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+46 46-222 00 00

Hypothermia as an adjunctive therapy in Acute Myocardial Infarction and Cardiogenic Shock

MATTHIAS GÖTBERG, MD

LUND UNIVERSITY
Faculty of Medicine

Doctoral Thesis
2010

Department of Cardiology
Lund University, Sweden

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“Whether you think you can or whether you think you can’t, you’re right”

Henry Ford

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1 List of Publications

This thesis is based on the following papers, which will be referred to by their Roman numerals in the text.

I. Götberg M, Olivecrona GK, Engblom H, Ugander M, van der Pals J, Heiberg E, Arheden H, Erlinge D. Rapid short-duration hypothermia with cold saline and endovascular cooling before reperfusion reduces microvascular obstruction and myocardial infarct size. *BMC Cardiovasc Disord*. 2008 Apr 10;8:7.

II. Götberg M, van der Pals J, Götberg M, Olivecrona GK, Kanski M, Koul S, Otto A, Engblom H, Ugander M, Arheden H, Erlinge D. Timing of hypothermia in relation to myocardial reperfusion. *Submitted*

III. Götberg M, van der Pals J, Götberg M, Olivecrona GK, Götberg M, Koul S, Erlinge D. Mild Hypothermia Reduces Acute Mortality and Improves Hemodynamic Outcome in a Cardiogenic Shock Pig Model. *Resuscitation.* 2010 Jun 1. Epub ahead of print

IV. Götberg M, Olivecrona GK Koul S, Carlsson M, Engblom H, Ugander M, van der Pals J, Algotsson L, Arheden H, Erlinge D. A pilot study of rapid cooling by cold saline and endovascular cooling before reperfusion in patients with ST-elevation myocardial infarction. *Circulation Cardiovascular Interventions. In press*

3 Abbreviations

AAR	Area At Risk
AMI	Acute Myocardial Infarction
CMR	Cardiac Magnetic Resonance
CS	Cardiogenic Shock
ECG	Electrocardiography
IS	Infarct Size
LAD	Left Anterior Descending Artery
LV	Left Ventricle
MaR	Myocardium at Risk
MRI	Magnetic Resonance Imaging
MO	Microvascular Obstruction
PCI	Percutaneous Coronary Intervention
SPECT	Single Photon Emission Computed Tomography
STEMI	ST-segment Elevation Myocardial Infarction

4 Abstract

INTRODUCTION: Reperfusion therapy in patients with an ongoing ST-elevation myocardial infarction (STEMI) is to re-establish coronary flow in the epicardial arteries as soon as possible in order to reduce infarct size and associated complications. Hypothermia has in experimental studies been shown to reduce infarct size. Clinical trials, however, have not been able to show this effect, possibly because a therapeutic temperature was not reached before reperfusion in the majority of the patients allocated to hypothermia treatment. We aimed to evaluate if hypothermia initiated before reperfusion would reduce infarct size. Furthermore, a protocol utilising a combination of an infusion of cold saline and endovascular cooling catheter was evaluated. Finally, the effects of hypothermia in cardiogenic shock were investigated.

MATERIAL and METHODS: For **paper I-III**, an experimental closed chest porcine model was used. Ischemia was induced by occlusion of the LAD using a PCI-balloon. Different hypothermia protocols using cold saline, endovascular cooling in combination or alone were tested. Infarct size and microvascular obstruction were evaluated using *ex-vivo* MRI. **In paper III**, endovascular cooling alone was investigated in a porcine model of cardiogenic shock. In **paper IV**, the safety and feasibility of the hypothermia protocol utilised in **paper I, II** was tested in a clinical trial in patients with STEMI.

RESULTS: **Paper I**: Combination hypothermia (a combination of an infusion of cold saline and endovascular cooling catheter), if initiated before reperfusion reduced infarct size by 39%, and abolished microvascular obstruction compared to normothermia. Furthermore, the hypothermia protocol achieved a reduction in core body temperature to < 35°C in <10 min. However, hypothermia induced at the onset of reperfusion reduced microvascular obstruction by 66%, but did not affect infarct size. In **Paper II**, combination hypothermia reduced infarct size by 18% and microvascular obstruction was virtually abolished despite prolonged ischemic time compared to normothermia. Furthermore, an infusion of cold saline alone did not reduce infarct size, but reduced microvascular obstruction by 74%. Prolonged post-reperfusion hypothermia did not offer any additional. In **Paper III**, endovascular hypothermia improved survival (8/8 vs. 3/8, hypothermia vs. control), improved hemodynamic parameters, and reduced acidosis in cardiogenic shock. **Paper IV:** Combination hypothermia in patients with STEMI was able to safely reach a core body temperature of < 35°C before reperfusion without delaying primary PCI, and resulted in a 38% reduction in infarct size.

CONCLUSIONS: In order for hypothermia treatment to reduce infarct size, it needs to be initiated before reperfusion. The results indicate that it is safe and clinically feasible to induce hypothermia by using a combination of cold saline infusion and endovascular cooling prior to reperfusion in awake STEMI patients without delaying time to reperfusion. Furthermore, hypothermia improves outcome in cardiogenic shock. Larger randomized clinical trials are needed to verify these findings and to assess possible long term clinical benefit for the patients.

5 Introduction

Acute myocardial infarction

Acute myocardial infarction (AMI) is the leading cause of mortality in the industrialized world today.[1] Modern therapy is aimed at opening the occluded coronary vessel and establishing reperfusion of the ischemic myocardium as soon as possible in order to reduce infarct size and associated complications. Infarct size is one of the main predictors for mortality in patients with acute myocardial infarction.[2-5] Despite modern reperfusion therapy, many patients suffer from extensive myocardial damage due to the infarct and die prematurely due to secondary complications such as congestive heart failure or arrhythmias. Therapies aimed at limiting infarct size are therefore an important objective of contemporary research.

Pathophysiology

The common cause of AMI is a rupture of an atherosclerotic plaque with platelet activation and aggregation, thrombin formation with formation of a thrombus causing a partial or total occlusion of the coronary artery.[6-13] Less common causes of an acute coronary occlusion involve spasm, spontaneous dissection or embolization.[14, 15] If the blood flow to a region of the heart becomes interrupted, it will be deprived of oxygen and nutrients (ischemia). If this interruption of blood flow is not quickly restored, myocardial necrosis (cell death) will occur within the ischemic area. If the occlusion is partial, the result is usually a Non-ST-segment Elevation Myocardial Infarction (NSTEMI), with limited myocardial damage. If the occlusion is total, a ST-segment Elevation Myocardial Infarction (STEMI) with extensive ischemia will occur within the region of the heart supplied by the occluded coronary artery. 20-30 min after onset of ischemia, an infarct starts to develop in the subendocardial tissue. If the occlusion persists, the infarct usually spreads in a wave-front manner towards the epicardium.[16, 17] Restoration of blood flow to the ischemic myocardium as soon as possible is paramount in limiting the infarct size and related complications.[18-20 21]

Therapy

In the 1980's, two pivotal trials showed that pharmacological reperfusion (thrombolytic therapy) aimed at dissolving the coronary thrombus reduced mortality in STEMI patients.[22, 23]. Furthermore, long term follow-up of the trials showed that the reperfusion therapy reduced infarct size and improved myocardial function and long term survival.[24, 25] A main drawback of thrombolytic therapy is that it only re-establishes normal coronary flow (TIMI III) in approximately 50 % of patients, with adequate myocardial tissue perfusion restored to an even lower extent.[26] Furthermore, patients subject to thrombolytic therapy have a 0.5-2% risk of lethal bleeding complications. Today, mechanical reperfusion therapy using percutaneous coronary intervention (PCI) has largely replaced thrombolytic therapy in STEMI. PCI has proven to be more effective in re-establishing coronary flow with fewer complications, resulting in a lower mortality compared to fibrinolytic therapy. [20, 27]

Reperfusion injury

Although reperfusion of the ischemic myocardium is a prerequisite for myocardial salvage, it has been described that the reperfusion in itself may cause additional damage to the myocardium (reperfusion injury).[28] The existence of this phenomenon is still debated. [29 30, 31] Upon reperfusion, the blood flow is increased by more than a 7-fold of the baseline flow, carrying oxygen and nutrients to the ischemic myocardium. [32] Several negative effects of reperfusion have been proposed.

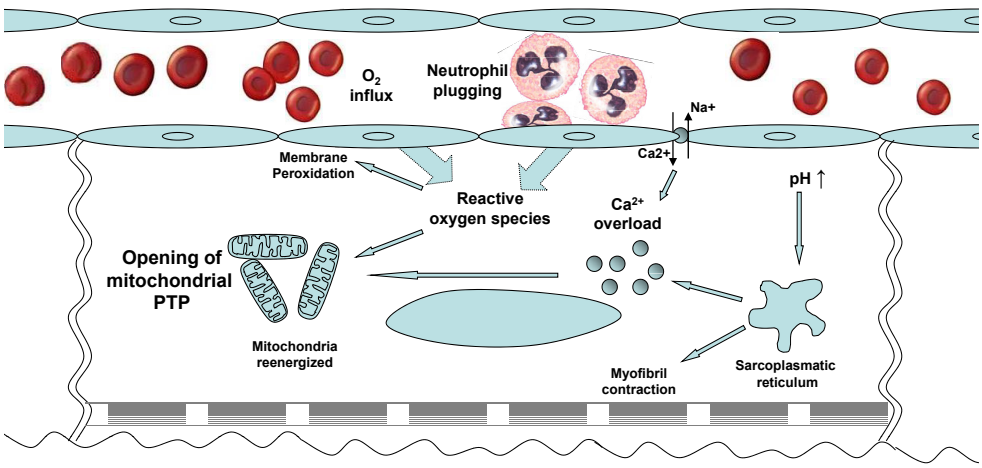


Figure 1. This figure illustrates the mechanisms considered to be involved in reperfusion injury

Oxygenation has been found to generate oxidative stress, which in turn can mediate myocardial injury. [33]. Reperfusion also causes an abrupt increase in intracellular Ca^{2+} which in turn causes myofibril contraction and subsequent apoptosis due to opening of the mitochondrial permeability transition pore.[34] An abrupt restoration of intracellular pH also facilitates Ca^{2+}-mediated myocardial injury.[35] Furthermore, inflammation is thought to play an important role in reperfusion injury. During the first day after an acute myocardial infarction, the reperfused myocardium is infiltrated with neutrophils who release reactive oxygen species and cause microvascular plugging.[36] Despite many potential targets for attenuation of reperfusion injury and numerous positive experimental studies, no therapy has yet reached clinical practice. [37]

Microvascular obstruction

Microvascular obstruction (MO) is a phenomenon related to reperfusion injury. MO represents a region with impaired microvascular blood flow within the infarcted myocardium, and illustrates that myocardial tissue perfusion has not occurred.

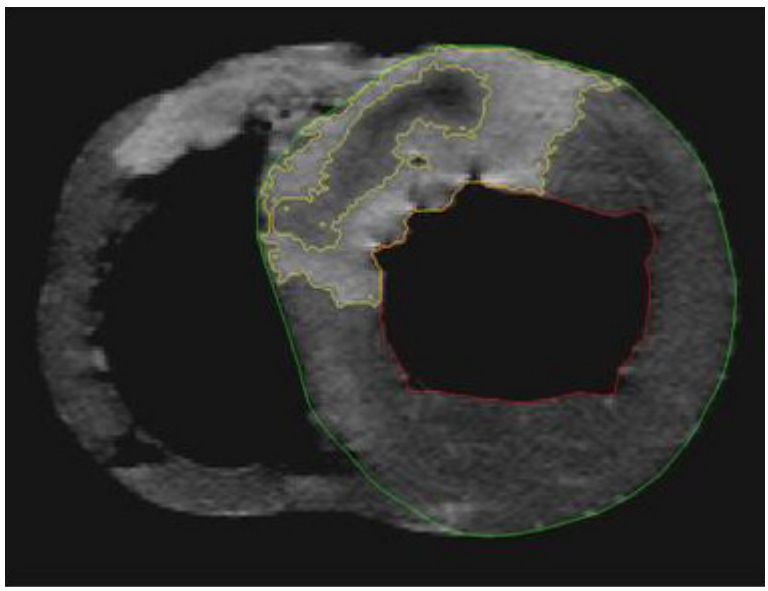


Figure 2. This figure shows a short-axis ex-vivo CMR image of an experimentally induced anterior AMI. The brighter area represents infarcted myocardium. Microvascular obstruction is seen as a darker area within the infarct.

The phenomenon is prevalent in larger myocardial infarctions and the extent of MO is correlated to the extent of the myocardial infarction.[38] MO is also a predictor of im-

paired recovery of left ventricular function and long-term mortality, independently of infarct size.[39, 40]

Hypotermia in myocardial ischemia

Over the past decade, several studies investigating the effects of therapeutic mild hypothermia on myocardial ischemia have been published. Duncker and co-workers demonstrated in an open chest porcine model that if external hypothermia was induced before ischemia was initiated, a reduction in infarct size was seen. The observed effect was also proportional to the reduction in temperature.[41] Furthermore, Hale and co-workers using an open chest rabbit model using topical cooling, found that induction of hypothermia treatment early after onset of ischemia reduced infarct size.[42, 43] Hypothermia could thus offer protective properties even after onset of ischemia. Miki and co-workers further investigated this effect and found that the earlier after onset of ischemia, hypothermia was induced, the greater reduction in infarct size was seen.[44] The protective effects of hypothermia seemed to be time-dependent. The drawback of these studies was that they were performed in an experimental setting, often using small animals with hypothermia protocols which could not easily be translated into a clinical application. In the clinical setting this is not feasible to induce hypothermia before or early after onset of ischemia. It will only be possible to induce hypothermia shortly before or after onset of reperfusion. Dae and co-workers found that hypothermia, using an endovascular cooling catheter caused an 80% reduction in infarct size in human sized pigs.[45] Hypothermia was in that study initiated after 20 min of ischemia. Furthermore, it took approximately 45 min to cool the pigs to a temperature < 35°C using endovascular cooling. The effect of hypothermia on infarct size observed in the experimental studies resulted in two clinical trials (ICE-IT and COOL-MI) investigating the effect of endovascular hypothermia in patients with STEMI.[46, 47] Unfortunately, the trials failed to show a reduction in infarct size in the hypothermia group. Post-hoc analysis of the COOL-MI-trial showed that in the minority of patients who reached a body temperature of < 35°C before reperfusion, a 49% reduction in infarct size (9.3% vs. 18.2%, p=0.05) was observed. In the ICE-IT trial, as similar trend was seen with a 43% reduction in infarct size in patients achieving a temperature of < 35°C before reperfusion (12.9% vs. 22.7%, p=0.09).
(Sätt in bild Cool-MI till vänster och bild ICE-IT till höger om denna (om det ser snyggt ut)

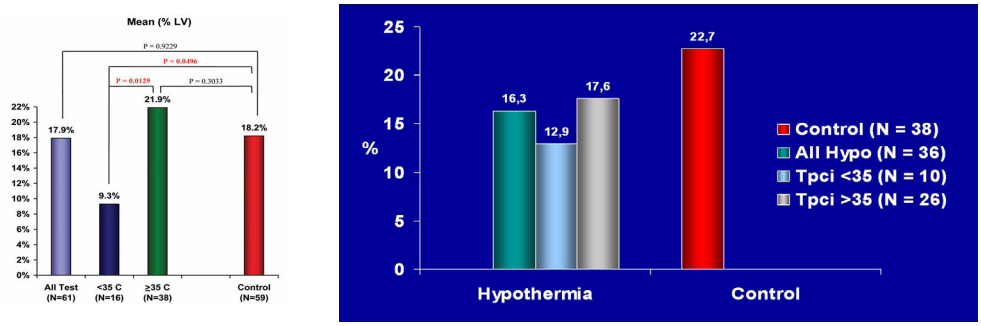


Figure 3: This left figure displays the results from the COOL-MI-trial, whereas the right figure displays the results from the ICE-IT-trial.

