden

[3]Wallenberg Laboratory for Cardiovascular Research, Sahlgrenska Academy, University of Gothenburg, Sweden

*Corresponding author

Corresponding author:

David Erlinge, MD, PhD

Dept. of Cardiology

Skåne University Hospital

Lund University

S-221 85 Lund, Sweden

Phone: +46 46 17 25 97

Fax: +46 46 15 78 57

david.erlinge@med.lu.se

Abstract

Therapeutic hypothermia has been found to improve hemodynamic and metabolic parameters in cardiogenic shock. Tissue plasminogen activator (t-PA) is a pro-thrombolytic enzyme, which also possesses pro-inflammatory properties. Interleukin 6 (IL-6) and tumour necrosis factor alpha (TNF-α) are pro-inflammatory cytokines; interleukin 10 (IL-10) and transforming growth factor beta 1 (TGF-β1) are anti-inflammatory cytokines. The aim of this experiment was to investigate the mechanism behind the protective effect of therapeutic hypothermia in cardiogenic shock. This was done by studying the effect of hypothermia on basal t-PA levels, peripheral t-PA release, and on the inflammatory response. Cardiogenic shock was induced by inflation of an angioplasty balloon in the proximal left anterior descending artery for 40 min in 16 pigs, followed by 110 minutes of reperfusion. The animals were randomized to hypothermia (33°C, n=8), or normothermia (n=8) at reperfusion. Hemodynamic parameters were continuously monitored. Plasma was sampled every 30 min for analysis of blood-gases and t-PA, and for analysis of inflammatory markers at baseline and at the end of the experiment. t-PA, IL-6 and TGF-β1 increased during cardiogenic shock. Apart from favourably affecting hemodynamic and metabolic variables, hypothermia was found to reduce basal arterial and venous t-PA levels, and to inhibit the release of t-PA from the peripheral vascular bed. Hypothermia did not alter the inflammatory response. In conclusion, mild hypothermia improves hemodynamic and metabolic parameters in cardiogenic shock. This is associated with a reduction in basal t-PA levels and t-PA release from the peripheral vascular bed, but not with an altered inflammatory response.

Key words: Cardiogenic chock, mild hypothermia, t-PA, inflammation.

Introduction

Cardiogenic shock is a major complication to acute myocardial infarction. The condition occurs in 7-10% of patients hospitalized due to myocardial infarction and is the main cause of death among these patients [1]. Despite early revascularization and circulatory support, the mortality rate remains around 50 % [2]. Cardiogenic shock is a state of inadequate tissue perfusion due to cardiac dysfunction, despite adequate left ventricular filling pressure. It is caused by extensive myocardial damage and appears to be aggravated by a systemic inflammatory response [3-6]. The result is hypotension with metabolic acidosis and often a fatal outcome.

Tissue plasminogen activator (t-PA) is a protease that initiates endogenous fibrinolysis in the vascular compartment via conversion of plasminogen to plasmin, and is important in controlling the coagulation process due to its thrombolytic properties. It is synthesized and stored in endothelial cells and vascular neurons [7,8], and is released in response to ischemia and other injurious stimuli [9-13]. Interestingly, t-PA has also been found to have pro-inflammatory properties that could contribute to tissue injury in ischemia-reperfusion injury [14,15].

Therapeutic hypothermia has been shown to offer tissue protection in myocardial ischemia-reperfusion injury [16,17]. In a porcine cardiogenic shock model, mild hypothermia has been demonstrated to reduce acute mortality and metabolic acidosis, and improve hemodynamic status [18]. In an experimental setting, mild hypothermia has a positive effect on contractility, but deep hypothermia has been associated with deterioration in cardiac function [19,20]. The tissue-protective mechanism of action is multi-factorial and partly unknown [20-27]. We recently found that hypothermia reduces ischemia related coronary t-PA release, suggesting a

possible role for t-PA as a mechanism for reperfusion injury that may be affected by hypothermia [28].

The primary objective of this study was to investigate the effect of therapeutic hypothermia in cardiogenic shock, on the basal levels of t-PA and on the release of t-PA from the peripheral vascular bed. The secondary objective was to investigate a possible effect on inflammation, as measured by the pro-inflammatory cytokines interleukin 6 (IL-6) and tumour necrosis factor alpha (TNF-α); and the anti-inflammatory cytokines interleukin 10 (IL-10) and transforming growth factor beta 1 (TGF-β1).

Methods

Experimental preparation

Healthy domestic male and female juvenile pigs weighing 40-50 kg were fasted overnight with free access to water. The animals were premedicated with Ketaminol (Ketamine, Intervet, Danderyd, Sweden), 100 mg/ml, 0.15 ml/kg, and Rompun (Xylazin, Bayer AG, Leverkusen, Germany), 20 mg/ml, 0.1 ml/kg intramuscularly 30 min before the procedure. After induction of anesthesia with thiopental 12.5 mg/kg (Pentothal, Abbott, Stockholm, Sweden) the animals were orally intubated with cuffed endotracheal tubes. A slow infusion of 1 ml/ml fentanyl (Fentanyl, Pharmalink AB, Stockholm, Sweden) in buffered glucose (25 mg/ml) was started at a rate of 2 ml/min and adjusted if needed. During balanced anaesthesia thiopental (Pentothal, Abbott, Stockholm, Sweden) was titrated towards animal requirements with small bolus doses. Mechanical ventilation was established with a Siemens-Elema 900B ventilator in a volume-controlled mode, adjusted in order to obtain normocapnia (PCO2: 5.0-6.0 kPa). The animals were ventilated with a mixture of nitrous oxide (70%) and oxygen

(30%). The pigs were continuously monitored by electrocardiography (ECG). Heparin (200 IU/kg) was given intravenously at the start of the catheterization. A 12 F introducer sheath (Boston Scientific Scimed, Maple Grove, MN, USA) was inserted into the surgically exposed left femoral vein. A 0.021-inch guide wire (Safe-T-J Curved™, Cook Medical Inc, Bloomington, IN, USA) was inserted into the proximal inferior vena cava through the introducer. Using the guide wire, a 10.7 F Celsius Control™ catheter (Innercool Therapies Inc, San Diego, CA, USA) was placed into the inferior vena cava with the tip of the catheter at the level of the diaphragm. Body temperature was measured with a temperature probe (TYCO Healthcare Norden AB, Solna, Sweden) placed in the distal part of the esophagus. The catheter and the temperature probe were connected to the Celsius Control and the system was set to maintain a normal pig body temperature of 38.0 °C. A 6 F introducer sheath (Boston Scientific Scimed, Maple Grove, MN, USA) was inserted into the surgically exposed left carotid artery upon which a 6 F FL4 Wiseguide™ (Boston Scientific Scimed, Maple Grove, MN, USA) was inserted into the left main coronary artery. The catheter was used to place a 0.014-inch PT Choice™ guide wire (Boston Scientific Scimed, Maple Grove, MN, USA) into the distal portion of the LAD. A 3.0-3.5 x 15 mm Maverick monorail™ angioplasty balloon (Boston Scientific Scimed, Maple Grove, MN, USA) was then positioned in the mid portion of the LAD, immediately distal to the first diagonal branch. A 9 F introducer sheath (Boston Scientific Scimed, Maple Grove, MN, USA) was inserted into the surgically exposed right jugular vein. A 7.5 F CCOmbo™ continuous cardiac output pulmonary artery catheter (Edwards Lifesciences, Irvine, CA, USA) was inserted into a pulmonary artery. Cardiac output was continuously recorded using a Vigilance™ monitor (Edwards Lifesciences, Irvine, CA, USA). Arterial blood pressure, pulmonary artery pressure, capillary wedge pressure and central venous pressure were continuously measured using separate transducers (ADInstruments Inc, Colorado Springs, CO, USA). Hemodynamic

parameters were digitally recorded using Chart v4.2 (ADInstruments Inc, Colorado Springs, CO, USA). All radiological procedures were performed using an Opescope Pleno™ imaging system (Shimadzu Corp., Kyoto, Japan).