Despite failure of the trials to meet their primary endpoint, the post-hoc analyses suggested the necessity of initiating hypothermia before reperfusion. Maeng and co-workers indirectly confirmed the findings from the clinical trials when observing that induction of hypothermia after onset of reperfusion in a porcine model did not affect infarct size.[48] There was thus a new need to establish a protocol which allowed for induction of hypothermia achieving a core body temperature of < 35°C without delaying reperfusion therapy.

Cardiogenic shock

Cardiogenic shock (CS) is a feared complication to acute myocardial infarction which occurs in 7-10% of STEMI-patients. [49] The condition is thought to be due to a depression in myocardial contractility due to ischemia, resulting in a vicious circle of hypotension, reduced tissue- and coronary perfusion, and resulting in metabolic acidosis, with a subsequent further reduction in cardiac output. The underlying cause is usually either a large myocardial infarction, or a moderate sized myocardial infarction in a patient with an already reduced ventricular function.[50] The condition, if untreated, has a mortality rate of up to 80%.[49, 51, 52] In the SHOCK-trial, patients were randomized between emergency revascularization and initial medical stabilization.[52] Early revascularization reduced 12-month mortality rate significantly (53% vs. 66%, p=0.025). The benefit of revascularization may have been underestimated in the study since 86% of patients in both groups received an intraaortic balloon pump which is a device designed to assist the circulation in case of acute heart failure. Furthermore, 25% of patients in the initial medical stabilization group underwent mechanical reperfusion therapy. The mortality rate in the initial medical stabilization group is also lower than typically seen in untreated patients. Based on the results from the SHOCK-trial, guidelines now state that patients in cardiogenic shock should undergo immediate revascularization. Nevertheless, despite early intervention, mortality rates still remain at around 50%.[53, 54]

Hypothermia and myocardial function

Experimental studies have demonstrated that mild hypothermia increases myocardial contractility in excised heart preparations as well as in the in situ heart.[55-57] Weisser and co-workers found that the positive inotropic effects were exerted by both prolonged contraction- and relaxation time.[55] Furthermore, the intracellular Ca^{2+}-content was not increased. This indicates that hypothermia acts in a cAMP-independent manner by an increased myofilament sensitivity to existing Ca^{2+}. Nishimura and co-workers confirmed the finding of an increase in myocardial contractility by mild hypothermia.[57] In the in-situ heart, an increase in myocardial contractility was observed, while myocardial oxygen consumption was reduced. Cardiac output decreased as a result of an increase in systemic vascular resistance due to hypothermia. When applying a vasodilator to the same setting, cardiac output was unchanged, while stroke volume increased. Thus, hypothermia increases myocardial contractility without increasing myocardial oxygen consumption, and decreases heart rate with a concomitant increase in stroke volume. Cardiac output may be unchanged or reduced depending on the systemic vascular resistance. [45, 55, 57]

Moriyama and co-workers applied external cooling together with intraaortic balloon pump support in a non-randomized fashion on 8 patients who had cardiogenic shock after open heart surgery.[58] They found unchanged heart rate, blood pressure, and cardiac output during cooling. However, tissue oxygen consumption was lowered from 231±30mlO_2/min before induction of hypothermia to 189±31mlO_2/min during hypothermia. Furthermore, SvO2 increased from 45±8% to 56±7%. Yahagi and co-workers performed a study on 10 patients in a similar setting as Moriyama.[59] They found an increase in cardiac index CI from 1.9±0.3l/min/m^2 to 2.2±0.3l/min/m^2, an increase in SvO2 from 55±7% to 64±6%, and an increase in urine output from 2.1± 1.1ml/kg/h to 3.4±2.2ml/kg/h. These small non-randomized studies suggest a potential use for hypothermia in reducing peripheral oxygen consumption and hemodynamic stabilization in cardiogenic shock after open heart surgery.

6 Hypotheses of the thesis

I. A combination of cold saline infusion and an endovascular cooling catheter will cause a rapid induction of hypothermia and will reduce infarct size if initiated before reperfusion.

II. Rapid cooling using a combination of cold saline and an endovascular cooling catheter will reduce infarct size by attenuation of reperfusion injury.

III. Cold saline alone, if initiated before reperfusion will reduce infarct size.

IV. Extending active hypothermia treatment beyond 15 min past reperfusion will confer myocardial protection compared to shorter post-reperfusion hypothermia treatment.

V. Endovascular hypothermia will stabilize circulation and improve outcome in cardiogenic shock.

VI. Rapid cooling using a combination of cold saline and an endovascular cooling catheter is safe and feasible in patients with ST-segment Elevation Myocardial Infarction and will reduce infarct size.

7 Materials and methods

Animals Paper I-III

All animals were healthy male or female pigs, weighing 40-50kg of Swedish landrace.

The pigs were fasted overnight with free access to water and were premedicated with Ketaminol (Ketamine, Intervet, Danderyd, Sweden), 100mg/ml, 1,5ml/10kg, and Rompun (Xylazin, Bayer AG, Leverkusen, Germany), 20mg/ml, 1ml/10kg 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 µl/ml fentanyl (Fentanyl, Pharmalink AB, Stockholm, Sweden) in buffered glucose (25 mg/ml) was started at a rate of 2 ml/min and adjusted as needed. During balanced anaesthesia, thiopental (Pentothal, Abbott, Stockholm, Sweden) was titrated against animal requirements with small bolus doses. Mechanical ventilation was established with a Siemens-Elema 900B ventilator in the volume-controlled mode, adjusted in order to obtain normocapnia (temperature corrected pCO_2: 5.0-6.0 kPa). The animals were ventilated with a mixture of nitrous oxide (70%) and oxygen (30%). Blood gases were analyzed every 30 minutes throughout the experiment in an automated bench top analyzer (Radiometer Medical ApS, Brønshsøj, Denmark). Heparin (200 IU/kg) was given intravenously at the start of the catheterization. The procedures were performed in an experimental catheterization laboratory (Shimadzu Corp., Kyoto, Japan).

Monitoring Paper I-II

The pigs were continuously monitored by electrocardiography (ECG) and defibrillations were performed using a Lifepak™ 12 (Medtronic Co., Minneapolis, MN, USA). Arterial blood pressure was measured using a blood pressure transducer (ADInstruments Inc, Colorado Springs, CO, USA).

Monitoring Paper III

ECG-monitoring was performed as described previously. Arterial blood pressure, pulmonary artery pressure, capillary wedge pressure, and central venous pressure were con-

tinuously measured using separate transducers (ADInstruments Inc, Colorado Springs, CO, USA). Cardiac output was continuously monitored through a Vigilance™ monitor (Edwards Lifesciences, Irvine, CA, USA). Hemodynamic parameters were digitally recorded using Chart v4.2 (ADInstruments Inc, Colorado Springs, CO, USA).

Insertion of an endovascular cooling catheterc Paper I-III

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.

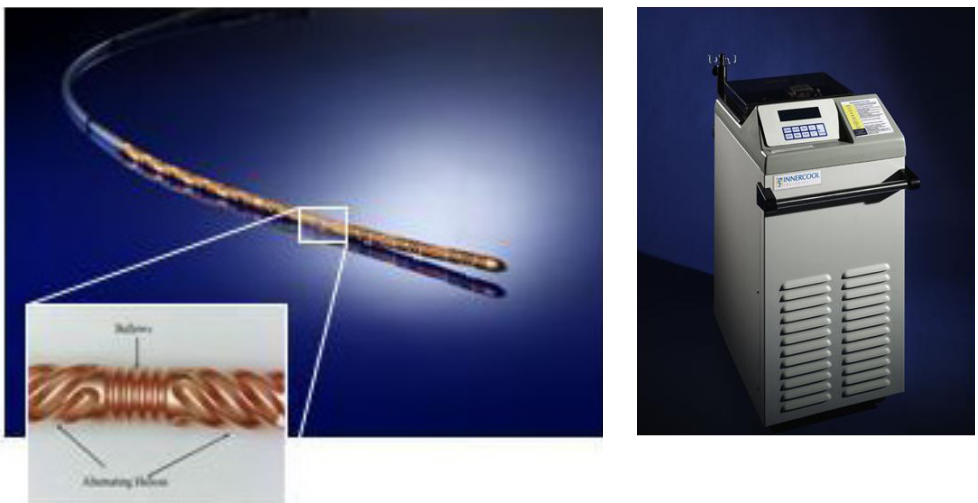


Figure 4. The left figure shows the endovascular cooling catheter through which cold saline is flushed. The catheter is placed in the v cava inferior through a catheter placed in a femoral vein. The right figure shows the heat exchange console to which the catheter is connected.

Core 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 then connected to the Celsius Control and the system was set to maintain a normal pig body temperature of 38.0° C.

General Catheterizations paper I-II

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. An angiogram was obtained using 8-10 ml of contrast (Omnipaque™ 300mg I/ml (Nycomed, Oslo, Norway) to ensure correct positioning of the catheter. The catheter was used to place a 0.014-inch PT Choice™ guide wire (Boston Scientific Scimed, Maple Grove, MN, USA) into the distal LAD. A 3.0-3.5 x 20 mm Maverick monorail™ angioplasty balloon (Boston Scientific Scimed, Maple Grove, MN, USA) was positioned in the LAD, immediately distal to the first diagonal branch.

General Catheterizations paper III

A 6 F introducer sheath (Boston Scientific Scimed, Maple Grove, MN, USA) was inserted into the surgically exposed left carotid artery and the left coronary artery was catheterized as described previously. A 0.014-inch PT Choice™ guide wire (Boston Scientific Scimed, Maple Grove, MN, USA) was placed into the distal LAD. A 3.0-3.5 x 20 mm Maverick monorail™ angioplasty balloon (Boston Scientific Scimed, Maple Grove, MN, USA) was positioned in the proximal LAD.

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 Continuous Cardiac Output Pulmonary Artery Catheter™ (Edwards Lifesciences, Irvine, CA, USA) was inserted into a pulmonary artery. The catheter was then connected to a Vigilance™ monitor (Edwards Lifesciences, Irvine, CA, USA).

Protocol

Study I: Rapid induction of hypothermia in pigs with acute myocardial infarction

Ischemia protocol

After a stable core body temperature of 38.0° C was achieved, ischemia was induced by inflation of the angioplasty balloon for 40 min. An angiogram was performed after inflation of the balloon and before deflation of the balloon in order to verify total occlusion of the coronary vessel 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.

Hypothermia protocol

The pigs were randomized to rapid hypothermia before reperfusion, (pre-reperfusion hypothermia, n=8) or immediately after reperfusion (post-reperfusion hypothermia, n=8). A normothermic group (n=6) was also studied in order to provide comparison between different hypothermia protocols and normothermia. Hypothermia was induced by a rapid intravenous infusion of 1000 ml of 4° C cold saline into a central vein together with the Celsius Control™ endovascular cooling system after 25 min of ischemia or immediately after reperfusion when coronary blood flow was restored

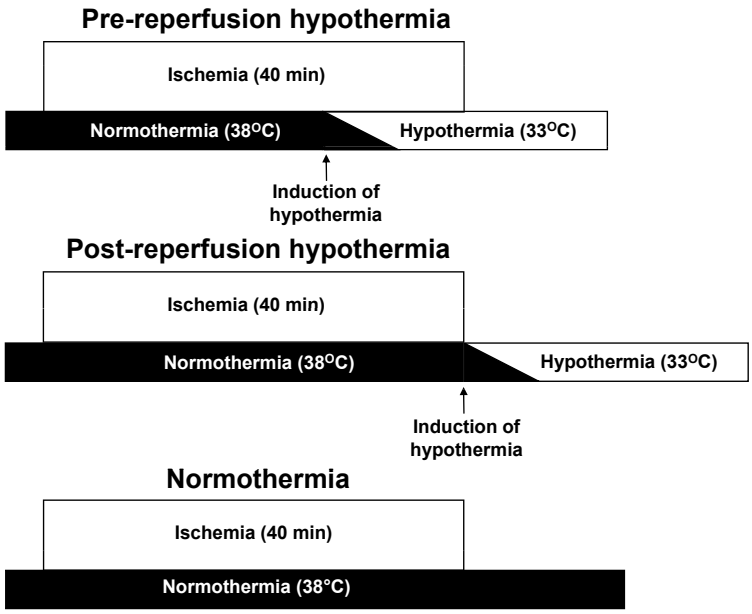


Figure 5: This figure shows the hypothermia protocol in study I. Protocol for induction of hypothermia. In the pre-reperfusion group, hypothermia was started after 25 min of ischemia (15 min before reperfusion) and in the post-reperfusion group, hypothermia was started immediately after reperfusion. Active hypothermia treatment continued for 30 min in both groups. The normothermia group was maintained at 38.0° C.

Target temperature was 33° C and successful cooling was defined as a temperature of ≤ 35° C. Hypothermia was then actively maintained for 30 min followed by passive rewarming with blankets.

Study II:

Ischemia protocol

After a stable core body temperature of 38.0° C was achieved, ischemia was induced by inflation of an angioplasty balloon in the LAD for 40 or 45 min depending on which hypothermia protocol which was utilised (see below). An angiogram was performed after inflation of the balloon and before deflation of the balloon in order to verify total occlusion of the coronary vessel 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.

Hypothermia protocol

The pigs were randomized to combination hypothermia (using cold saline and endovascular cooling (n=8)) or to normothermia (n=8), **(figure 6a**). Furthermore, we examined the effect of hypothermia induced by cold saline alone (n=8), **(figure 6b),** and extended hypothermia for 75 minutes (n=8) compared to shorter active hypothermia (30 minutes) (n=7) (**figure 6c).**

The normothermia group (control) had duration of ischemia of 40 min, followed by reperfusion.

Combination hypothermia

In the combination hypothermia group, hypothermia was induced after 40 min of ischemia by a rapid intravenous infusion of 1000 ml of 4° C cold saline into a central vein together with the Celsius Control™ endovascular cooling system. Target temperature was 33° C and successful cooling was defined as a temperature of ≤ 35° C. Total ischemia time was extended by 5 min to a total of 45 min in this group, achieving equal normothermic ischemic time compared to controls. Following reperfusion, hypothermia was actively maintained for 30 min followed by passive warming with blankets.

Cold saline hypothermia

In the cold saline hypothermia group, hypothermia was induced after 40 min of ischemia by a rapid intravenous infusion of 1000 ml of 4° C saline into a central vein. Total ischemic time was 45 min. The temperature was then continuously monitored but no further active cooling was undertaken.

Extended hypothermia

In the extended hypothermia group, hypothermia was induced after 25 min of ischemia using a combination of cold saline and endovascular cooling as described above. Hypothermia was initially maintained for the final 15 minutes of ischemia, thus yielding a total ischemic time of 40 min. Following reperfusion, hypothermia was con-

tinued and actively maintained for 60 min followed by passive warming. The shorter active hypothermia group was identical to the extended hypothermia group, with the exception that hypothermia following reperfusion was only actively maintained for 30 minutes, followed by passive warming. Data from the shorter active hypothermia group originate from paper I.

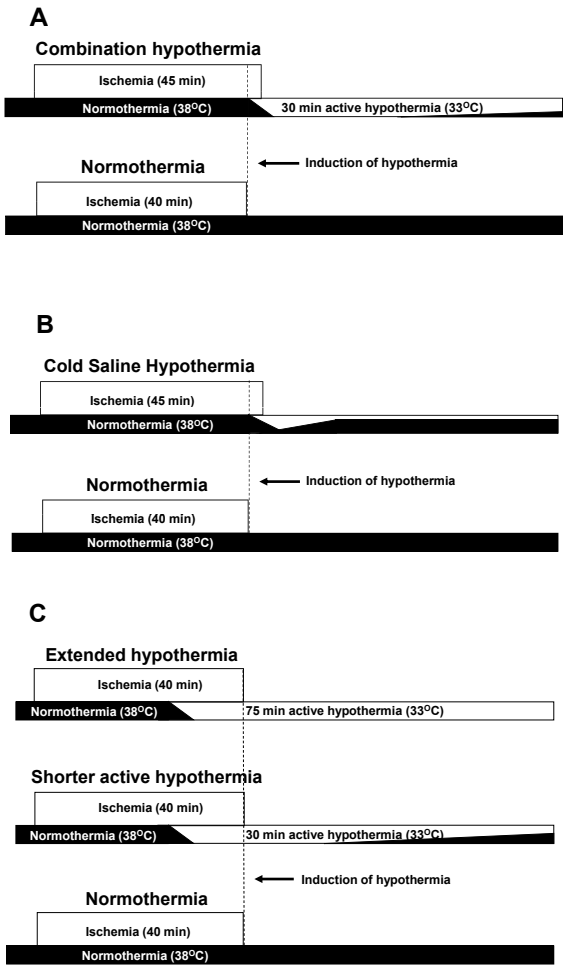


Figure 6: This figure shows the hypothermia protocols in study II. a) Combination hypothermia was started after 40 min of ischemia and the ischemic time was then prolonged by 5 min in order to achieve the same normothermic ischemic time. b) Cold saline hypothermia was started after 40 min of ischemia by a single infusion of 1000ml of cold saline solution. The ischemic time was then prolonged by 5 min in order to achieve the same normothermic ischemic time. c) Extended hypothermia was initiated after 25 min of ischemia, continued for 15 minutes of ischemia, and maintained during 60 min after the onset of reperfusion. Shorter active hypothermia was also initiated after 25 min of ischemia and continued for 15 minutes of ischemia, but was only maintained during 15 min after the onset of reperfusion.

Study III:

Ischemia protocol

After a stable core body temperature of 38.0° C was achieved, ischemia was induced by inflation of the angioplasty balloon in the proximal LAD for 40 min. 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.

Hypothermia protocol

After a stable core body temperature of 38.0° C was achieved, ischemia was induced by inflation of the angioplasty balloon in the proximal LAD for 40 min. 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. The animals were included in the study if they fulfilled our prespecified criterion of a high risk of developing sustained cardiogenic shock (<90 mm Hg for at least 15 min) immediately before reperfusion. If the animal fulfilled this criteria, it was randomized to hypothermia (n=8) or to normothermia (n=8), by drawing folded notes which read "cool" or "warm" out of a box. Hypothermia was then induced after reperfusion 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 normothermia group, the endovascular catheter was used to maintain a normal pig body temperature of 38° C. After 4 hours the experiment ended and surviving pigs were sacrificed. Pigs that died during ischemia or did not meet the criterion for cardiogenic shock (< 90 mm Hg during at least 15 minutes) before randomization were excluded from the study. In order to exclude any positive effects on volume-loading, cold fluids during the experiment were excluded from the hypothermia protocol. No additional fluids in either group were permitted except for administration of anaesthetic drugs.

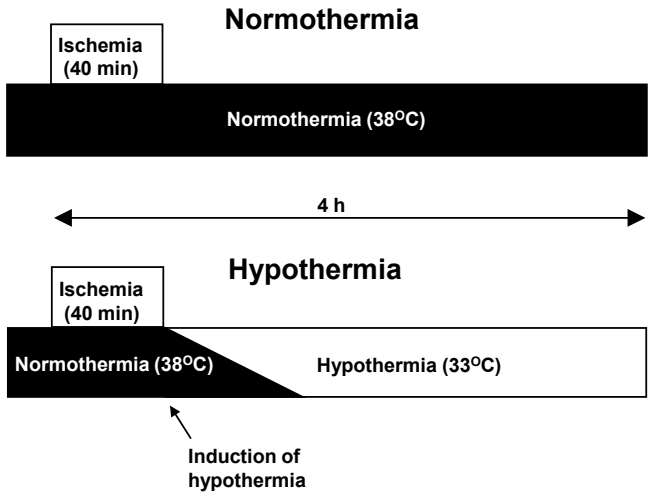


Figure 7. This figure shows the hypothermia protocol in study III. Endovascular hypothermia was induced after randomization, at the onset of reperfusion and was then maintained throughout the duration of the experiment.

Study IV:

Study population

From March 2007 to October 2009, patients were enrolled in this prospective randomized single center study to test the feasibility and safety of an infusion of cold saline together with endovascular hypothermia, using the Celsius Control™ System (Innercool Therapies Inc, San Diego, CA, USA) as an adjunct therapy in patients with an acute STEMI eligible for primary PCI. Men and women between 18 and 75 years of age who presented with an anterior or inferior STEMI with ST-segment elevation of > 0.2mV in two or more anatomically contiguous leads and a duration of symptoms of < 6h were included. Patients with cardiac arrest, previous AMI, previous PCI or CABG, known congestive heart failure, end stage kidney disease or hepatic failure, recent stroke, coagulopathy, pregnancy or Killip class II-IV at presentation were excluded from the study.

Protocol

Figure 8 gives a timeline of the protocol. Eligible patients were randomized to hypothermia or to the control group in the cardiac catheterization laboratory before angiography. Sealed opaque envelopes that contained the study group assignment were opened after informed consent was obtained. Patients assigned to the hypothermia group were given 30mg of oral buspirone. Meperidine was given as an intravenous loading dose of

1mg/kg. The loading dose was reduced to 0.5mg/kg if the patient had been given morphine prior to enrollment in the study. The loading dose was followed by a continuous infusion of meperidine at 30mg/h. Additional 25mg intravenous boluses doses of meperidine was given if the patient started to shiver. Shivering was further suppressed at the ward by skin warming using a forced-air warming blanket. Hypothermia was induced by forced infusion of 4°C cold saline using pressure bags. Volume administered was 1000-2000 ml at the physician's discretion. A 14 F introducer was inserted in the right femoral vein. Through the introducer, a 14 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. The target temperature was then set to 33°C. Core body temperature was measured using an integrated temperature probe in the cooling catheter. Cooling was maintained for 3h followed by passive warming to 36-37 °C during 3h. Loading doses of 500mg of Aspirin, and 600mg of clopidogrel were given to all patients before cardiac catheterization. After induction of hypothermia, a coronary angiogram was performed. Patients underwent PCI according to current standard of practice.

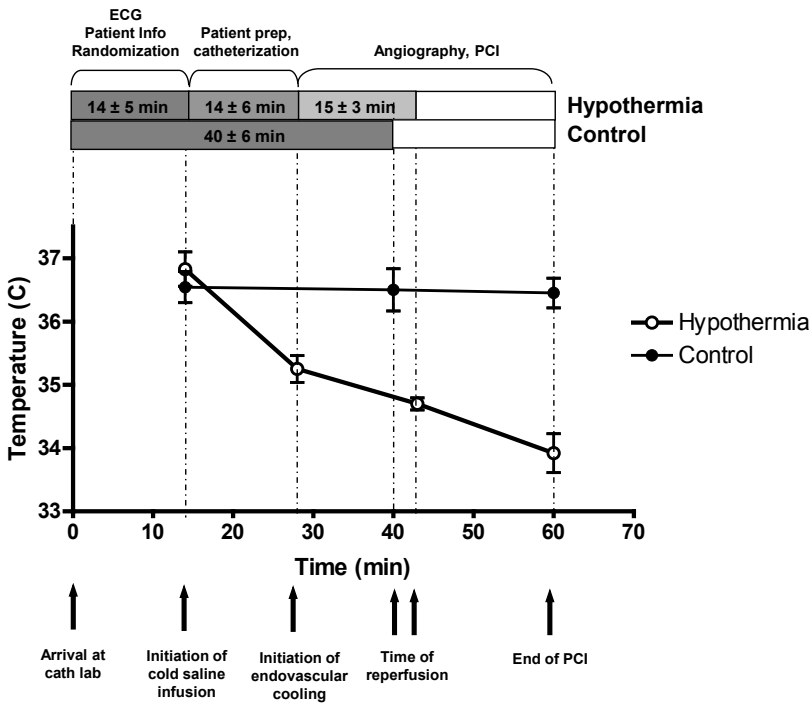


Figure 8. The timeline at the catheterization laboratory.

Imaging

Assessment of area at risk by in vivo SPECT paper I

Single photon emission computed tomography (SPECT) was used to assess the area at risk (AAR). Five hundred MBq of ^{99m}Tc-tetrofosmin was administered intravenously ten minutes before deflation of the angioplasty balloon. The anesthetized pigs were then imaged in a supine position with a dual head camera (ADAC Vertex, Milpitas, CA, USA) at 32 projections (40 s per projection) with a 64 X 64 matrix yielding a digital resolution of 5 X 5 X 5 mm. Iterative reconstruction using maximum likelihood-expectation maximization (MLEM) was performed with a low-resolution Butterworth filter with a cut-off frequency set to 0.6 of Nyquist and order 5.0. No attenuation or scatter correction was applied. Finally short and long-axis images were reconstructed. Quantification of the size of AAR in ml was performed automatically as the extent of the perfusion defect as determined by commercially available software (Auto QUANT™ 4.3.1 and a standard database; ADAC, Milpitas, CA, USA).[60] AAR was expressed as percent of the left ventricular volume, and this was determined by dividing the AAR (ml) from SPECT by the left ventricular wall volume (ml) determined by ex vivo MRI as described below. This was performed due to the known limitations in accuracy for determining left ventricular wall volume by SPECT.[61]

Assessment of area at risk by ex vivo SPECT paper II

Single photon emission computed tomography (SPECT) was used to assess the AAR as percent of left ventricular myocardium. 1000 MBq of ^{99m}Tc-tetrofosmin was administered intravenously ten minutes before deflation of the angioplasty balloon. Ex vivo imaging was performed with a dual head camera (Skylight, Philips, Best, the Netherlands) at 32 projections (40 s per projection) with a 64 X 64 matrix yielding a digital resolution of 5 X 5 X 5 mm. Iterative reconstruction using maximum likelihood-expectation maximization (MLEM) was performed with a low-resolution Butterworth filter with a cut-off frequency set to 0.6 of Nyquist and order 5.0. No attenuation or scatter correction was applied. Finally short and long-axis images were reconstructed. The endocardial and epicardial borders of the left ventricle that were manually delineated in the MR images were copied to the co-registered SPECT images. A SPECT defect was defined as a region within the MRI-determined myocardium with counts lower than 55% of the maximum counts in the myocardium.[62]

Infarct size and microvascular obstruction assessed by ex vivo MRI paper I, II

Ex vivo imaging of the heart was undertaken using a 1.5 T Philips Intera CV MR scanner (Philips, Best, the Netherlands) according to a previous described protocol.[63] In brief, a commercially available gadolinium-based contrast agent (Magnevist, *gadopentetate dimeglumine*, Gd-DTPA, Schering Nordisker AB, Järfälla, Sweden) was administered intravenously (0.2 mmol/kg) both 60 and 15 minutes prior to removal of the

heart. After removal, the heart was immediately rinsed in cold saline and the ventricles were filled with balloons containing deuterated water. Three dimensional acquisition of T1-weighted images (TR = 20ms, TE = 3.2ms, flip angle = 70° and 2 averages) yielded a stack of approximately 200 images with an isometric resolution of 0.5 mm covering the entire heart. Images were then acquired using a head coil and the duration of acquisition was typically 45 minutes.

The MR images were analyzed using freely available software (Segment 1.457, paper I), or (Segment 1.700, paper II), (http://segment.heiberg.se). The endocardial and epicardial borders of the left ventricular myocardium were manually delineated in short-axis *ex vivo* images. This defined the volume of left ventricular myocardium (cm^3 = ml). The infarct size (IS) was first determined as the volume of infarcted myocardium (cm^3). The infarct volume was calculated as the product of the slice thickness (cm) and the area of hyperenhanced pixels (cm^2) with a signal intensity above the infarction threshold defined as > 8 SD above the mean intensity of non-affected remote myocardium. Microvascular obstruction was defined as hypointense regions in the core of the infarction which had signal intensity less than the threshold for infarction. These regions were manually included in the infarct volume. The volume of microvascular obstruction (cm^3) was calculated as the difference between the infarct volume before and after manual inclusion of regions of microvascular obstruction. Furthermore, the size of microvascular obstruction was expressed as percent of infarct size. Ultimately, the infarct size was expressed as percent of left ventricular myocardium.

Finally, infarct size was expressed as a percentage of the area at risk (IS/AAR) in order to adjust for any difference in area at risk between the groups. [21, 64]

Patchiness index paper I

Infarct homogeneity was assessed by a patchiness index based on infarct surface area. The high resolution *ex vivo* MR images allowed quantification of the surface area of the infarct. Infarct surface area (cm^2) was automatically determined as the product of the slice thickness (cm) and the distance along the pixel border between infarcted and non-infarcted pixels (cm) in each slice. For equally homogeneous infarcts, a larger infarct volume will yield a larger surface area. A patchiness index (cm^{-1}) was therefore calculated as the infarct surface area (cm^2) divided by the infarct volume (cm^3). Thus, the patchiness index provided a method for estimating the homogeneity of the myocardial infarction adjusted for infarct size.

CMR imaging paper IV

After 4±2 days, patients underwent a CMR examination in supine position using a 1.5T system (Philips Intera CV, Philips, Best, the Netherlands) with a five element cardiac synergy coil. Initial scout images were acquired to locate the heart and the standard imaging planes. For visualization of the initial ischemic myocardium, T2-weighted short tau inversion recovery (STIR) turbo spin echo images with a double inversion pulse for blood suppression were acquired in the short-axis view, covering the left ventricle from

the base to apex. Image parameters for T2-weighted imaging were: echo time, 100 ms; repetition time, 2 heart beats; number of averages, 2; inversion time, 180 ms; image resolution, 1.5 x 1.5 x 10 mm with no gap. Parallel imaging with SENSE=1 was used to minimize signal inhomogeneities due to differences in coil sensitivity. For infarct visualisation, late gadolinium enhancement (LGE) images were acquired 15-20 minutes after administration of 0.2mmol per kilogram of body weight of an extracellular gadolinium-based contrast agent (*gadoteric acid*, Gd-DOTA, Guerbet, Gothia Medical AB, Billdal, Sweden). The LGE images were acquired in the short-axis view, from base to apex, and in the three standard long-axis views (two-chamber, four-chamber and left ventricular outflow tract views), during breath-hold, using an ECG-triggered segmented inversion-recovery gradient recalled echo (IR-GRE) image sequence. Typical IR-GRE sequence parameters were: echo time, 1.2 ms; repetition time, 3.9 ms; image resolution, 1.5 x 1.5 x 8 mm with no gap; flip angle, 15º; acquisition every heartbeat. The inversion time of typically 230-270 ms was manually adjusted to null the signal from remote myocardium.

Image analysis

The analysis of MaR and infarct size was performed using a freely available post-processing software (Segment, v.1.8 R0795; http://segment.heiberg.se).[65] For assessment of MaR, the endocardial and epicardial borders were manually traced in each T2-weighted short-axis image. The myocardium with increased signal intensity was manually delineated, as previously described, by an experienced observer blinded to all other data.[66] The MaR was expressed as a percentage of the left ventricular myocardium. The infarct size was assessed from the short-axis images and quantified using a previously described and validated semiautomatic method.[67] The assessment of infarct size was performed by an observer blinded to all other data. In short, the endocardial and epicardial borders were manually traced in each LGE short-axis images. Thereafter, the hyperenhanced myocardium was automatically quantified using a computer algorithm, taking partial volume effects in the periphery of the infarction into account. Regions where the computer algorithm was clearly wrong, manual adjustments were made. Infarct size was expressed as a percentage of the left ventricular myocardium. Infarct size was subsequently expressed as percent of myocardium at risk. Microvascular obstruction was defined as hypo-intense regions in the core of the infarction which had signal intensity less than the threshold for infarction. These regions were manually included in the infarct volume. The volume of microvascular obstruction (cm^3) was calculated as the difference between the infarct volume before and after manual inclusion of regions of microvascular obstruction. The size of microvascular obstruction was expressed as percent of infarct size.