Experimental protocol

After a stable core body temperature of 38.0° C was established, ischemia was induced by inflation of the angioplasty balloon in the proximal LAD for 40 min, followed by 110 minutes of reperfusion (Fig. 1a). An angiogram was performed after inflation of the balloon and before deflation of the balloon in order to verify total occlusion of the LAD and correct balloon positioning. After deflation of the balloon a subsequent angiogram was performed to verify restoration of blood flow in the previously occluded artery. Immediately before reperfusion the pigs were randomized to hypothermia (n=8) or to normothermia (n=8), (Fig. 1a). Hypothermia was induced and maintained by using the Celsius Control™ endovascular cooling system. After reaching the target temperature of 33°C, hypothermia was maintained throughout the experiment. In the normothermic group, the endovascular catheter was used to maintain a normal pig body temperature of 38°C. Pigs that died during ischemia or did not meet the criterion for cardiogenic shock (systolic blood pressure < 90 mm Hg during at least 15 minutes) before randomization were excluded from the study.

Plasma samples were collected from the carotid artery and the inferior vena cava for analysis of t-PA at baseline and every 30 minutes until the experiment ended. Venous plasma samples were collected for analysis of IL-6, IL-10, TNF-α and TGF-β1 at baseline and at the end of the experiment. Blood gases were analyzed every 30 minutes throughout the experiment in an automated bench top analyzer (Radiometer Medical ApS, Brønshsøj, Denmark). The blood

gas values were corrected for core body temperature at the time the samples were taken from the animals.

Biochemical analysis of t-PA and inflammatory markers

Plasma concentrations of t-PA were determined by commercial ELISA kits (TriniLIZE t-PA Antigen, Trinity Biotech, Bray, Ireland). All samples from one experiment were assayed in duplicate on the same microtest plate. The intra-assay variation coefficient was 2.7%. Levels of secreted TGF-β1, TNF-α, IL-6 and IL-10 were measured by quantitative sandwich enzyme-linked antibody immunoassay technique (R&D System Europe Ltd, Abingdon, UK). Optical density was determined on a microplate reader; Spectramax Plus 384 (Molecular Devices Corporation, Sunnyvale, CA, USA).

Calculation and statistics

The net release of t-PA from the peripheral vascular bed was calculated as the difference between arterial and venous samples. Total t-PA release was calculated by factoring net t-PA release against changes in cardiac output compared to baseline (and was measured in relative units consisting of (ng/ml – ng/ml) x (l/min / l/min)). The effects on inflammatory markers were calculated in proportion to baseline values. In order to test significance for hemodynamic, t-PA and blood gas variables, the mean value of the tested variable was calculated from the time of randomization (at reperfusion) until the end of experiment. The sample size was chosen in order to achieve a statistical power level of 0.8 for an intermediate effect size (>1.5, hence clinically relevant) on the measured parameters. Mann-Whitney's test was performed to test for differences in mean values. Linear regression was used to test for correlations. Regression analysis was performed post hoc, in an exploratory manner. Calculations and statistics were performed using the GraphPad Prism 4.0 software (GraphPad

Software Inc., La Jolla, CA, USA). Statistical significance was accepted when $p < 0.05$. Data are presented as mean ± SEM.

Ethics

The study conforms to the Guide for the Care and Use of Laboratory Animals, US National Institute of Health (NIH Publication No. 85-23, revised 1996) and was approved by the local animal research ethics committee.

Results

A total of 25 pigs were studied. Five pigs died prior to randomization due to intractable ventricular fibrillation or pulseless electrical activity and were excluded from the study. Four pigs were excluded due to lack of hypotension. Sixteen pigs were randomized into the two study-groups with eight animals in each group. One animal in the control group died in pulseless electrical activity 100 minutes from baseline.

Measurements of core body temperature are shown in figure 1b. At the time of initiation of ischemia and at reperfusion there was no difference in temperature between the groups. Hypothermia was induced at the time of reperfusion by the endovascular catheter. 45 minutes after initiation of hypothermia treatment, the mean temperature in the hypothermia group was 33.6 ± 0.7° C. The mean cooling rate was 5.9 ° C/h.

Detailed hemodynamic and blood-gas data are shown in figure 2 and table 1. Briefly, hypothermia resulted in an increased mean arterial pressure. Heart rate was decreased but stroke volume increased leading to a slightly increased cardiac output in the hypothermic

group. Mixed venous saturation, arterial pH and base excess were higher in the hypothermic group. All these differences were statistically significant.

Detailed information on t-PA is given in table 1 and figure 3. In brief, cardiogenic shock resulted in increased basal levels of venous and arterial t-PA, as well as in net- and total t-PA release (factorial correction for blood flow, see above). Hypothermia inhibited any increase in t-PA, both in basal levels and in net- and total release. All these differences were statistically significant.

There was a statistically significant inverse correlation between arterial baseline and end-experimental levels of t-PA; and mean arterial pressure, systemic vascular resistance, stroke volume, pH and mixed venous oxygen saturation. There was a strong trend towards statistical significance for an inverse correlation between arterial t-PA and cardiac output. A statistically significant positive correlation was observed for heart rate (Fig. 4). The results for arterial values were valid also for venous values, apart from cardiac output, heart rate (both clearly non-significant) and stroke volume (trend towards significance): mean arterial pressure (r sq=0.34, P<0.01), systemic vascular resistance (r sq=0.28, P<0.01), stroke volume (r sq=0.08, P=0.13), pH (r sq=0.36, P<0.01), mixed venous saturation (r sq=0.23, P<0.01), cardiac output (r sq=0.04, P=0.27) and heart rate (r sq=0.08, P=0.12). Subgroup analysis revealed that the correlations were dependent on observations in the normothermic group (table 2 and 3).

Cardiogenic shock triggered a statistically significant increase in TGF-β1 and IL-6 but not in TNF-∝ or IL-10 at 150 minutes (Fig. 5). However, there were no statistically significant differences between the hypothermic and normothermic groups in any of the measured cytokines; IL-6 (1758±534 vs 3184±1953 %baseline, hypothermia vs normothermia, NS), IL-

10 (98±41 vs 132±58 %baseline, hypothermia vs normothermia, NS), TNF-∝ (78±30 vs 77±24 %baseline, hypothermia vs normothermia, NS) and TGF-β1 (1063±418 vs 472±251 %baseline, hypothermia vs normothermia, NS) (Fig. 5).

Discussion

The main findings of this experiment were that cardiogenic shock resulted in increased basal levels of venous and arterial t-PA, as well as in net- and total t-PA release. Hypothermia inhibited any increase in t-PA. t-PA levels were found to correlate with both metabolic and hemodynamic parameters. Cardiogenic shock also induced an inflammatory response with increased levels of TGF-β1 and IL-6, but hypothermia did not affect this response. As reported earlier, hypothermia was found to favourably affect hemodynamic and metabolic variables [18].

The ability of hypothermia to reduce infarct size during ischemia has been demonstrated in several experimental studies [16,29-32]. However, induction of hypothermia after the onset of reperfusion does not reduce infarct size [32,16]. In order to avoid possible bias from a reduction in infarct size, hypothermia was induced after the onset of reperfusion.

Previous experimental studies have demonstrated that mild hypothermia increases myocardial contractility in excised heart preparations as well as in the in situ heart [33,34,20]. The increase in contractility is considered to be mediated by an increased myofilament sensitivity to existing Ca^{2+} [20], without a corresponding increase in myocardial oxygen consumption [34,33]. A decrease in heart rate, combined with a larger stroke volume and an increased cardiac output has also been reported previously [20]. This is in line with the observations in the current experiment. Hypothermia has also been demonstrated to reduce the metabolic

demand of the cells [22,35,36,23]. In the current experiment, hypothermia treatment resulted in a significantly higher mixed venous saturation, pH and base excess. An explanation to the observed results could be that hypothermia lowered the peripheral oxygen demand, resulting in less tissue hypoxia and no development of metabolic acidosis. The hemodynamic and metabolic effects of hypothermia in cardiogenic shock are further discussed separately [18].

Reduced oxygen demand does not fully explain the positive effects of hypothermia and several additive tissue protective effects have been suggested [24]. It has been shown that mild hypothermia can prevent ischemic cells from entering apoptosis through prevention of mitochondrial dysfunction and inhibition of caspase release [25-27]. Moreover, hypothermia has been shown to improve ion homeostasis, decrease free radical formation, stabilize cellular membranes and prevent intracellular acidosis [21]. It has also been shown to reduce ischemia related coronary t-PA release [28]. In the current experiment hypothermia was found to inhibit a shock-related increase in basal arterial and venous levels of t-PA and also the release of t-PA from the peripheral vascular bed.

The release of t-PA is triggered by various injurious stimuli including ischemia, and is mediated by a variety of substances including thrombin, ADP, serotonin, bradykinin, and epinephrine [9,12,13,10,11,37-41]. It is possible that the release of t-PA was triggered by the shock related metabolic compromise. This hypothesis is supported by the observed inverse correlations between pH, base excess, mixed venous saturation and t-PA. However, even though the hypothermic animals were less metabolically compromised, they were still in shock and not metabolically unaffected. As hypothermia completely abolished t-PA release above basal levels, a more selective effect on the endothelial t-PA release is indicated. This

hypothesis is further supported by the fact that significant correlations between t-PA and metabolic parameters were confined to the normothermic group.

Although t-PA is important in keeping vessel walls free from thrombi under physiologic circumstances, it has been found to have proinflammatory properties that could contribute to reperfusion injury [15,14,42,43]. In this experiment, cardiogenic shock was found to increase the anti-inflammatory TGF-β1 and pro-inflammatory IL-6, to a similar degree in both groups. The effect of shock on the pro-inflammatory TNF-∝ and anti-inflammatory IL-10 was neutral in both groups. Thus, a possible positive effect of t-PA on hemodynamic parameters does not involve these cytokines. However, t-PA has been reported to activate inducible nitric oxide synthase (iNOS) [44], which acts to increase the levels of nitric oxide (NO). In turn, NO has been associated with a reduction in myocardial contractility by uncoupling of calcium metabolism [45,46], through effects on glucose metabolism [46], and through a reduction in β-adrenergic responsiveness [47]. Furthermore, NO induce vasodilatation, thereby decreasing coronary perfusion pressure and systemic perfusion [3]. Mild hypothermia has been demonstrated to reduce iNOS [48,49]. In the current experiment, there was an inverse correlation between t-PA and systemic vascular resistance, mean arterial pressure and stroke volume. Possibly, the reduction in t-PA is a contributing link between hypothermia and improved hemodynamic status, via reduced iNOS levels. As judged by the analysis of correlations between t-PA and hemodynamic parameters, the effect attributable to such a mechanism appear to be mild to moderate in magnitude. Further research is warranted to test this hypothesis.

Limitations

In an experimental model with hemodynamic compromise and temperature related influence on the circulation, changes in hepatic blood flow may arise. By sampling venous blood in the inferior vena cava, there may be influence of changes in hepatic clearance on venous t-PA levels.

Conclusion

Mild hypothermia improves hemodynamic and metabolic parameters in cardiogenic shock. This is associated with a reduction in basal t-PA levels and t-PA release from the peripheral vascular bed, but not with an altered inflammatory response as measured by IL-6, IL-10, TNF-α and TGF-β1.

Acknowledgements

We would like to thank Boston Scientific Cardiology, Nordic AB (Helsingborg, Sweden) for their generosity in unrestricted donations of catheters and guide wires for use in animal research and Innercool therapies Inc, San Diego, CA, USA for unrestricted loan of the Celsius Control™ cooling console.

Competing interests

All authors declare that they have no competing interests.

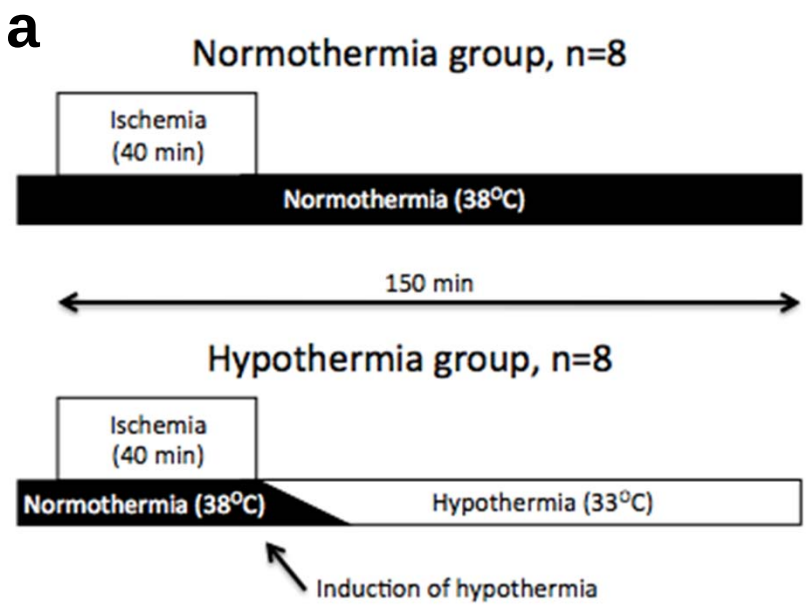
References

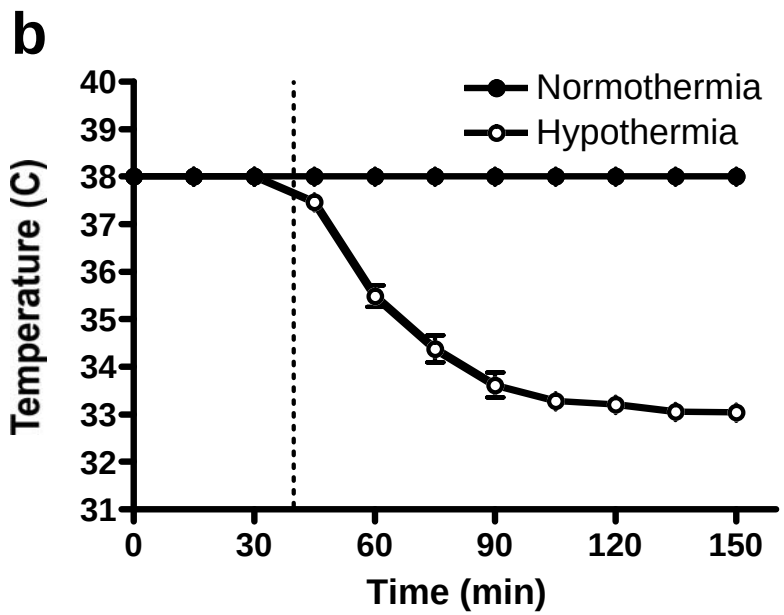
1. Holmes DR, Jr., Bates ER, Kleiman NS, Sadowski Z, Horgan JH, Morris DC, Califf RM, Berger PB, Topol EJ (1995) Contemporary reperfusion therapy for cardiogenic shock: The GUSTO-I trial experience. The GUSTO-I investigators. Global utilization of streptokinase and tissue plasminogen activator for occluded coronary arteries. J Am Coll Cardiol 26:668-674.