Biochemical markers paper IV

CKMB and Troponin T were sampled on admission to the catheterization laboratory, and at 12h, and 24h after admission. Peak values were defined as the highest measured value within 24h. Area under the curve (AUC) was calculated from the measurements. NT-proBNP was sampled at day 1.

8 Ethics and Statistics

Ethics

Study I-III 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. Study IV was performed in accordance with the Declaration of Helsinki and the ethics committee of Lund University approved the study.

Statistics

Paper I

Calculations and statistics were performed using the GraphPad Prism 4.0 software. Values are presented as mean ± SEM. Statistical significance was accepted when $P < 0.05$ (Mann-Whitney's test).

Paper II

Calculations and statistics were performed using the GraphPad Prism 4.0 software (GraphPad Software Inc., La Jolla, CA, USA). Significance for the primary end point was tested using the unpaired Student's t-test. Multiple comparisons between variants of cooling protocols were tested with ANOVA. Values are presented as mean ± SEM. Statistical significance was accepted when $p < 0.05$.

Paper III

Calculations and statistics were performed using the GraphPad Prism 5.0 software (GraphPad Software Inc., La Jolla, CA, USA). Significance for survival was tested using the Fischer's exact test. In order to test significance for hemodynamic and blood gas variables in a conservative manner, the mean value of the tested variable was calculated

from the time of randomization (at 40 min) until the end of experiment (4h) in the respective groups. Mann-Whitney's test was then performed to test for any difference in mean values. Statistical significance was accepted when $p < 0.05$.

Paper IV

Calculations and statistics were performed using the GraphPad Prism 5.0 software (GraphPad Software Inc., La Jolla, CA, USA). Fisher's exact test was performed on categorical variables. Continuous variables were tested using Mann-Whitney U test with exact inference. Statistical significance was accepted when $p < 0.05$.

9 Results

Induction of hypothermia

The rationale for adding an infusion of cold saline to the use of an endovascular cooling catheter (combination hypothermia) was to cause a more rapid induction of hypothermia than with the cooling catheter alone. In studies I-II, combination hypothermia achieved a reduction in core body temperature from 38°C to <35°C in 5-10 min (**Figure 9a-c**). An infusion of cold saline alone achieved a similar rapid reduction in core body temperature, but as soon as the infusion was finished, a quick rebound in core body temperature was seen (**Figure 9d**). At the time of reperfusion, core body temperature was 35.2±0.3 °C, but 25 min after onset of reperfusion, the temperature was 36.3±0.1 °C. Cold saline alone was thus able to achieve a fast induction of hypothermia but was unable to sustain hypothermia. In study III, an endovascular cooling catheter alone was used to induce hypothermia (**Figure 9e**). Hypothermia was initiated at reperfusion, and 45 minutes after initiation of hypothermia, the mean temperature in the hypothermia group was 33.6 ± 0.7° C. The mean cooling rate in the study was 5.9° C/hr. Endovascular cooling alone had a slower induction phase than combination hypothermia or cold saline alone but was able to sustain hypothermia.

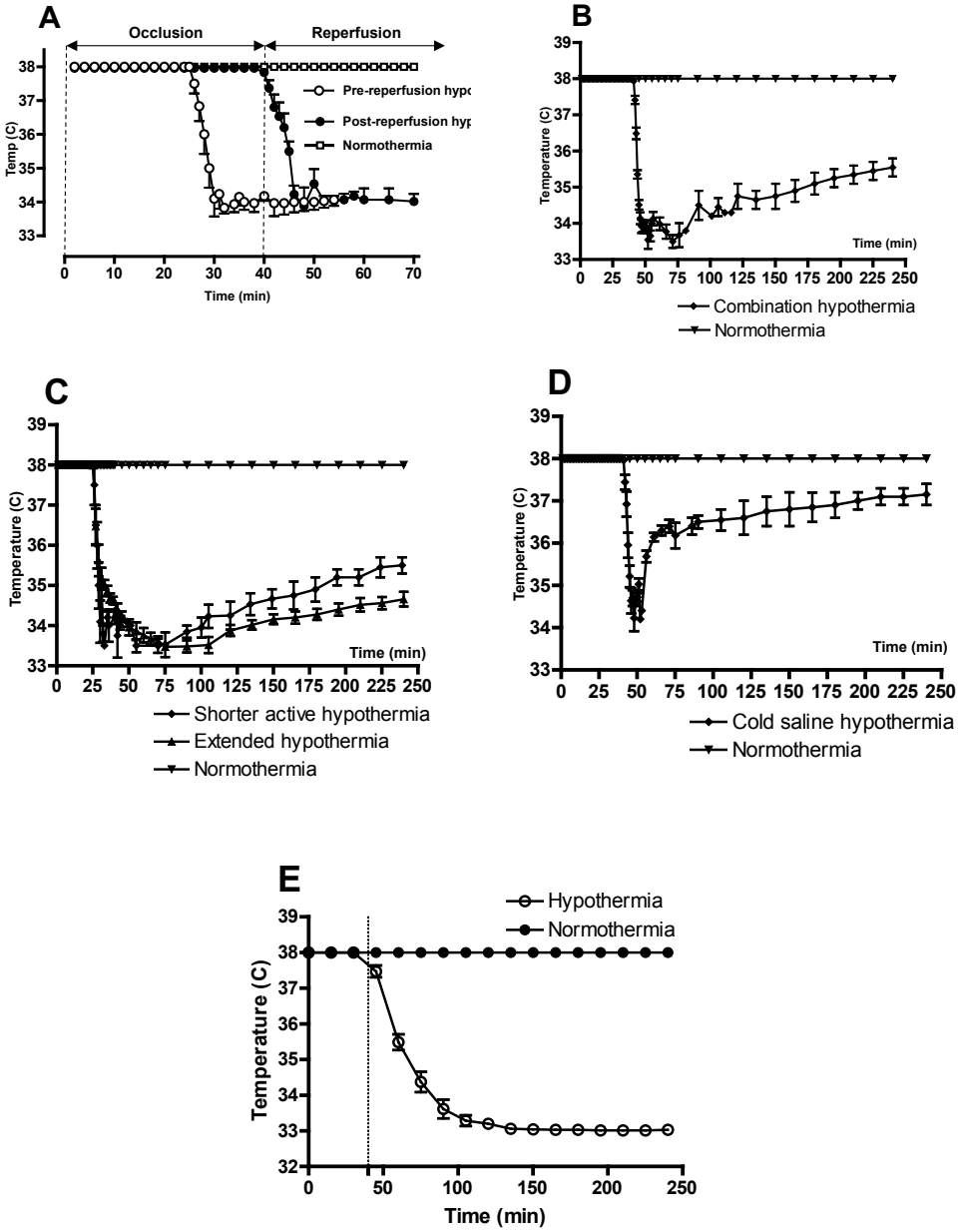


Figure 9. This figure shows the reduction in core body temperature caused by the different experimental hypothermia protocols. a) Combination hypothermia caused a rapid reduction in core body temperature to <35°C in approximately 5-10 min (study I). b,c) Similar effects of combination hypothermia were seen in study II. d) Cold saline alone caused a rapid induction of hypothermia, but was unable to sustain hypothermia in the early reperfusion phase. e) Endovascular hypothermia alone resulted in a mean cooling rate of 5.9° C/hr.

Safety and feasibility of hypothermia

Twenty patients were enrolled in the study. There were no significant differences in baseline characteristics between the hypothermia and the control group (**Table 1**). **(Jag har satt in tabell 1 nedan, kan självklart sättas var som helst i texten)** One patient in the normothermia group had a visible thrombus in the left main coronary artery and underwent emergency CABG after angiography had been performed, and was therefore excluded from further analysis. One patient in the hypothermia group was prevented from immediate angiography due to another STEMI-patient at the catheterization laboratory, delaying cooling beyond the pre-specified 6 hours duration of ischemia, and was therefore excluded from further analysis. Clinical and angiographic data are shown in Table 1. The time from onset of symptoms to reperfusion did not differ between the two groups (174±51min vs. 174±62min, hypothermia vs. control, p=1.00). Successful revascularization was performed in all patients. All patients who underwent PCI were stented. TIMI 3 flow was established in all patients. Thrombectomy was performed in 15/18 patients with no difference between the groups.

Variable	Hypothermia (n=9)	Control (n=9)
Age	62 ± 10	58 ± 7
Women	2	2
Hypertension	3	2
Diabetes	1	2
Hyperlipidemia	1	0
Current smoker	3	5
Infarct related artery		
LAD	6	7
RCA	3	2
Onset of symptoms to reperfusion (min)	174 ± 51	174 ± 62
Door-to-balloon time (min)	43 ± 7	40 ± 6
Initial TIMI flow		
0/1	7	8
2/3	2	1
Successful revascularisation	9	9
TIMI 3 flow post PCI	9	9
Thrombectomy	8	7
Procedural time (min)	55 ± 24	40 ± 18
Abciximab	6	6
Bivalirudin	3	3

Table 1: This table shows the baseline clinical and angiographic data for the patients. P = NS for all comparisons. Data are presented as mean ± SD.

Hypothermia treatment

Baseline temperature did not differ among the groups (hypothermia 36.8 ± 0.7°C vs control 36.5 ± 0.6°C, p = 0.87). The timeline at the catheterization laboratory and measurements of core body temperature is shown in figure 10.

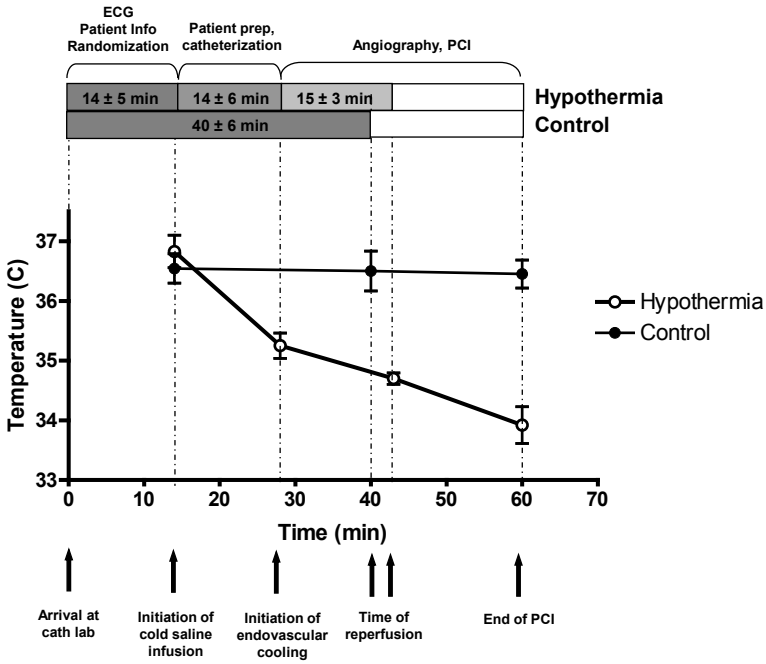


Figure 10. This figure shows the time line at the catheterization laboratory and the reduction in core body temperature.

Door to balloon time was 43 ± 7 min in the hypothermia group and 40 ± 6 min in the normothermia group, indicating that initiation of hypothermia did not markedly delay reperfusion (p=0.12) (**Figure 11**).

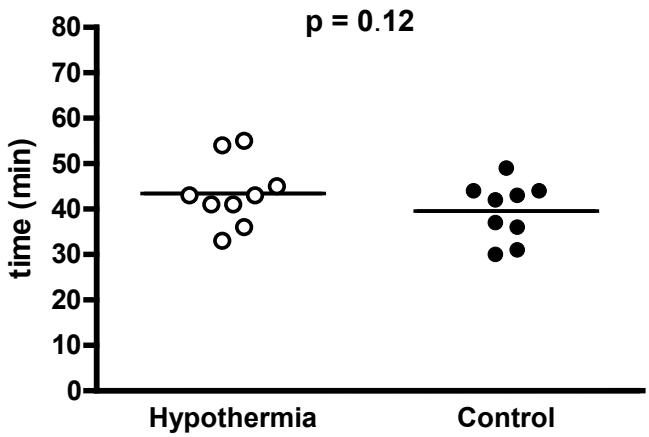


Figure 11. This figure shows the time from arrival at the hospital until reperfusion (door to balloon time). There was no marked difference between the groups (p=0.12). Values are presented as mean ± S.E.M.

Cold saline was given after randomization to the hypothermia arm 29 ± 6 min before reperfusion. An endovascular cooling catheter was then inserted and endovascular hypothermia treatment was initiated 15 ± 3 min before reperfusion. A core body temperature of < 35°C was achieved in 10 ± 7 min after onset of endovascular hypothermia treatment. Temperature at reperfusion was 34.7 ± 0.3°C (**Figure 10**). In one patient, cold saline was administered but with no endovascular cooling due to technical problems. The volume of 4°C cold saline given was 1540 ± 430 ml.

Clinical events

There were no significant differences between the groups with regard to clinical events (**Table 2**). **(Jag har satt in tabell 2 nedan, kan självklart sättas var som helst i texten)** Combination hypothermia was well tolerated in all patients, and no heart failure was seen in the hypothermia group whereas three patients in the control group had clinical signs of heart failure (p=0.21). NT-proBNP was analyzed on day 1 after the infarct. There was no difference among the groups (hypothermia 1275 ± 651 ng/l vs control 1350 ± 930 ng/l, p=0.99 (**Figure 12**). Three patients in the hypothermia group underwent antibiotic treatment due to infections while no infections were seen in the normothermia group (p=0.21) The 30-day MACE-rate was 0% in both groups.

Table 2: This table shows the clinical event data for the patients. P = NS for all comparisons. Data are presented as mean ± SD.

Variable	Hypothermia (n=9)	Control (n=9)
30 day mortality	0	0
Re-infarction	0	0
CABG	0	0
30 day MACE	0	0
Heart failure	0	3
VT/VF	0	2
Stroke	0	0
Infection	3	0
Hematoma	0	0
Bradycardia	0	0

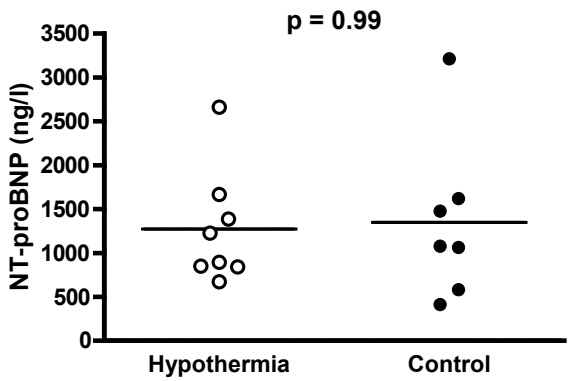


Figure 12. The NT-proBNP levels at day 1. There was no difference between the groups.

Effect of hypothermia on infarct size.

In pigs, myocardial infarct development is much faster than in humans.[68] A for experimentally induced infarction typical ischemia time of 40-45 min was thus chosen in order to correct for the difference between species. In study I, combination hypothermia was induced after 25min of a 40 min ischemia time. Active hypothermia treatment was sustained for 30 min followed by passive warming. Combination hypothermia reduced infarct size corrected for area at risk (IS/AAR) by 39% compared to normothermia (46 ± 8 % vs. 75 ± 5, p=0.048). When the same hypothermia protocol was applied at the onset of reperfusion, no reduction in infarct size was seen (80 ± 6 %)

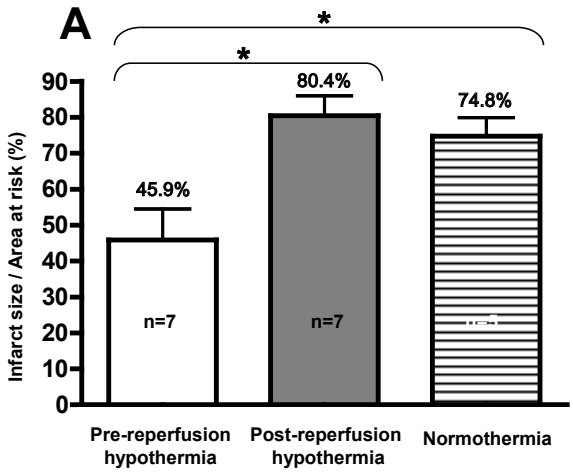


Figure 13. Pre-reperfusion hypothermia causes a 43% relative reduction in infarct size compared to post-reperfusion hypothermia and by 39% compared to normothermia. Values are presented as mean ± S.E.M.

It seemed as if hypothermia had to be initiated before reperfusion in order to have an effect on infarct size, though the protective effects could also be have been attributed to active hypothermia treatment during the last 10 min of ischemia time by means of slowing infarct progression.

Since combination hypothermia achieved such a rapid reduction in core body temperature in study I, the mechanism was further elucidated by introducing a study protocol where total ischemic time was prolonged by 5 min in the hypothermia group in order to have time to induce hypothermia before reperfusion (study II). Combination hypothermia, initiated during the last 5 min of a prolonged ischemia time reduced infarct size (IS/AAR) by 18 % compared to normothermic controls (61±5 % vs 74±4 %; p=0.03) (**Figure 14a**). Thus, despite prolonged ischemic time, combination hypothermia could reduce infarct size, as long as core body temperature was <35°C at the time of reperfusion. Cold saline alone failed to reduce infarct size compared to normothermic control (73±4 % vs 74±4 %; p>0.05) (**Figure 14b**)

The temperature in the cold saline group was 35.2±0.3 º C at reperfusion and 36.3±0.1 º C at 25 min after onset of reperfusion. It seemed as if a low core body temperature at reperfusion, but also after onset of reperfusion was important if a reduction in infarct size could be achieved. Prolonging active hypothermia treatment for 60 minutes past onset of reperfusion did however not confer any additional benefit compared to 15 min past onset of reperfusion (IS/AAR: extended hypothermia (48±7 %), compared to shorter active hypothermia (46±8 %, p>0.05), **(Figure 14c).** Active hypothermia treatment could thus be limited to 15 minutes past reperfusion.

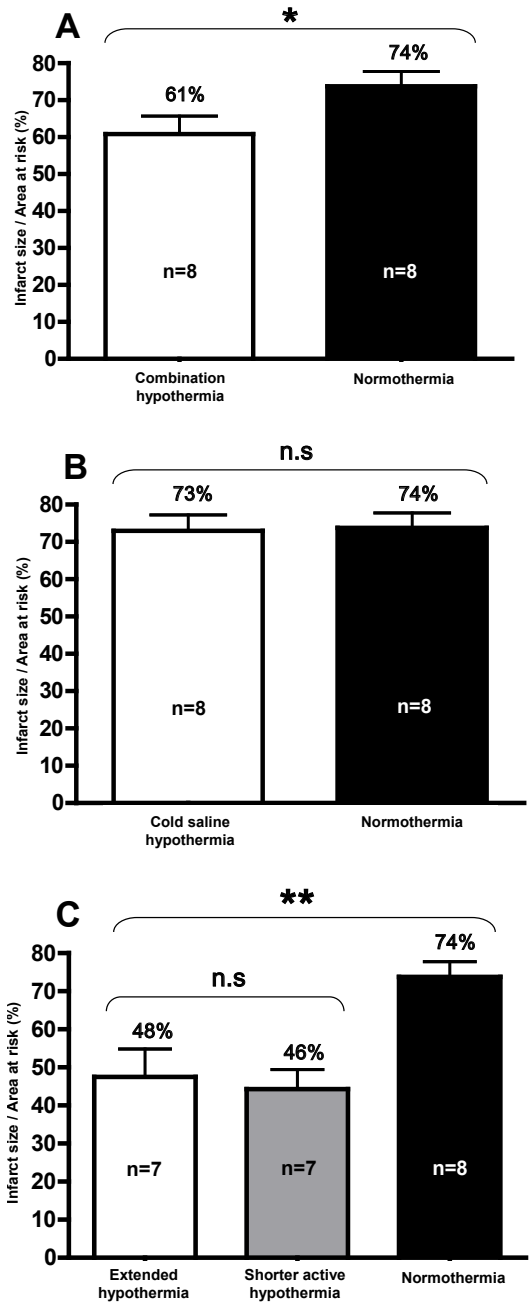


Figure 14. a) Combination hypothermia reduces infarct size by 18% despite longer duration of ischemia. b) Cold saline alone does not reduce infarct size. c) Extended hypothermia does not confer any additional benefit compared to shorter active hypothermia. Values are presented as mean ± S.E.M.

In study IV, myocardium at risk measured by MRI using T2 weighted stir was 44 ± 8 % of left ventricular mass in the hypothermia group and 43 ± 8 % in the normothermia group, and did not differ among the groups (p=0.65) (**Figure 15a**). This indicates that the groups were well matched. In one patient in each group, myocardium at risk measurements were uninterpretable. Combination hypothermia caused a 38% reduction in infarct size corrected for myocardium at risk (hypothermia 29.8 ± 12.6 % vs control 48.0 ± 21.7 %, p=0.04) (**Figure 15b**). Infarct size uncorrected for myocardium at risk displayed a trend towards a reduction in infarct size (hypothermia 13.7 ± 6 % vs control 20.5 ± 10 %, p=0.08) (**Figure 15c**).

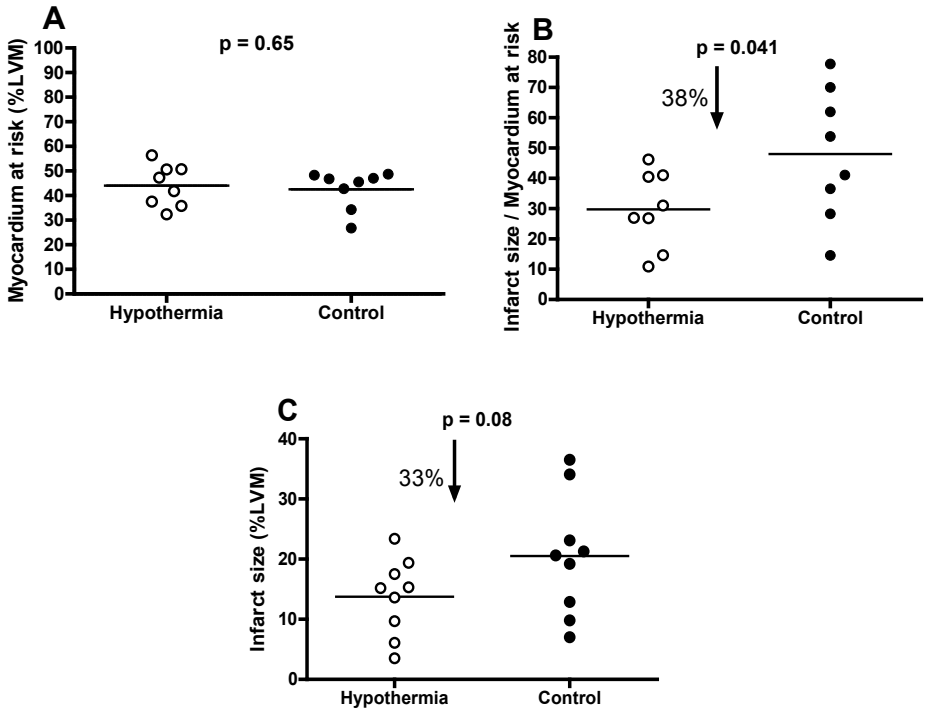


Figure 15. Myocardium at risk and infarct size. a) Myocardium at risk as percentage of left ventricular mass (%LVM), which showed no difference between the groups. b) Infarct size normalized to myocardium at risk, which was significantly reduced in the hypothermia group. c) Infarct size alone showed no statistically significant difference between the groups, although a trend for reduction was seen in the hypothermia group.

Biochemical markers (study IV)

As part of the protocol to assess the effect of hypothermia on myocardial damage, CKMB and Troponin T were sampled. Peak Troponin T was reduced by 43% in the hypothermia group compared to the control group (3.9±2.5µg/l vs. 6.9±2.8µg/l, p=0.01) (**Figure 16a**). Furthermore, the area under the curve (AUC) for Troponin T was re-

duced by 41 % in the hypothermia group compared to control group (67.7±40.3 vs. 113.8±47.2, p=0.03) (**Figure 16b**). Peak CKMB, however, did not differ between the groups even though a trend was observed (273±196µg/l vs. 343±153µg/l, hypothermia vs. control, p=0.17) (**Figure 16c**). Nor did the AUC for CKMB showed a significant difference between the groups, even though similar trend was observed (hypothermia 3978± 3083 vs control 5358 ± 2544, p=0.15) (**Figure 16d**). Several patients in both groups had a peak CKMB >500 µg/l which was the upper limit of detection in the analysis. No patient reached the upper limit for Troponin T, which may explain the disparity between the reduction in CKMB vs. Troponin T in the hypothermia group.

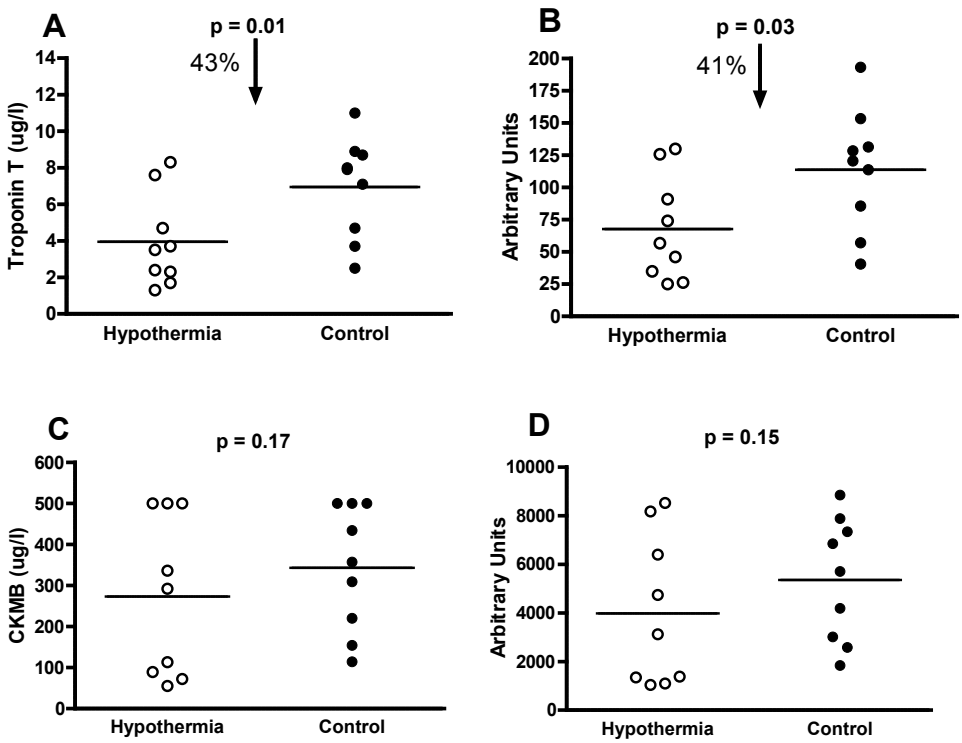


Figure 16. Biochemical marker release. Both peak and cumulative release of Troponin T were significantly reduced in the hypothermia group compared to the control group (a,b). Neither peak, nor cumulative release of CKMB differed significantly between the two groups, even though an trend toward a reduction in the hypothermic group was observed (c,d).

Effect of hypothermia on microvascular obstruction

CMR evaluation allowed the extent of microvascular obstruction to be quantified. In study I, pre-reperfusion hypothermia obliterated microvascular obstruction, whereas it was prevalent in the normothermia group (0% vs. 30.2±5%, p<0.001), (**figure 17**). Post-reperfusion hypothermia reduced the extent of microvascular obstruction with

66% compared to normothermia (10.3±5% vs 30.2±5%, p<0.05), (**figure 17**). Post-reperfusion hypothermia caused a reduction in microvascular obstruction without any concomitant reduction in infarct size.

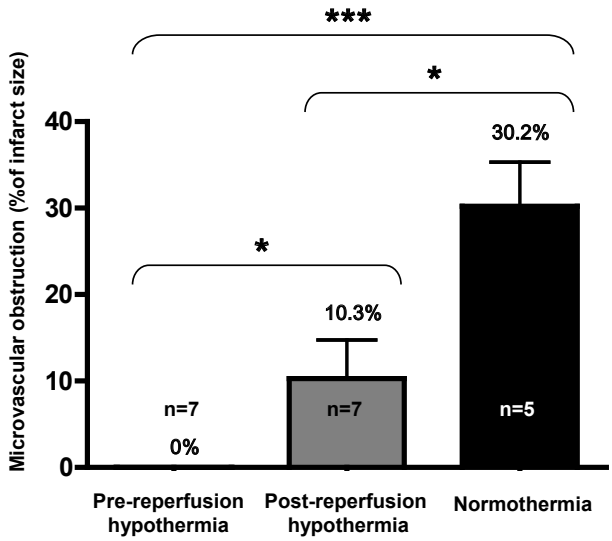


Figure 17. Pre-reperfusion hypothermia obliterates microvascular obstruction compared to normothermia. Post-reperfusion hypothermia reduces the extent of microvascular obstruction compared to normothermia. Values are presented as mean ± S.E.M.

In study II, MO was present in 7 out of 8 pigs in the normothermia group, whereas it was present in only 1 of 8 pigs in the combination hypothermia group. Combination hypothermia reduced the size of microvascular obstruction by 98% compared to normothermia (0.5±0.5 % vs 21.5±5.2 %), p<0.001) (**figure 18a**). Cold saline alone reduced the size of MO by 74% compared to normothermia (5.5±2.5 % vs 21.5±5.2 %), p<0.005) (**Figure 18b**). Thus, both in the post-reperfusion hypothermia group (study I), and in the cold saline group (study II), hypothermia reduced microvascular obstruction without affecting infarct size. This suggests separate mechanisms for infarct development and microvascular obstruction. In the extended hypothermia group, microvascular obstruction was present in 1 out of 8 pigs (0.2±0.2 %). Extended hypothermia reduced microvascular obstruction compared to normothermia (p<0.01) and was similar to shorter active hypothermia (0 %), p>0.05, (**Figure 18c**).