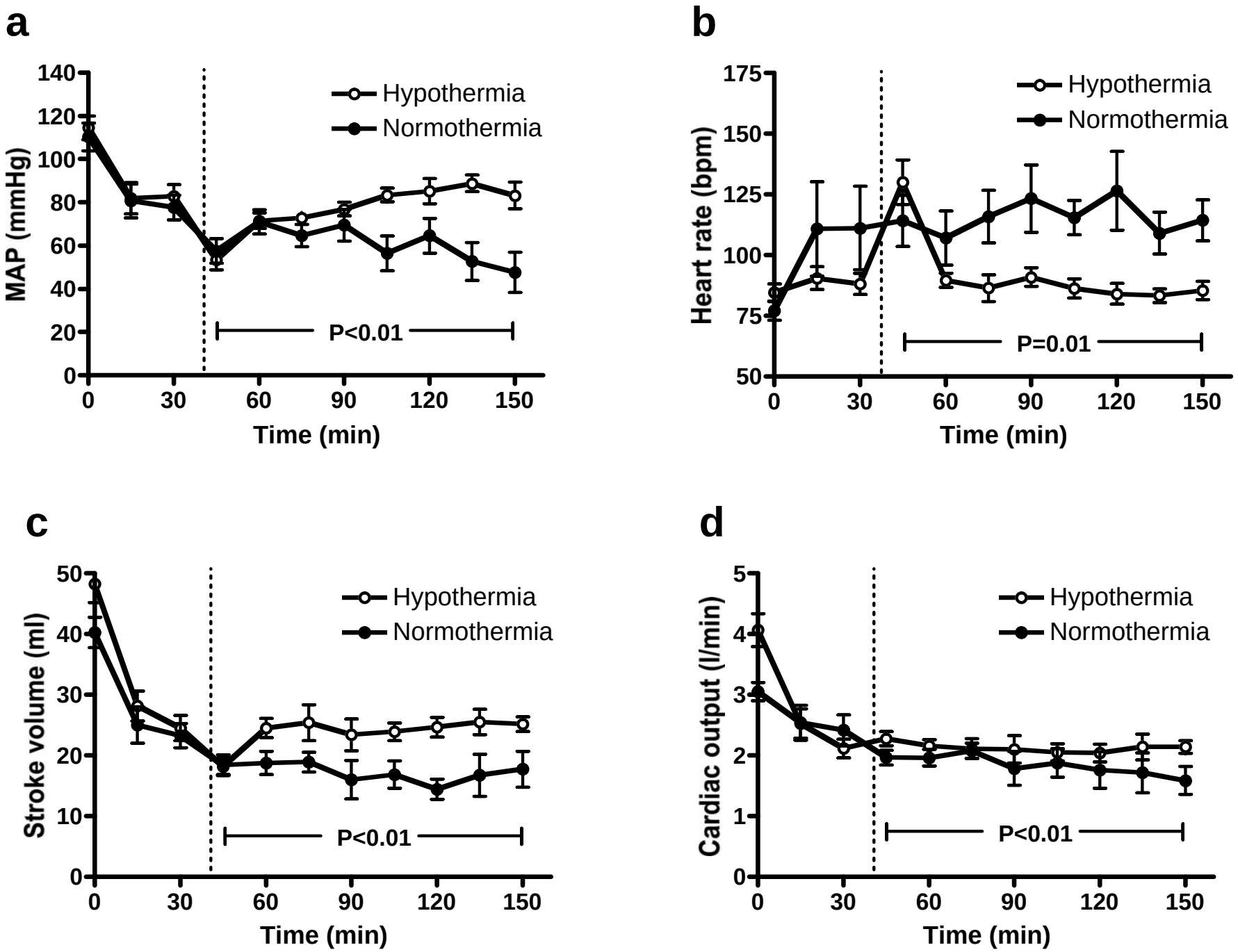
2. Hochman JS, Sleeper LA, Webb JG, Sanborn TA, White HD, Talley JD, Buller CE, Jacobs AK, Slater JN, Col J, McKinlay SM, LeJemtel TH (1999) Early revascularization in acute myocardial infarction complicated by cardiogenic shock. SHOCK investigators. Should we emergently revascularize occluded coronaries for cardiogenic shock. N Engl J Med 341:625-634.
3. Hochman JS (2003) Cardiogenic shock complicating acute myocardial infarction: Expanding the paradigm. Circulation 107:2998-3002.
4. Kaluski E, Hendler A, Blatt A, Uriel N (2006) Nitric oxide synthase inhibitors in post-myocardial infarction cardiogenic shock -- an update. Clin Cardiol 29:482-488.
5. Cotter G, Berger PB (2006) Cardiogenic shock -- beyond the large infarction. Crit Care Med 34:2234-2235.
6. Geppert A, Dorninger A, Delle-Karth G, Zorn G, Heinz G, Huber K (2006) Plasma concentrations of interleukin-6, organ failure, vasopressor support, and successful coronary revascularization in predicting 30-day mortality of patients with cardiogenic shock complicating acute myocardial infarction. Crit Care Med 34:2035-2042.
7. van Hinsbergh VW, Binnema D, Scheffer MA, Sprengers ED, Kooistra T, Rijken DC (1987) Production of plasminogen activators and inhibitor by serially propagated endothelial cells from adult human blood vessels. Arteriosclerosis 7:389-400.
8. Wang Y, Hand AR, Wang YH, Mina M, Gillies C, Peng T, Cone RE, O'Rourke J (1998) Functional and morphologic evidence of the presence of tissue-plasminogen activator in vascular nerves: Implications for a neurologic control of vessel wall fibrinolysis and rigidity. J Neurosci Res 53:443-453.
9. Bjorkman JA, Jern S, Jern C (2003) Cardiac sympathetic nerve stimulation triggers coronary t-PA release. Arterioscl, Thromb and Vasc Biol 23:1091-1097.
10. Osterlund B, Andersson B, Haggmark S, Jern C, Johansson G, Seeman-Lodding H, Biber B (2002) Myocardial ischemia induces coronary t-PA release in the pig. Acta Anaest Scand 46:271-278.
11. Osterlund B, Jern C, Seeman-Lodding H, Johansson G, Haggmark S, Broome M, Biber B (2003) Intracoronary beta2 receptor activation induces dynamic local t-PA release in the pig. Thromb and Haemost 90:796-802.
12. Hrafnkelsdottir T, Erlinge D, Jern S (2001) Extracellular nucleotides ATP and UTP induce a marked acute release of tissue-type plasminogen activator in vivo in man. Thromb and Haemost 85:875-881.
13. Hrafnkelsdottir T, Gudnason T, Wall U, Jern C, Jern S (2004) Regulation of local availability of active tissue-type plasminogen activator in vivo in man. J Thromb Haemost 2:1960-1968.
14. Strbian D, Karjalainen-Lindsberg ML, Kovanen PT, Tatlisumak T, Lindsberg PJ (2007) Mast cell stabilization reduces hemorrhage formation and mortality after administration of thrombolytics in experimental ischemic stroke. Circulation 116:411-418.
15. Hermann DM, Matter CM (2007) Tissue plasminogen activator-induced reperfusion injury after stroke revisited. Circulation 116:363-365.
16. Gotberg M, Olivecrona GK, Engblom H, Ugander M, van der Pals J, Heiberg E, Arheden H, Erlinge D (2008) Rapid short-duration hypothermia with cold saline and endovascular cooling before reperfusion reduces microvascular obstruction and myocardial infarct size. BMC Cardiovasc Disord 8:7.
17. Gotberg M, Olivecrona GK, Koul S, Carlsson M, Engblom H, Ugander M, van der Pals J, Algotsson L, Arheden H, Erlinge D (2010) A pilot study of rapid cooling by cold saline and endovascular cooling before reperfusion in patients with st-elevation myocardial infarction. Circ Cardiovasc Interv 3:400-407.