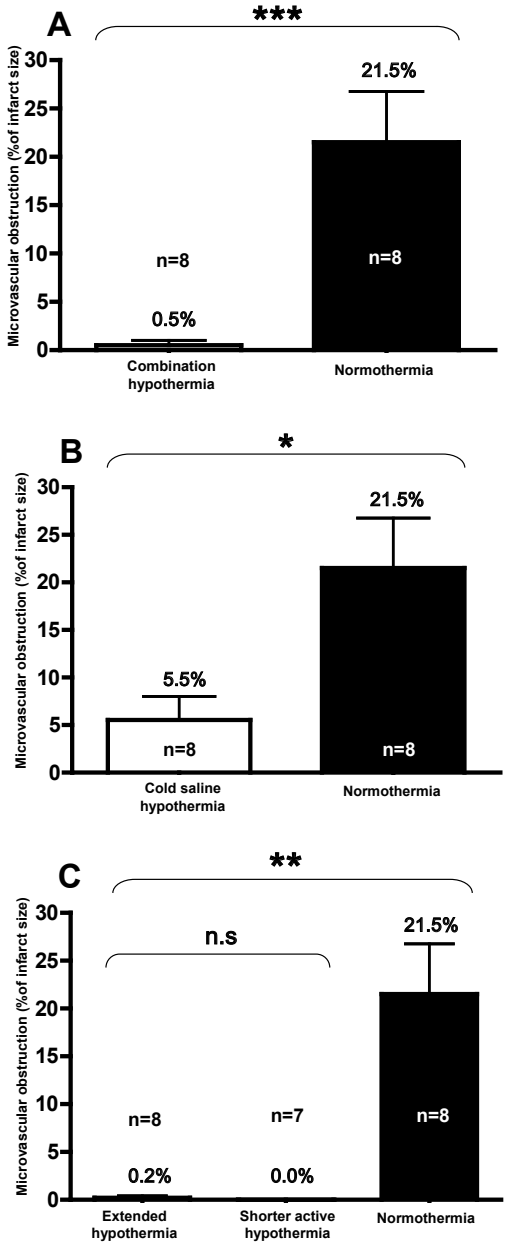


Figure 18. Microvascular obstruction (MO) as a percentage of infarct size (%IS). a) Combination hypothermia significantly reduced MO. b) Cold saline significantly reduced MO, although to a lesser extent compared to combination hypothermia. c) Different duration of hypothermia did not have an impact on the reduction of MO. Data are expressed as mean ± SEM.

In study IV, MO occurred in 4/9 patients in the hypothermia group and 5/9 patients in the control group. The size of microvascular obstruction was 0.8±1.5% vs. 1.9±3.4%, hypothermia vs. control, p=0.24, and did not differ between the groups. The extent of microvascular obstruction was lower in the control group in study IV compared to in study I and II.

Effect of hypothermia in cardiogenic shock

Using an endovascular cooling catheter, the effects of hypothermia in cardiogenic shock was studied (study I). A total of 25 pigs were studied. Five pigs died during ischemia before randomization due to intractable ventricular fibrillation or pulseless electrical activity despite repeated resuscitation attempts and where excluded from the study. Four pigs were excluded due to failure to meet the criterion for cardiogenic shock (< 90 mm Hg during at least 15 minutes). The occurrence of ventricular tachycardia/fibrillation during ischemia and at the onset of reperfusion was recorded. VT/VF occurred in 4/8 pigs in the hypothermia group and in 5/8 pigs in the normothermia group.

Survival

From the time of reperfusion until the end of experiment, 5/8 pigs in the normothermia group died while none of the pigs in the hypothermic group died (p<0.01). (**Figure 19**) The pigs in the normothermia group died at a mean time of 1h 53 ± 38 minutes after onset of reperfusion. All pigs died due to progressive circulatory failure, no pigs included in the study died due to arrhythmias.

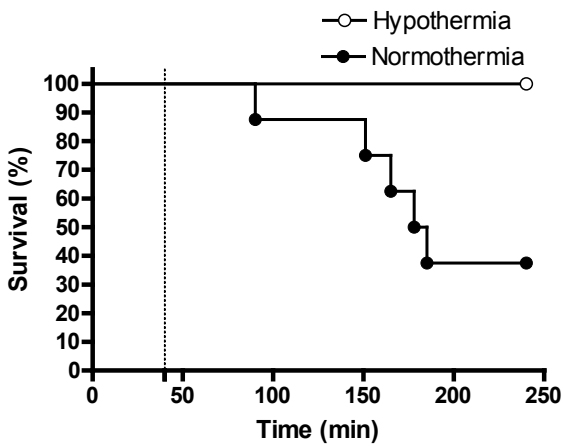


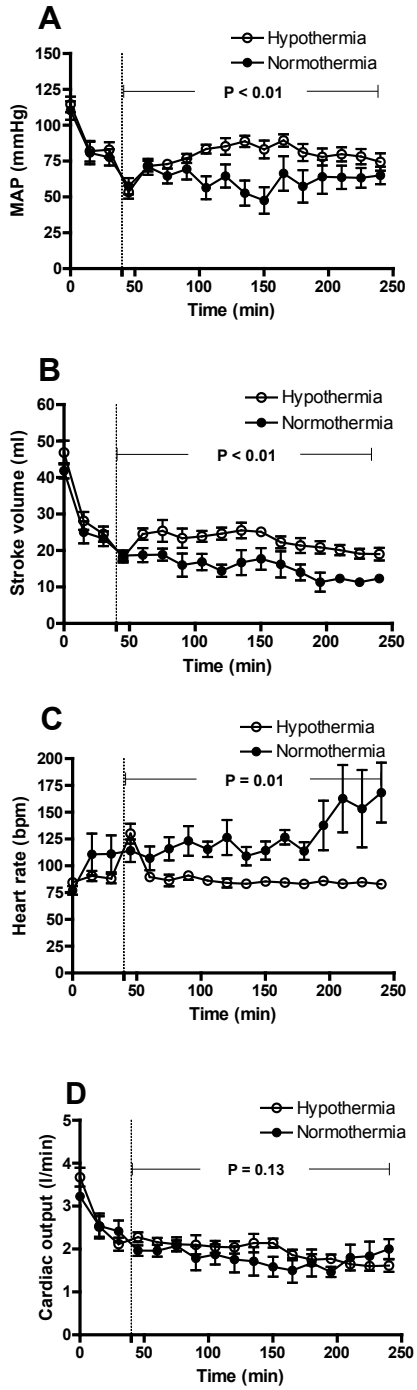
Figure 19. Kaplan-Meier curve displaying the outcome among the groups. Approximately 150 min after onset of reperfusion, 5/8 pigs in the normothermia group had died due to circulatory failure while none of the pigs in the hypothermia group died during the experiment (p<0.01).

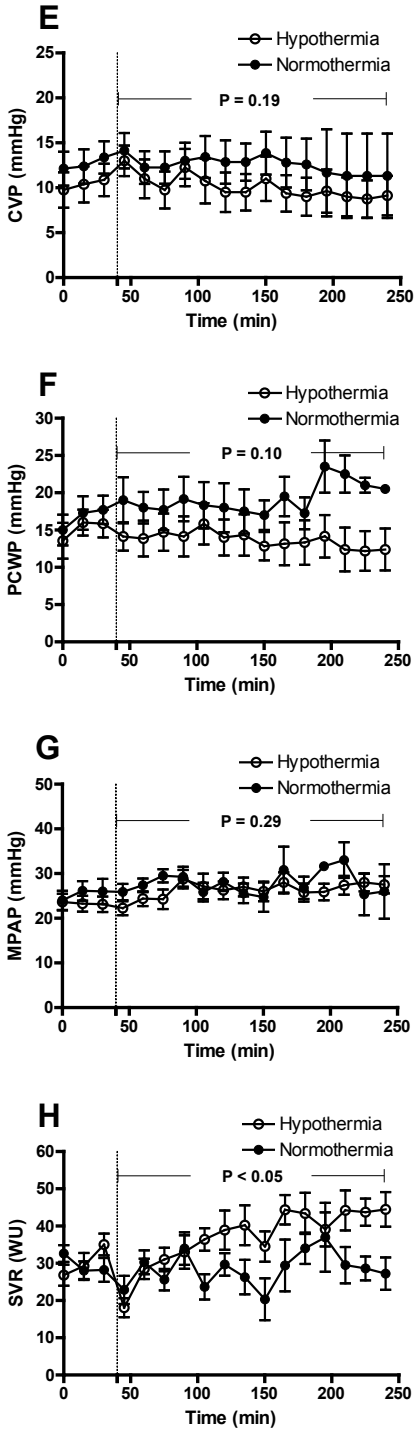
Hemodynamic measurements

Outcome of hemodynamic and blood gas variables are shown in table 3. Heart rate and mean arterial pressure were continuously recorded. Furthermore, using a Swan-Ganz-catheter, recordings of hemodynamic parameters were performed. Hypothermia resulted in an increase in mean arterial pressure and stroke volume in the hypothermia group compared to normothermia (**Figure 20a-b**). Heart rate in the hypothermia group was lower and with less variability compared to normothermia (**Figure 20c**). Cardiac output did not differ among the groups (**Figure 20d**). Furthermore, central venous pressure and pulmonary capillary wedge pressure did not differ among the groups (**Figure 20e-f**). Systemic vascular resistance was higher in the hypothermia group, whereas mean pulmonary artery pressure and pulmonary vascular resistance did not differ among the groups (**Figure 20g-i**).

Table 3. Hemodynamic and blood gas variables Data are presented as mean ± SD.

Variable	Hypothermia (n=8)	Normothermia (n=8)	P-value
MAP (mmHg)	78 ± 9	61 ± 4	$p < 0.01$
Stroke volume (ml)	22.5 ± 3.5	16.6 ± 4.0 ml	$p < 0.01$
Heart rate (bpm)	89 ± 9	119 ± 28	$p = 0.01$
Cardiac output (l/min)	2.0 ± 0.2	1.8 ± 0.4 l/min	$p = 0.13$
CVP (mmHg)	10 ± 6	12 ± 5	$p = 0.19$
PCWP (mmHg)	13 ± 6	18 ± 6	$p = 0.10$
MPAP (mmHg)	26 ± 5	28 ± 4	$p = 0.29$
SVR (WU)	37 ± 9	28 ± 8	$p = 0.03$
PVR (WU)	6.9 ± 1.4	6.9 ± 2.2	$p = 0.50$
SvO_2 (%)	30 ± 10	16 ± 9	$p < 0.01$
Arterial pH	7.49 ± 0.04	7.36 ± 0.09	$p < 0.001$
Arterial pO_2 (kPa)	22.0 ± 4.1	18.1 ± 5.1	$p = 0.07$
Arterial pCO_2 (kPa)	4.9 ± 0.3	6.1 ± 1.9	$p = 0.04$
Arterial BE (mmol/l)	5.1 ± 1.6	-1.0 ± 4.9	$p = 0.002$


A
Hypothermia
Normothermia
P < 0.01
MAP (mmHg)
Time (min)
B
Hypothermia
Normothermia
P < 0.01
Stroke volume (ml)
Time (min)
C
Hypothermia
Normothermia
P = 0.01
Heart rate (bpm)
Time (min)
D
Hypothermia
Normothermia
P = 0.13
Cardiac output (l/min)
Time (min)


E
Hypothermia
Normothermia
P = 0.19
CVP (mmHg)
Time (min)
F
Hypothermia
Normothermia
P = 0.10
PCWP (mmHg)
Time (min)
G
Hypothermia
Normothermia
P = 0.29
MPAP (mmHg)
Time (min)
H
Hypothermia
Normothermia
P < 0.05
SVR (WU)
Time (min)

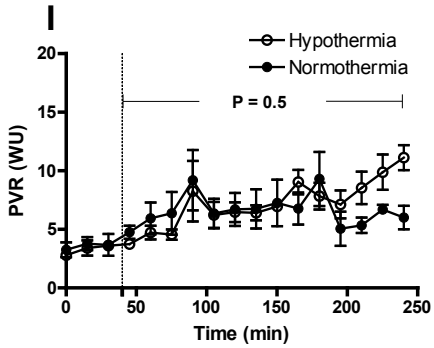
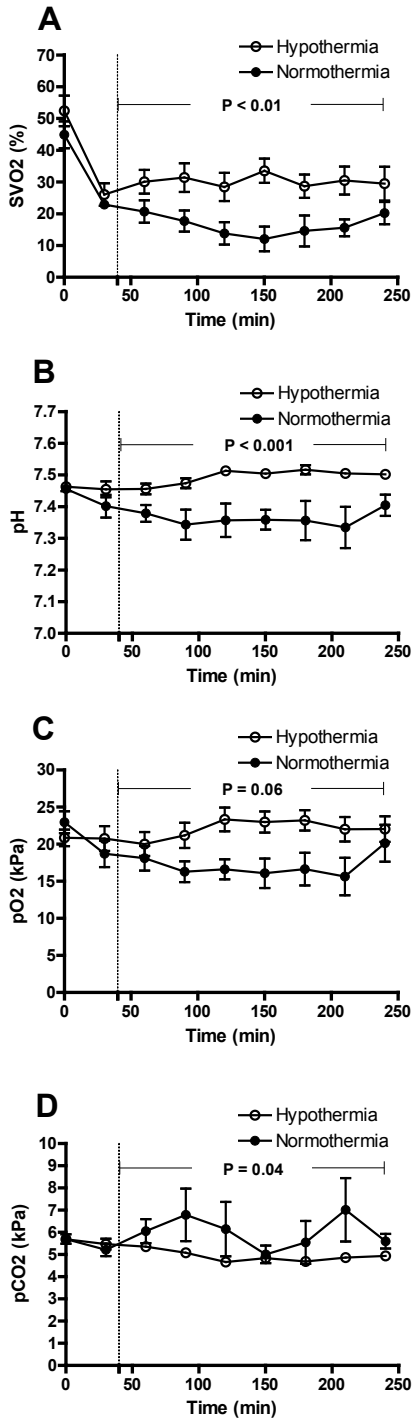


Figure 20. The dotted line illustrates the time of randomization. Please note that in the normothermia group, only three pigs survived beyond 190 min. Mean values in the normothermia group beyond this time point should be interpreted with caution. a) Mean arterial pressure was significantly higher in the hypothermia group ($p<0.01$). b) Stroke volume was significantly higher in the hypothermia group ($p<0.001$). c) Heart rate was significantly lower with less variability in the hypothermia group ($p=0.01$). d) Cardiac output did not differ among the groups ($p=0.13$). e) Central venous pressure did not differ among the groups ($p=0.19$). f) Pulmonary capillary wedge pressure did not differ among the groups ($p=0.10$). g) Mean pulmonary artery pressure did not differ among the groups ($p=0.29$). h) Systemic vascular resistance was significantly higher in the hypothermia group ($p<0.05$). i) Pulmonary vascular resistance did not differ among the groups ($p=0.50$). Data are expressed as mean ± S.E.M.

Blood gas measurements

Arterial blood-gas samples and mixed venous saturation were recorded every 30 minutes. Mixed venous saturation was higher in the hypothermia group indicating lower peripheral oxygen consumption (**Figure 21a**). Arterial pH was significantly higher in the hypothermia groug (**Figure 21b**). PO_2 did not differ between the groups (**Figure 21c**). Furthermore, pCO_2 was lower in the hypothermia group (**Figure 21d**). PCO_2 often became deranged in the normothermic pigs prior to death due to circulatory failure affecting gas exchange in the lungs. This is reflected in the large variations in pCO_2 seen. Finally, base excess was negative and significantly lower in the normothermia group indicating less development of metabolic acidosis in the hypothermia group (**Figure 21e**).


A
Hypothermia
Normothermia
P < 0.01
SVO2 (%)
Time (min)
B
Hypothermia
Normothermia
P < 0.001
pH
Time (min)
C
Hypothermia
Normothermia
P = 0.06
pO2 (kPa)
Time (min)
D
Hypothermia
Normothermia
P = 0.04
pCO2 (kPa)
Time (min)

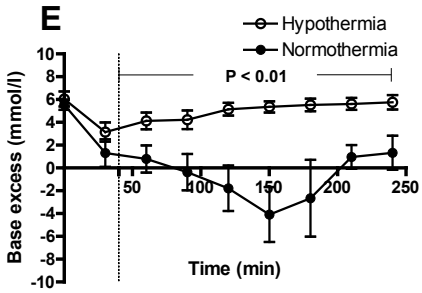


Figure 21. The dotted line illustrates the time of randomization. Please note that in the normothermia group, only three pigs survived beyond 190 min. Mean values in the normothermia group beyond this time point should be interpreted with caution. a) Mixed venous saturation was significantly higher in the hypothermia group indicating lower metabolic demand in peripheral tissue ($p<0.01$). b) Arterial pH was significantly lower, indicating no development of metabolic acidosis in the hypothermia group ($p<0.001$). c) PO2 did not differ among the groups ($p=0.06$) d) PCO2 was significantly lower in the hypothermia group indicating less oxygen demand ($p=0.04$). e) Base excess was significantly lower in the normothermia group indicating development of metabolic acidosis due to the cardiogenic shock ($p<0.01$). Data are expressed as mean ± S.E.M.