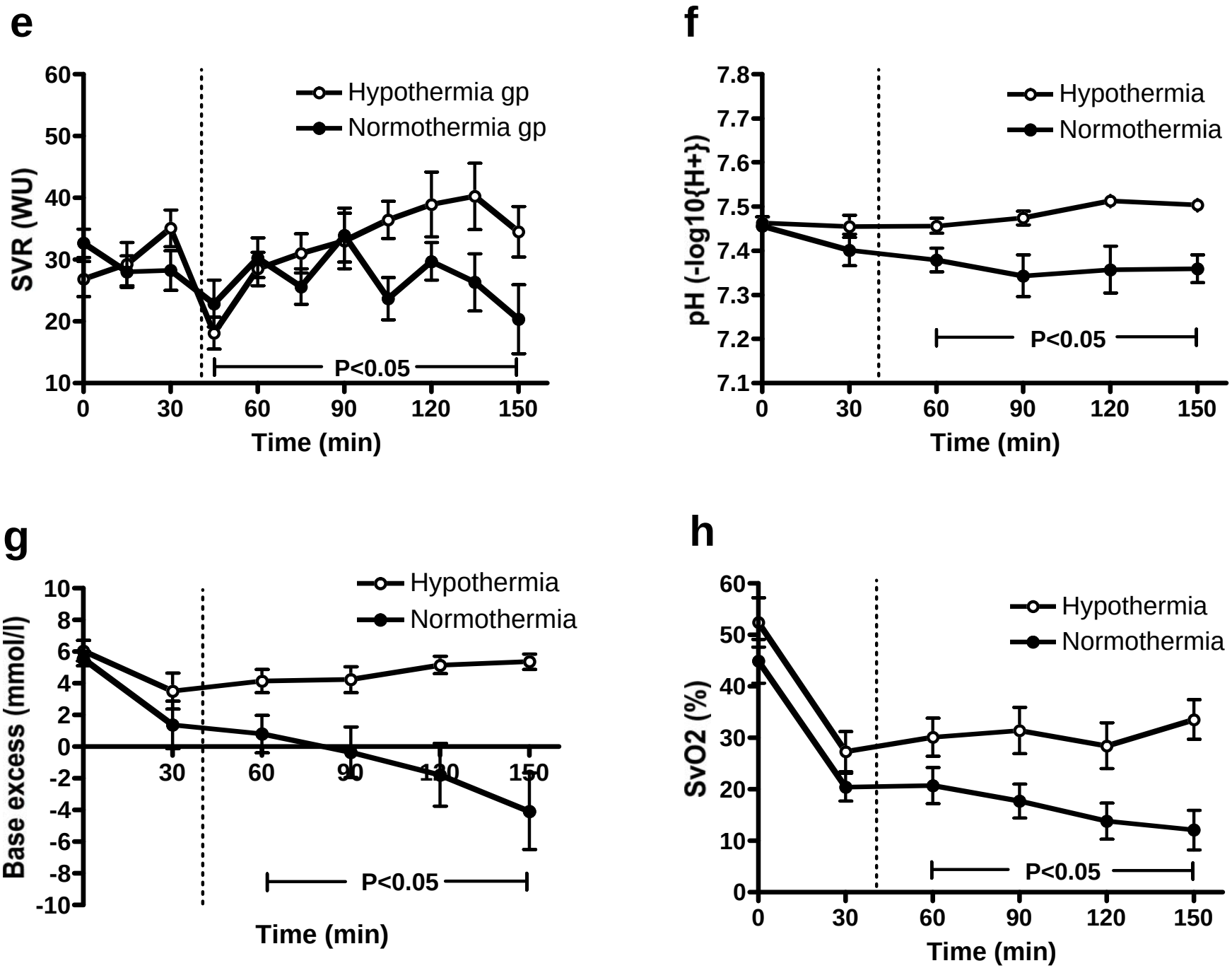
18. Gotberg M, van der Pals J, Olivecrona GK, Koul S, Erlinge D (2010) Mild hypothermia reduces acute mortality and improves hemodynamic outcome in a cardiogenic shock pig model. Resuscitation 81:1190-1196.
19. Lauri T, Leskinen M, Timisjarvi J (1997) Effects of surface-induced hypothermia and rewarming on canine cardiac contraction-relaxation cycle. Int J Circumpolar Health 56:40-48
20. Weisser J, Martin J, Bisping E, Maier LS, Beyersdorf F, Hasenfuss G, Pieske B (2001) Influence of mild hypothermia on myocardial contractility and circulatory function. Basic Res Cardiol 96:198-205.
21. Polderman KH (2009) Mechanisms of action, physiological effects, and complications of hypothermia. Crit Care Med 37 (7 Suppl):S186-202.
22. Ohta S, Yukioka T, Wada T, Miyagatani Y, Matsuda H, Shimazaki S (1995) Effect of mild hypothermia on the coefficient of oxygen delivery in hypoxemic dogs. J Appl Physiol 78:2095-2099.
23. Badeer H (1956) Effect of hypothermia on oxygen consumption and energy utilization of heart. Circulation Research 4:523-526.
24. Milde LN (1992) Clinical use of mild hypothermia for brain protection: A dream revisited. J Neurosur Anest 4:211-215.
25. Xu L, Yenari MA, Steinberg GK, Giffard RG (2002) Mild hypothermia reduces apoptosis of mouse neurons in vitro early in the cascade. J Cereb Blood Flow Metab 22:21-28.
26. Adachi M, Sohma O, Tsuneishi S, Takada S, Nakamura H (2001) Combination effect of systemic hypothermia and caspase inhibitor administration against hypoxic-ischemic brain damage in neonatal rats. Pediatric Research 50:590-595.
27. Ning XH, Chen SH, Xu CS, Li L, Yao LY, Qian K, Krueger JJ, Hyyti OM, Portman MA (2002) Hypothermic protection of the ischemic heart via alterations in apoptotic pathways as assessed by gene array analysis. J Appl Physiol 92:2200-2207.
28. van der Pals J, Gotberg M, Olivecrona GK, Brogren H, Jern S, Erlinge D (2010) Mild hypothermia markedly reduces ischemia related coronary t-PA release. J Thromb Thrombolys 29:289-295.
29. Dae MW, Gao DW, Sessler DI, Chair K, Stillson CA (2002) Effect of endovascular cooling on myocardial temperature, infarct size, and cardiac output in human-sized pigs. Am J Physiol 282:H1584-1591
30. Duncker DJ, Klassen CL, Ishibashi Y, Herrlinger SH, Pavek TJ, Bache RJ (1996) Effect of temperature on myocardial infarction in swine. Am J Physiol 270 (4 Pt 2):H1189-1199.
31. Hale SL, Dave RH, Kloner RA (1997) Regional hypothermia reduces myocardial necrosis even when instituted after the onset of ischemia. Basic Res Cardiol 92:351-357.
32. Maeng M, Mortensen UM, Kristensen J, Kristiansen SB, Andersen HR (2006) Hypothermia during reperfusion does not reduce myocardial infarct size in pigs. Basic Res Cardiol 101:61-68.
33. Nishimura Y, Naito Y, Nishioka T, Okamura Y (2005) The effects of cardiac cooling under surface-induced hypothermia on the cardiac function in the in situ heart. Interact Cardiovasc Thorac Surg 4:101-105.
34. Suga H, Goto Y, Igarashi Y, Yasumura Y, Nozawa T, Futaki S, Tanaka N (1988) Cardiac cooling increases emax without affecting relation between O2 consumption and systolic pressure-volume area in dog left ventricle. Circ Res 63:61-71.
35. Gerola A, Feinberg H, Katz LN (1959) Myocardial oxygen consumption and coronary blood flow in hypothermia. Am J Physiol 196:719-725.
36. Edwards WS, Tuluy S, Reber WE, Siegel A, Bing RJ (1954) Coronary blood flow and myocardial metabolism in hypothermia. Ann Surg 139:275-281.