10 Main findings

I. Combination hypothermia (an infusion of cold saline and endovascular cooling catheter) causes a rapid induction of hypothermia, achieving a core body temperature of <35°C in < 5 min.

II. Combination hypothermia induced before reperfusion reduces infarct size and microvascular obstruction.

III. Combination hypothermia induced before reperfusion during prolonged ischemic time reduces infarct size and microvascular obstruction indicating an effect on reperfusion injury per se.

IV. Combination hypothermia induced at the onset of reperfusion reduces microvascular obstruction but not infarct size.

V. An infusion of cold saline alone before reperfusion has a rapid rebound in temperature and reduces microvascular obstruction but not infarct size.

VI. Prolonging active hypothermia treatment beyond 15 min post-reperfusion does not confer any additional benefit.

VII. Combination hypothermia is safe and feasible in patients with acute myocardial infarction.

VIII. Combination hypothermia reduces infarct size by 38% in patients with acute myocardial infarction.

IX. Hypothermia improves survival, improves hemodynamic parameters and reduces acidosis in cardiogenic shock.

11 Discussion

Efficacy of combination hypothermia in reaching and maintaining hypothermia

Two clinical trials (COOL-MI and ICE-IT) investigating therapeutic hypothermia in patients with acute myocardial infarction failed to reduce infarct size.[46, 47] Furthermore, post hoc analysis of the data from the clinical trials indicated that only a minority of the patients were cooled to a temperature <35°C before onset of reperfusion, and in that subgroup of patients, a significant reduction in infarct size was demonstrated. The indication from an experimental study and the clinical trials was that it took approximately 45 min to cool subjects below 35°C.[45-47] By combining two different cooling methods (an infusion of cold saline together with an endovascular cooling catheter), we aimed to achieve a more rapid induction of hypothermia than with endovascular cooling alone. Indeed, in study I, core body temperature was reduced from 38°C to <35°C in 5-10 min. In study II, the infusion rate of cold saline was further increased, and a reduction in temperature to <35°C was now achieved in 5 min. Cold saline alone could rapidly reduce core body temperature, but was not able to sustain hypothermia. A rebound in temperature was seen as soon as the infusion of cold saline was discontinued.

In order for hypothermia to be induced and maintained properly, shivering needs to be maintained. The animals in the experimental studies were sedated using fentanyl and thiopental together with N_2O. Shivering was further maintained using intermittent bolus doses of thiopental. In study IV, the combination hypothermia protocol was to be investigated in awake patients with acute myocardial infarction. Awake patients are more prone to shiver when subject to hypothermia. If no anti-shivering drug would be administered, then the efficiency of the hypothermia method would be diminished due to heat generation from shivering. Meperidine and buspirone were chosen to suppress shivering since they act synergistically without causing additive respiratory depression.[69]. The same anti-shivering protocol was utilised in the clinical trials with a high tolerability to hypothermia treatment.[46, 47] In study IV, hypothermia was well tolerated, and treatment was not discontinued in any patients due to shivering. The combination hypothermia protocol reduced core body temperature to <35°C in all patients without significantly delaying reperfusion therapy. The cold saline infusion caused a rapid induction of hypothermia synergistically with the endovascular cooling catheter.

Safety of hypothermia

Endovascular cooling alone has previously been proven to be safe and well tolerated in patients with acute myocardial infarction.[46, 47, 70] By introducing an infusion of cold saline as part of the hypothermia regimen there is a theoretical risk of fluid overloading and pulmonary oedema. In study IV, no increase in acute heart failure in the hypothermia group was seen. Furthermore, the groups had similar NT-proBNP values at day one indicating that the administration of cold saline did not adversely affect left ventricular load the day after intervention. Finally, the clinical event rate was low and did not differ between the groups. Three patients in the hypothermia group were treated with antibiotics due to infections. However, the difference to the control group was not statistically significant. In the HACA-trial, investigating hypothermia in comatose survivors after cardiac arrest, a non-significant trend towards an increase in septicaemia and pneumonia was seen.[71] This trend was not seen in another large hypothermia trial, nor in a hypothermia registry.[72, 73] Whether the short duration of hypothermia treatment in awake patients carries an increased risk of infections remains to be established.

Effect of hypothermia on infarct size

The scientific foundation for performing the clinical trials (COOL-MI and ICE-IT) at the time was animal data indicating that hypothermia reduced infarct size.[41-43, 45, 74, 75] However, in these studies, hypothermia was induced and maintained during a substantial portion of the ischemic period, limiting clinical applicability. Furthermore, the analysis from the negative clinical trials indicated that only in the subgroup of patients that were cooled before reperfusion, a reduction in infarct size was seen. Thus, based on these results, we aimed to investigate whether hypothermia needed to be induced during ischemia, or could be initiated after onset of reperfusion (study I). We found a reduction in infarct size in the pre-reperfusion hypothermia group, but not in the post-reperfusion hypothermia group. We thus confirmed the results by Maeng and co-workers that inducing hypothermia after the onset of reperfusion using a coronary perfusion catheter did not reduce infarct size. In study I, hypothermia was induced after 25 min of 40 min of ischemia time. Since the efficiency of the combination hypothermia protocol achieved such a rapid reduction in infarct size, we then aimed to investigate whether the protective properties of hypothermia were attributed to a reduction in myocardial injury during ischemia or due to attenuation of reperfusion induced myocardial injury. In the protocol for study II, reperfusion was delayed by 5 min in the combination hypothermia group in order to induce hypothermia. A reduction in infarct size of 18% was seen despite prolonged ischemia. The results confirmed the existence of lethal reperfusion injury, and that hypothermia could attenuate the effects of the process. We also speculated whether cold saline alone would affect infarct size. Possibly, this could have been a poor man's way of inducing hypothermia. However,

cold saline alone did not reduce infarct size in this setting. Finally, extending active hypothermia treatment beyond 15 min past reperfusion did not confer any additional benefit. These findings could be important for the design of new clinical hypothermia trials. The previous studies used 3 or 6 hours post-reperfusion hypothermia.[46, 47] During hypothermia treatment, the patient is mildly sedated and immobilized. Prolonged immobilization could potentially lead to complications such as infections, thrombosis or bleeding. Since there was no benefit of prolonging the post-reperfusion time from 15 to 60 min, it would probably be possible to limit the duration of active post-reperfusion hypothermia time to 1 h (instead of 3-6 h) in a future clinical trial. This would simplify the protocol, would be more comfortable for the patient, and could possibly reduce complications.

In study IV, a 38% significant reduction in infarct size normalized to myocardium at risk was seen in patients with acute myocardial infarction who were treated with hypothermia. There was a clear trend, although statistically not significant for a reduction in infarct size alone (uncorrected for myocardium at risk). This reflects the importance and strength of being able to assess infarct size in relation to myocardium at risk in order to reduce interindividual variability in infarct size. By using CMR for assessment of myocardium at risk, the sample size in a future clinical trial could thus be reduced. CMR assessment of myocardium at risk is a novel method which only recently has been validated.[66]

The observed reduction in infarct size measured by CMR was further supported by the reduction in Troponin T release. A European multi-centre clinical trial (CHILL-MI) is planned to verify the treatment effect observed in the pilot study (study IV).

Effect of hypothermia on microvascular obstruction

Microvascular obstruction (MO) is the area within the infarct with impaired microvascular reperfusion. The occurrence and extent of MO is correlated to the extent of the myocardial infarction.[38] The cellular alterations associated with microvascular obstruction consist of swollen endothelium with intraluminal protrusions, tightly packed erythrocytes and increased neutrophil adherence.[76, 77] These ultrastructural changes within the infarcted myocardium makes it more difficult to re-establish coronary flow to the myocytes. MO has also shown to be associated with a worse clinical long term outcome independently of infarct size.[39, 40, 78] A reduction in microvascular obstruction could therefore hypothetically be beneficial. Hale and co-workers have previously demonstrated that hypothermia during ischemia reduced microvascular obstruction in a rabbit model.[79] In study I, pre-reperfusion hypothermia caused a reduction in microvascular obstruction to a greater extent that a reduction in infarct size. Furthermore, post-reperfusion hypothermia caused a marked reduction in microvascular obstruction without any associated reduction in infarct size. In study II, cold saline hypothermia failed to reduce infarct size, but a significant reduction in microvascular obstruction was observed. The experimental data suggests disparate mechanisms behind infarct size

development and MO. Furthermore, it links MO to the injury incurred upon the microvasculature during reperfusion. In study IV, no significant reduction in microvascular obstruction was seen. There could be several potential reasons for the difference in reduction in infarct size and MO when comparing the experimental studies to the clinical trial. Hypothetically, a species based difference in the development of MO between man and pig could explain the observed difference. Secondly, the control animals in the experimental studies had a larger uncorrected infarct size (31% of left ventricular mass) compared to the control patients 21% of left ventricular mass). Since the extent of MO is correlated with the extent of the infarct size, the smaller infarct size in the control group in the clinical study could explain the difference in the results. Whether hypothermia will reduce the extent of MO in patients with AMI and the clinical relevance of the potential finding needs to be investigated further in the planned multi-centre trial.

Effect on hypothermia on infarct appearance

In study I, in the pre-reperfusion hypothermia group, a patchy appearance of the myocardial infarcts in the animals was observed. In the post-reperfusion hypothermia and normothermia groups, the infarcts were more homogeneous in appearance. (**Figure 22**) In study II, some of the infarcts in the combination hypothermia group displayed the same phenomenon, although to a lesser extent compared to extended hypothermia, or to pre-reperfusion hypothermia (study I). In study IV, three of the patients in the hypothermia group displayed a similar patchy appearance as seen in the experimental studies. (**Figure 23**) Dae and co-workers described scattered islands of reduced Sestamibi uptake in pig hearts when assessing infarct size with SPECT in pigs subject to endovascular hypothermia.[45] It may be the same phenomenon, although described by two different imaging modalities. The infarct appearance differs from the classical wave-front pattern in infarct progression previously described by Reimer and Jennings.[16] The long term significance of the observed patchy infarct pattern is not known.

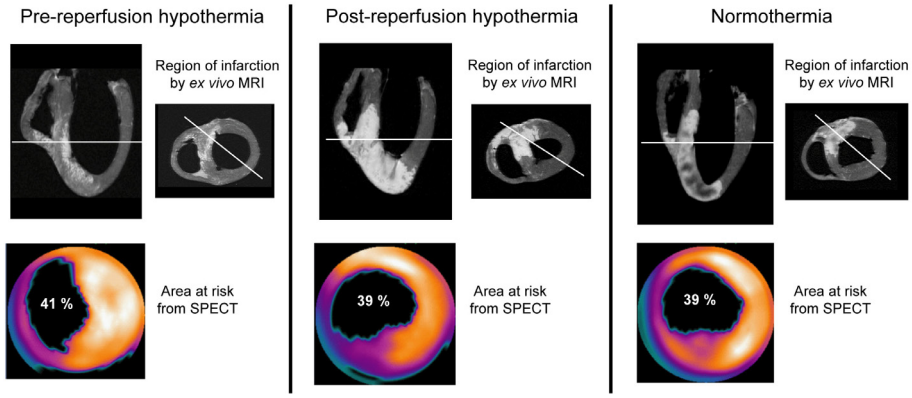


Figure 22. This figure illustrates the visual comparison between typical examples of region of infarction from ex vivo MRI from the respective groups. White lines denote the slice position of the two ex vivo MRI slices in relation to each other. Note the patchy pattern of the myocardial infarction in the MRI image from the pre-reperfusion hypothermia group. In the MRI images from the post-reperfusion hypothermia group and most notably, the normothermia group, hypointense zones of microvascular obstruction are seen within the area of infarction.

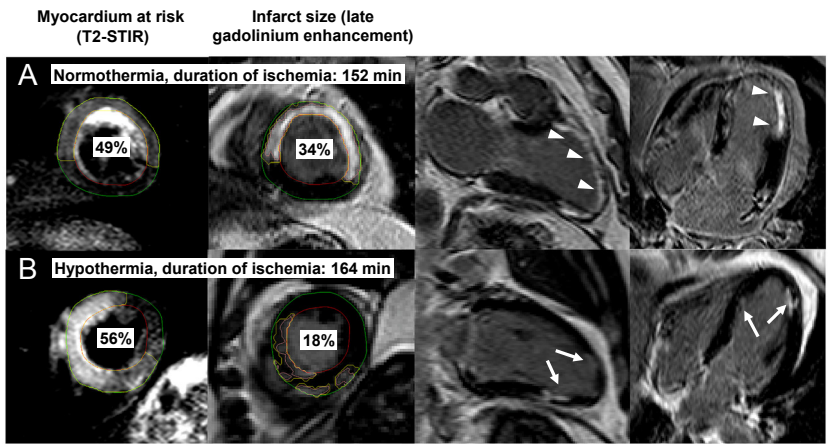


Figure 23. Two patients with infarction due to acute occlusion of the left anterior descending artery. In the **first column** from the left, midventricular T2-STIR short-axis images are shown, where the epicardium is delineated in green, the endocardium in red and the ischemic myocardium in yellow. In the **second column** from the left, corresponding midventricular LGE short-axis images are shown, where the epicardium is delineated in green, the endocardium in red and the infarcted myocardium in pink within the hyper-enhanced region (yellow). The **third and fourth column** show LGE long-axis images in the two-chamber view and four-chamber view, respectively. **A**) A control patient with 152 min between symptom onset and primary PCI performed at normothermia. This patient had a myocardium at risk (MaR) of 49 % of the left ventricle and an infarct size of 34 % of the left ventricle, resulting in 70 % infarct size normalized to MaR. **B**) A patient with 164 min between symptom onset and primary PCI, undertaken after induction of hypothermia. This patient had a MaR of 56 % and an infarct size of 18 %, resulting in 32 % infarct size normalized to MaR. Note the patchy appearance of the infarction in **B** (arrows) compared to the solid transmural infarction in **A** (arrowheads).

Effects of hypothermia in cardiogenic shock

In study III, the effects of hypothermia in experimentally induced cardiogenic shock were investigated. In order to exclude any possible positive effects on infarct size, hypothermia was induced after onset of reperfusion. Furthermore, the infusion of cold saline was excluded in order to exclude differences in volume loading as a potential bias between the groups. Hypothermia resulted in a stabilization of the circulation, prevention of acidosis, and outcome was improved. Previous experimental studies have demonstrated that mild hypothermia increases myocardial contractility in excised heart preparations as well as in the in situ heart. [55-57] The observed positive inotropic effects were not associated with a corresponding increase in myocardial oxygen consumption. [56, 57] The hemodynamic effects in the cardiogenic shock study was similar to the effects observed in other experimental studies without cardiogenic shock (unchanged cardiac output, lowered heart rate, an increase in stroke volume, mean arterial pressure, and in systemic vascular resistance). A notable effect of hypothermia was the observed 85% increase in mixed venous saturation (SvO_2). SvO_2 is a marker of peripheral oxygen consumption. The finding also correlates with the lower pH and base excess seen in the normothermia group. Despite similar cardiac output, no metabolic acidosis was seen in the hypothermia group. One explanation of the observed results could be that hypothermia lowered the peripheral oxygen demand which in turn resulted in less tissue hypoxia and prevention of development of metabolic acidosis. This may also explain the significant difference in acute mortality between the two groups. Furthermore, there was a trend towards increased pO_2, while pCO_2 was significantly lower in the hypothermia group. When hypothermia was induced, in order to keep pCO_2 within normal levels, ventilation had to be systematically reduced by approximately 30-40% reflecting the decrease in metabolism and oxygen demand. No randomized trials evaluating hypothermia in cardiogenic shock have been performed, but two small observational non-randomized studies investigating hypothermia and cardiogenic shock in postoperative patients have been performed.[58, 59] They found that hypothermia resulted in stabilization of circulation with an unchanged or increased cardiac output, an increase in mixed venous O_2 saturation and urine output. The pathogenesis of cardiogenic shock is an imbalance between a diminished cardiac output and unchanged peripheral oxygen tissue consumption resulting in tissue hypoxia with subsequent metabolic acidosis. While hypothermia does have a mild inotropic effect without an increase in cardiac oxygen consumption, the major benefit seems to be in the lowered peripheral oxygen demand and prevention of metabolic acidosis.