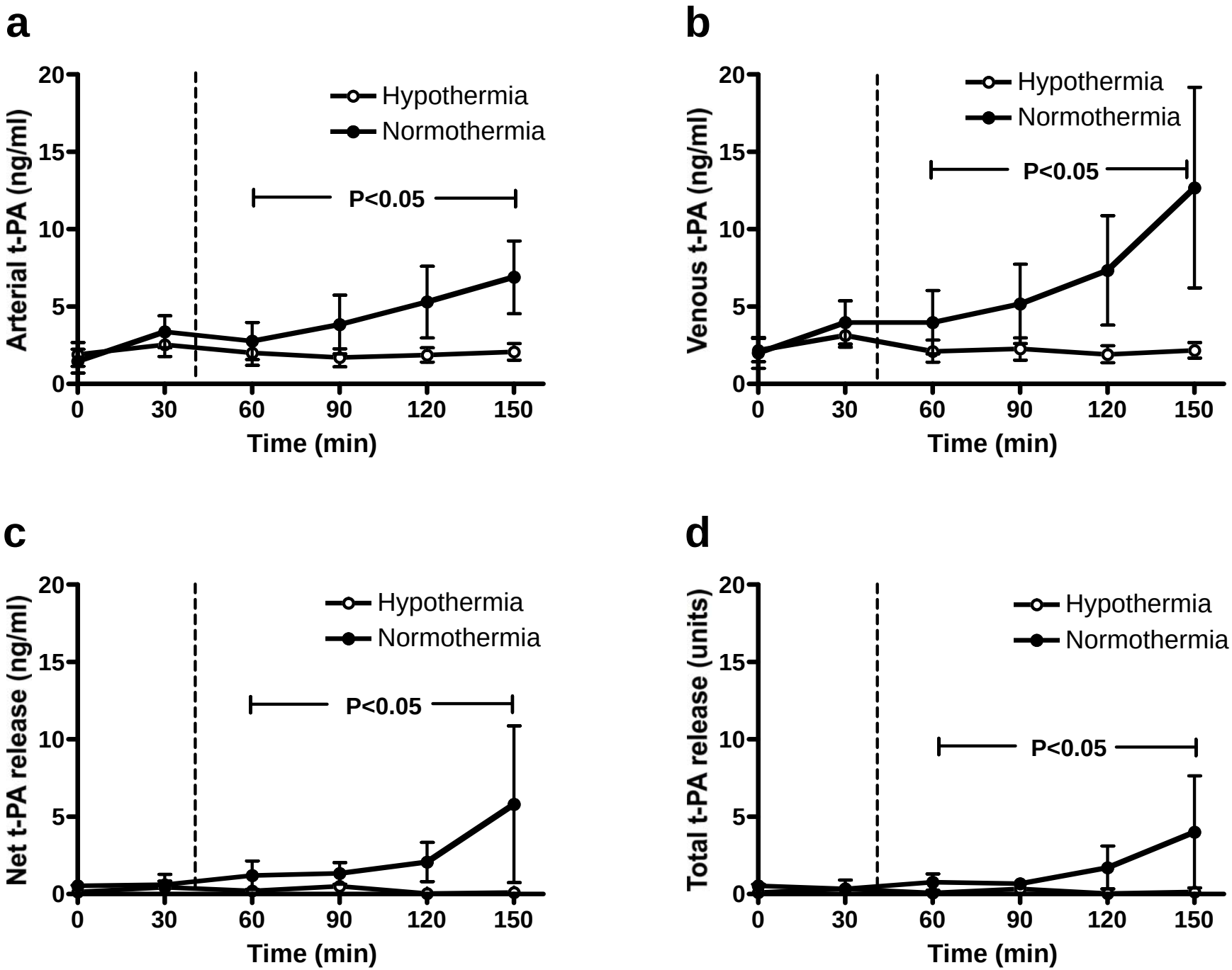
37. Smalley DM, Fitzgerald JE, O'Rourke J (1993) Adenosine diphosphate stimulates the endothelial release of tissue-type plasminogen activator but not von Willebrand factor from isolated-perfused rat hind limbs. Thromb Haemost 70:1043-1046.
38. Tranquille N, Emeis JJ (1990) The simultaneous acute release of tissue-type plasminogen activator and von Willebrand factor in the perfused rat hindleg region. Thromb Haemost 63:454-458.
39. Witherow FN, Dawson P, Ludlam CA, Fox KA, Newby DE (2002) Marked bradykinin-induced tissue plasminogen activator release in patients with heart failure maintained on long-term angiotensin-converting enzyme inhibitor therapy. J Am Coll Cardiol 40:961-966.
40. Stein CM, Brown N, Vaughan DE, Lang CC, Wood AJ (1998) Regulation of local tissue-type plasminogen activator release by endothelium-dependent and endothelium-independent agonists in human vasculature. J Am Coll Cardiol 32:117-122.
41. Yamamoto C, Kaji T, Sakamoto M, Kozuka H, Koizumi F (1994) Calcium regulation of tissue plasminogen activator and plasminogen activator inhibitor-1 release from cultured human vascular endothelial cells. Thromb Res 74:163-168.
42. Kilic E, Kilic U, Matter CM, Luscher TF, Bassetti CL, Hermann DM (2005) Aggravation of focal cerebral ischemia by tissue plasminogen activator is reversed by 3-hydroxy-3-methylglutaryl coenzyme a reductase inhibitor but does not depend on endothelial no synthase. Stroke 36:332-336.
43. Wang YF, Tsirka SE, Strickland S, Stieg PE, Soriano SG, Lipton SA (1998) Tissue plasminogen activator (tPA) increases neuronal damage after focal cerebral ischemia in wild-type and tPA-deficient mice. Nature medicine 4:228-231.
44. Kilic E, Kilic U, Reiter RJ, Bassetti CL, Hermann DM (2005) Tissue-plasminogen activator-induced ischemic brain injury is reversed by melatonin: Role of inos and akt. J Pineal Res 39:151-155.
45. Schulz R, Wambolt R (1995) Inhibition of nitric oxide synthesis protects the isolated working rabbit heart from ischaemia-reperfusion injury. Cardiovasc Res 30:432-439.
46. Depre C, Vanoverschelde JL, Goudemant JF, Mottet I, Hue L (1995) Protection against ischemic injury by nonvasoactive concentrations of nitric oxide synthase inhibitors in the perfused rabbit heart. Circulation 92:1911-1918.
47. Ziolo MT, Katoh H, Bers DM (2001) Expression of inducible nitric oxide synthase depresses beta-adrenergic-stimulated calcium release from the sarcoplasmic reticulum in intact ventricular myocytes. Circulation 104:2961-2966.
48. Scumpia PO, Sarcia PJ, Kelly KM, DeMarco VG, Skimming JW (2004) Hypothermia induces anti-inflammatory cytokines and inhibits nitric oxide and myeloperoxidase-mediated damage in the hearts of endotoxemic rats. Chest 125:1483-1491.
49. Hassoun HT, Kozar RA, Kone BC, Safi HJ, Moore FA (2002) Intraischemic hypothermia differentially modulates oxidative stress proteins during mesenteric ischemia/reperfusion. Surgery 132:369-376.

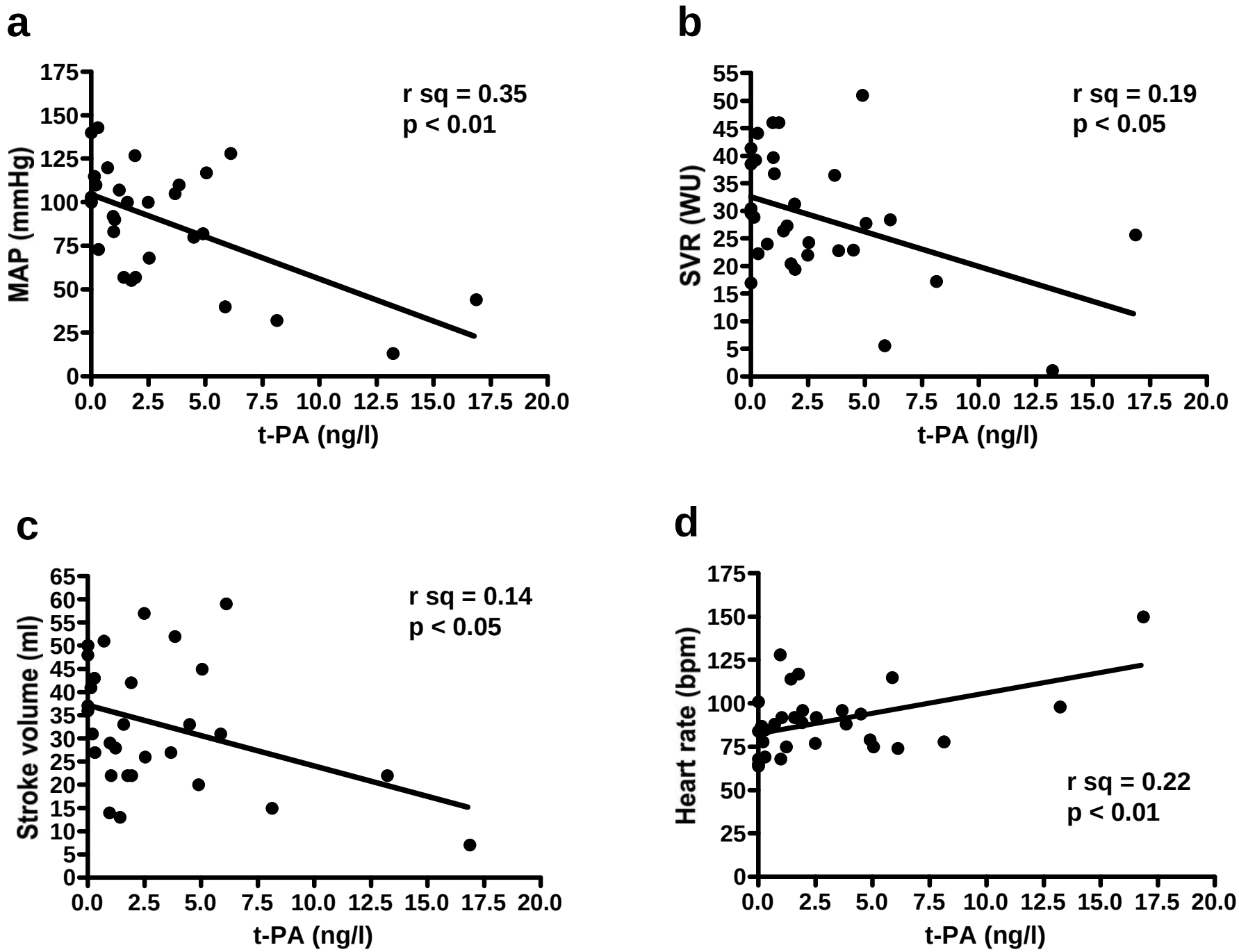

a
Normothermia group, n=8
Ischemia
(40 min)
Normothermia (38°C)
150 min
Hypothermia group, n=8
Ischemia
(40 min)
Normothermia (38°C)
Hypothermia (33°C)
Induction of hypothermia

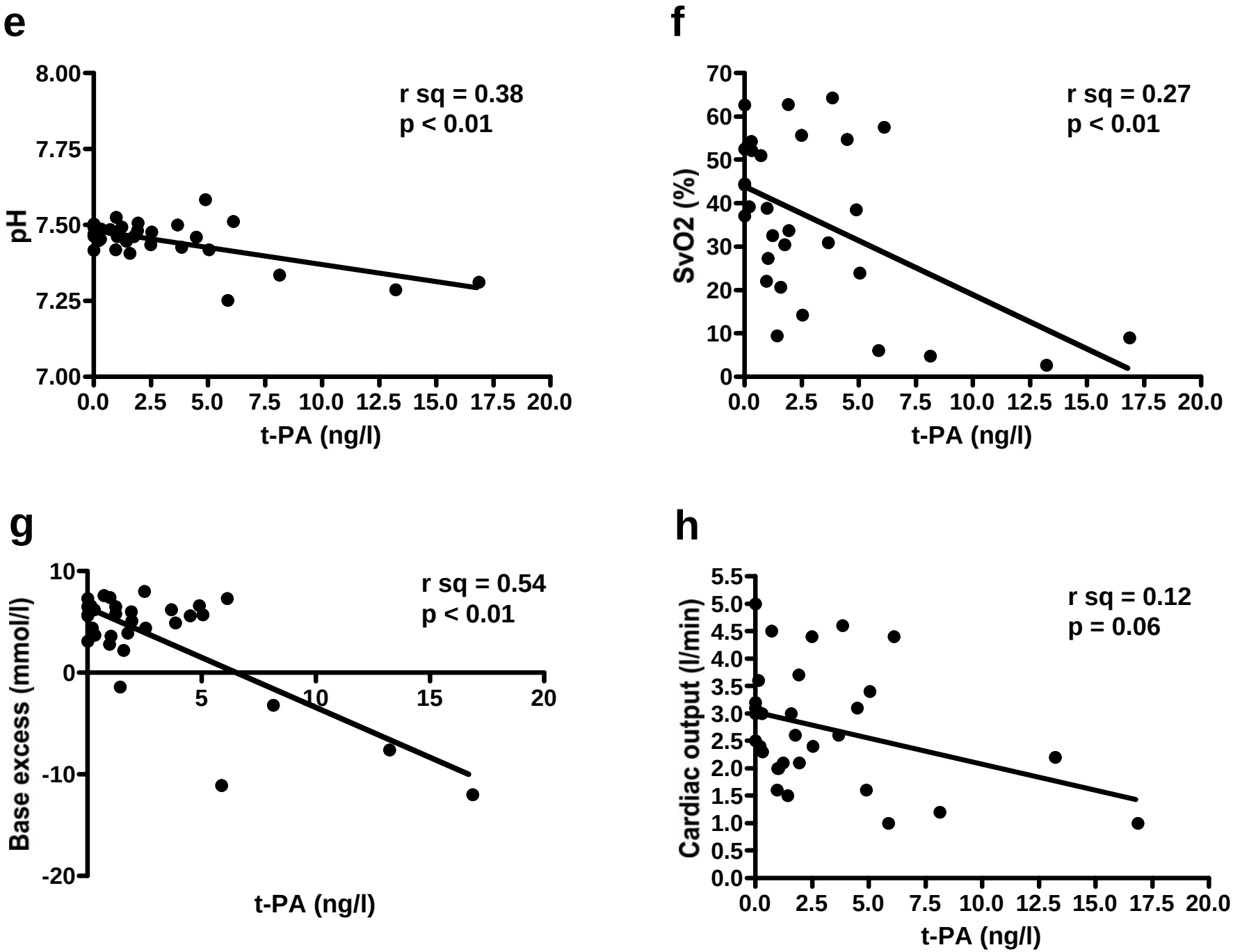

b
Normothermia
Hypothermia
Temperature (C)
40
39
38
37
36
35
34
33
32
31
0
30
60
90
120
150
Time (min)

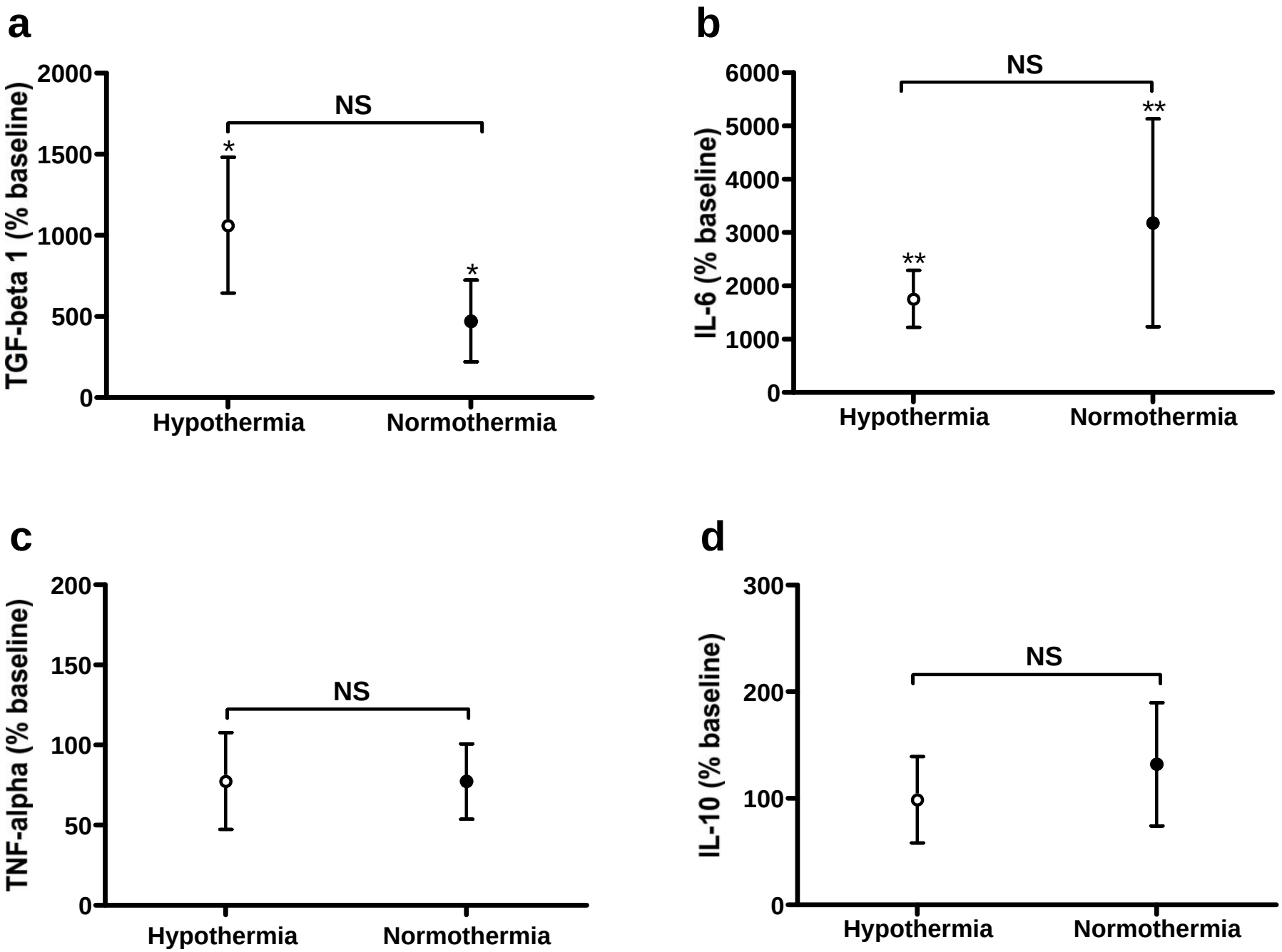

a
MAP (mmHg)
140
120
100
80
60
40
20
0
0
30
60
90
120
150
Time (min)
Hypothermia
Normothermia
P<0.01
b
Heart rate (bpm)
175
150
125
100
75
50
0
30
60
90
120
150
Time (min)
Hypothermia
Normothermia
P=0.01
c
Stroke volume (ml)
50
40
30
20
10
0
0
30
60
90
120
150
Time (min)
Hypothermia
Normothermia
P<0.01
d
Cardiac output (l/min)
5
4
3
2
1
0
0
30
60
90
120
150
Time (min)
Hypothermia
Normothermia
P<0.01


e
SVR (WU)
Time (min)
Hypothermia gp
Normothermia gp
P<0.05
f
pH (-log10{H+})
Time (min)
Hypothermia
Normothermia
P<0.05
g
Base excess (mmol/l)
Time (min)
Hypothermia
Normothermia
P<0.05
h
SvO2 (%)
Time (min)
Hypothermia
Normothermia
P<0.05


a
Arterial t-PA (ng/ml)
Hypothermia
Normothermia
P<0.05
Time (min)
b
Venous t-PA (ng/ml)
Hypothermia
Normothermia
P<0.05
Time (min)
c
Net t-PA release (ng/ml)
Hypothermia
Normothermia
P<0.05
Time (min)
d
Total t-PA release (units)
Hypothermia
Normothermia
P<0.05
Time (min)


a
MAP (mmHg)
175
150
125
100
75
50
25
0
r sq = 0.35
p < 0.01
0.0 2.5 5.0 7.5 10.0 12.5 15.0 17.5 20.0
t-PA (ng/l)
b
SVR (WU)
55
50
45
40
35
30
25
20
15
10
5
0
r sq = 0.19
p < 0.05
0.0 2.5 5.0 7.5 10.0 12.5 15.0 17.5 20.0
t-PA (ng/l)
c
Stroke volume (ml)
65
60
55
50
45
40
35
30
25
20
15
10
5
0
r sq = 0.14
p < 0.05
0.0 2.5 5.0 7.5 10.0 12.5 15.0 17.5 20.0
t-PA (ng/l)
d
Heart rate (bpm)
175
150
125
100
75
50
25
0
r sq = 0.22
p < 0.01
0.0 2.5 5.0 7.5 10.0 12.5 15.0 17.5 20.0
t-PA (ng/l)


e
8.00
7.75
7.50
7.25
7.00
pH
r sq = 0.38
p < 0.01
0.0 2.5 5.0 7.5 10.0 12.5 15.0 17.5 20.0
t-PA (ng/l)
f
70
60
50
40
30
20
10
0
SvO2 (%)
r sq = 0.27
p < 0.01
0.0 2.5 5.0 7.5 10.0 12.5 15.0 17.5 20.0
t-PA (ng/l)
g
10
0
-10
-20
Base excess (mmol/l)
r sq = 0.54
p < 0.01
5 10 15 20
t-PA (ng/l)
h
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Cardiac output (l/min)
r sq = 0.12
p = 0.06
0.0 2.5 5.0 7.5 10.0 12.5 15.0 17.5 20.0
t-PA (ng/l)


a
TGF-beta 1 (% baseline)
2000
1500
1000
500
0
NS
*
*
Hypothermia
Normothermia
b
IL-6 (% baseline)
6000
5000
4000
3000
2000
1000
0
NS
**
**
Hypothermia
Normothermia
c
TNF-alpha (% baseline)
200
150
100
50
0
NS
Hypothermia
Normothermia
d
IL-10 (% baseline)
300
200
100
0
NS
Hypothermia
Normothermia