Conclusions

This thesis provides novel insight in the protective properties of hypothermia in acute myocardial infarction and in cardiogenic shock. We have demonstrated the importance

of using a combination of a cold saline infusion and an endovascular cooling catheter in achieving a rapid induction of hypothermia. Furthermore, we have found that hypothermia has to be initiated before onset of reperfusion in order to reduce infarct size. We also found that it is safe and feasible to induce hypothermia in awake patients with an acute myocardial infarction without delaying reperfusion, and that hypothermia treatment reduces infarct size by 38%. The results indicate a potential role of hypothermia as an adjunctive therapy in patients with acute myocardial infarction. Finally, hypothermia reduces acute mortality, improves hemodynamic parameters, and reduces metabolic acidosis in cardiogenic shock.

12 Populärvetenskaplig sammanfattning (Summary in Swedish)

Hjärtinfarkt och dess komplikationer är den främsta orsaken till död i västvärlden. Vid en akut hjärtinfarkt stängs blodförsörjningen till en del av hjärtat av pga en blodpropp i ett av hjärtats kranskärl. Den delen av hjärtat som försörjdes av det nu stängda kranskärlet drabbas då av brist på syre och näringsämnen och riskerar att skadas. Skadans storlek på hjärtat beror på hur länge stoppet i kranskärlet varar och hur stor del av hjärtat som det stängda kranskärlet försörjde med blod. Modern hjärtinfarktbehandling syftar till att öppna kranskärlet så fort som möjligt med ballongvidgning (PCI). Ju större skada en hjärtinfarkt orsakar på hjärtat, desto större är också risken att utveckla hjärtsvikt och för död. Ibland är hjärtinfarkten så stor att hjärtat inte orkar pumpa runt blodet i kroppen. Då går kroppen in i ett akut chocktillstånd (kardiogen chock) och ca hälften av alla patienter avlider trots behandling.

Många känner till att människor som drunknat i iskallt vatten kan återupplivas igen. Kylan ifrån vattnet har skyddat kroppen och hjärnan mot den syrebrist som uppkommer vid drunkning. Idag används kontrollerad kylbehandling för att skydda hjärnan hos patienter som överlevt hjärtstopp. De patienterna kyls då ned till 33°C under 24h tid för att skydda hjärnan mot den syrebrist som uppkom då hjärtat stannade. Flera experimentella djurstudier har visat att även skadan på hjärtat i samband med hjärtinfarkt kan minska om man kyler ner hjärtat och/eller hela kroppen. Två kliniska studier på patienter med akut hjärtinfarkt där man kylde ner kroppen med en slang i ett blodkärl via ljumsken har utförts. I de studierna kunde man dock inte se någon minskad hjärtskada vid kylbehandlingen. Vid ytterligare analys fann man dock att de patienter som hann kylas ned till <35°C innan kranskärlet öppnades hade effekt av kylbehandlingen. Ett stort problem i studierna var dock att det tog lång tid att kyla ned patienterna och att fördröja behandling med ballongvidgning skulle också öka skadan på hjärtat.

Övergripande syften med denna avhandling var att studera om kylbehandling skyddar hjärtat vid akut hjärtinfarkt och dess effekter vid kardiogen chock.

I **arbete I** testade vi i en djurmodell om det var viktigt att påbörja kylbehandling före eller efter att det stängda kranskärlet öppnats. Dessutom prövade vi att kombinera två olika kylbehandlingsmetoder för att kunna kyla kroppen snabbare. Vi fann att kylbehandling som påbörjas efter att kärlet öppnats inte hade någon effekt på skadan på hjärtat. Kylbehandling innan kärlet öppnats minskade dock skadan på hjärtat med 39

% mätt med MR-kamera. Vi fann också att kombinationen av ett snabbt dropp av kall vätska tillsammans med kylbehandling via slang i kärl i ljumsken var ett mycket snabbt sätt att få ned kroppstemperaturen.

I **arbete II** studerade vi om kylbehandling kunde minska den skada som uppkommer på hjärtat man öppnar upp blodkärlet (reperfusionsskada). Vi fann att skadan på hjärtat minskade med 18% mätt med MR-kamera. Vi fann även att kylbehandling med kall koksalt i sig inte minskade skadan på hjärtat. Dessutom såg vi att det räckte med att kylbehandla under kort tid efter att kärlet öppnats.

I **arbete III** studerade vi effekterna av kylbehandling vid kardiogen chock. Vi fann att kylbehandling förbättrade överlevnaden vid kardiogen chock. Vi kunde även se att kylbehandlingen hade positiva effekter på blodtryck, hjärtats pumpkraft och minskade kroppens behov av syrgas.

I **arbete IV** som var en klinisk studie testade vi om de positiva effekter på hjärtat som vi sett i **arbete I** och **II** även kunde minska skadan på hjärtat hos patienter med akut hjärtinfarkt. Dessutom testades säkerheten och effektiviteten på att kombinera två kylbehandlingar. Tio patienter med akut hjärtinfarkt som skulle genomgå akut ballongvidgning fick kylbehandling som tillägg till standardbehandling. De patienterna jämfördes i studien med tio patienter som fick standardbehandling. Vi såg att kylbehandlingen inte gav några komplikationer och lyckades på ett effektivt sätt snabbt få ned kroppstemperaturen trots att patienterna var vakna under hela behandlingen. Vi fann även att skadan på hjärtat minskade med 38 % till följd av kylbehandlingen.

Sammanfattningsvis visar avhandlingen när kylbehandling måste initieras för att få effekt, att kombinationen av två olika kylmetoder ger en snabb sänkning av kroppstemperaturen samt att det på ett säkert och effektivt sätt går att applicera detta på patienter med minskad skada på hjärtat som följd. Vi visar dessutom att kylbehandling kan ha positiva effekter vid kardiogen chock. Den minskning av hjärtskadan som vi såg kan om resultaten bekräftas i en större studie ge stora möjligheter att minska följdverkningarna vid denna svåra sjukdom.

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15 References

1. Risk stratification and survival after myocardial infarction. *N Engl J Med.* 1983;309(6):331-336.
2. Burns RJ, Gibbons RJ, Yi Q, Roberts RS, Miller TD, Schaer GL, Anderson JL, Yusuf S. The relationships of left ventricular ejection fraction, end-systolic volume index and infarct size to six-month mortality after hospital discharge following myocardial infarction treated by thrombolysis. *J Am Coll Cardiol.* 2002;39(1):30-36.
3. Miller TD, Christian TF, Hopfenspirger MR, Hodge DO, Gersh BJ, Gibbons RJ. Infarct size after acute myocardial infarction measured by quantitative tomographic 99mTc sestamibi imaging predicts subsequent mortality. *Circulation.* 1995;92(3):334-341.
4. Lamas GA, Pfeffer MA. Left ventricular remodeling after acute myocardial infarction: clinical course and beneficial effects of angiotensin-converting enzyme inhibition. *Am Heart J.* 1991;121(4 Pt 1):1194-1202.
5. Van de Werf F, Arnold AE. Intravenous tissue plasminogen activator and size of infarct, left ventricular function, and survival in acute myocardial infarction. *BMJ.* 1988;297(6660):1374-1379.
6. Naghavi M, Libby P, Falk E, Casscells SW, Litovsky S, Rumberger J, Badimon JJ, Stefanadis C, Moreno P, Pasterkamp G, Fayad Z, Stone PH, Waxman S, Raggi P, Madjid M, Zarrabi A, Burke A, Yuan C, Fitzgerald PJ, Siscovick DS, de Korte CL, Aikawa M, Juhani Airaksinen KE, Assmann G, Becker CR, Chesebro JH, Farb A, Galis ZS, Jackson C, Jang IK, Koenig W, Lodder RA, March K, Demirovic J, Navab M, Priori SG, Rekhter MD, Bahr R, Grundy SM, Mehran R, Colombo A, Boerwinkle E, Ballantyne C, Insull W, Jr., Schwartz RS, Vogel R, Serruys PW, Hansson GK, Faxon DP, Kaul S, Drexler H, Greenland P, Muller JE, Virmani R, Ridker PM, Zipes DP, Shah PK, Willerson JT. From vulnerable plaque to vulnerable patient: a call for new definitions and risk assessment strategies: Part I. *Circulation.* 2003;108(14):1664-1672.
7. Ross R. The pathogenesis of atherosclerosis--an update. *N Engl J Med.* 1986;314(8):488-500.
8. Davies MJ, Thomas AC. Plaque fissuring--the cause of acute myocardial infarction, sudden ischaemic death, and crescendo angina. *Br Heart J.* 1985;53(4):363-373.
9. Malek AM, Alper SL, Izumo S. Hemodynamic shear stress and its role in atherosclerosis. *JAMA.* 1999;282(21):2035-2042.
10. Davies MJ. The pathophysiology of acute coronary syndromes. *Heart.* 2000;83(3):361-366.
11. Maseri A, Chierchia S, Davies G. Pathophysiology of coronary occlusion in acute infarction. *Circulation.* 1986;73(2):233-239.
12. Dahlback B. Blood coagulation. *Lancet.* 2000;355(9215):1627-1632.
13. Braunwald E, Antman EM, Beasley JW, Califf RM, Cheitlin MD, Hochman JS, Jones RH, Kereiakes D, Kupersmith J, Levin TN, Pepine CJ, Schaeffer JW, Smith EE, 3rd, Steward DE, Theroux P, Alpert JS, Eagle KA, Faxon DP, Fuster V, Gardner TJ, Gregoratos G, Russell RO, Smith SC, Jr. ACC/AHA guidelines for the management of patients with unstable angina and non-ST-segment elevation myocardial infarction. A report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Committee on the Management of Patients With Unstable Angina). *J Am Coll Cardiol.* 2000;36(3):970-1062.
14. Lip GY, Gupta J, Khan MM, Singh SP. Recurrent myocardial infarction with angina and normal coronary arteries. *Int J Cardiol.* 1995;51(1):65-71.
15. Gersh BJ, Bassendine MF, Forman R, Walls RS, Beck W. Coronary artery spasm and myocardial infarction in the absence of angiographically demonstrable obstructive coronary disease. *Mayo Clin Proc.* 1981;56(11):700-708.

16. Reimer KA, Lowe JE, Rasmussen MM, Jennings RB. The wavefront phenomenon of ischemic cell death. 1. Myocardial infarct size vs duration of coronary occlusion in dogs. *Circulation.* 1977;56(5):786-794.
17. Bogaert J, Maes A, Van de Werf F, Bosmans H, Herregods MC, Nuyts J, Desmet W, Mortelmans L, Marchal G, Rademakers FE. Functional recovery of subepicardial myocardial tissue in transmural myocardial infarction after successful reperfusion: an important contribution to the improvement of regional and global left ventricular function. *Circulation.* 1999;99(1):36-43.
18. Andersen HR, Nielsen TT, Rasmussen K, Thuesen L, Kelbaek H, Thayssen P, Abildgaard U, Pedersen F, Madsen JK, Grande P, Villadsen AB, Krusell LR, Haghfelt T, Lomholt P, Husted SE, Vigholt E, Kjaergard HK, Mortensen LS. A comparison of coronary angioplasty with fibrinolytic therapy in acute myocardial infarction. *N Engl J Med.* 2003;349(8):733-742.
19. Kastrati A, Mehilli J, Dirschinger J, Schricke U, Neverve J, Pache J, Martinoff S, Neumann FJ, Nekolla S, Blasini R, Seyfarth M, Schwaiger M, Schomig A. Myocardial salvage after coronary stenting plus abciximab versus fibrinolysis plus abciximab in patients with acute myocardial infarction: a randomised trial. *Lancet.* 2002;359(9310):920-925.
20. Keeley EC, Boura JA, Grines CL. Primary angioplasty versus intravenous thrombolytic therapy for acute myocardial infarction: a quantitative review of 23 randomised trials. *Lancet.* 2003;361(9351):13-20.
21. Hedstrom E, Engblom H, Frogner F, Astrom-Olsson K, Ohlin H, Jovinge S, Arheden H. Infarct evolution in man studied in patients with first-time coronary occlusion in comparison to different species - implications for assessment of myocardial salvage. *J Cardiovasc Magn Reson.* 2009;11(1):38.
22. Intravenous streptokinase given within 0-4 hours of onset of myocardial infarction reduced mortality in ISIS-2. *Lancet.* 1987;1(8531):502.
23. Effectiveness of intravenous thrombolytic treatment in acute myocardial infarction. Gruppo Italiano per lo Studio della Streptochinasi nell'Infarto Miocardico (GISSI). *Lancet.* 1986;1(8478):397-402.
24. Califf RM. Ten years of benefit from a one-hour intervention. *Circulation.* 1998;98(24):2649-2651.
25. Franzosi MG, Santoro E, De Vita C, Geraci E, Lotto A, Maggioni AP, Mauri F, Rovelli F, Santoro L, Tavazzi L, Tognoni G. Ten-year follow-up of the first megatrial testing thrombolytic therapy in patients with acute myocardial infarction: results of the Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto-1 study. The GISSI Investigators. *Circulation.* 1998;98(24):2659-2665.
26. Gibson CM, Cannon CP, Murphy SA, Marble SJ, Barron HV, Braunwald E. Relationship of the TIMI myocardial perfusion grades, flow grades, frame count, and percutaneous coronary intervention to long-term outcomes after thrombolytic administration in acute myocardial infarction. *Circulation.* 2002;105(16):1909-1913.
27. Stenestrand U, Lindback J, Wallentin L. Long-term outcome of primary percutaneous coronary intervention vs prehospital and in-hospital thrombolysis for patients with ST-elevation myocardial infarction. *JAMA.* 2006;296(14):1749-1756.
28. Jennings RB, Sommers HM, Smyth GA, Flack HA, Linn H. Myocardial necrosis induced by temporary occlusion of a coronary artery in the dog. *Arch Pathol.* 1960;70:68-78.
29. Hausenloy DJ, Yellon DM. Time to take myocardial reperfusion injury seriously. *N Engl J Med.* 2008;359(5):518-520.
30. Ambrosio G, Tritto II. Lethal Myocardial Reperfusion Injury: Does it Exist, Should We Treat It? *J Thromb Thrombolysis.* 1997;4(1):69-70.
31. Kloner RA. Does reperfusion injury exist in humans? *J Am Coll Cardiol.* 1993;21(2):537-545.
32. Olsson RA, Gregg DE. Myocardial Reactive Hyperemia in the Unanesthetized Dog. *Am J Physiol.* 1965;208:224-230.
33. Hearse DJ, Humphrey SM, Chain EB. Abrupt reoxygenation of the anoxic potassium-arrested perfused rat heart: a study of myocardial enzyme release. *J Mol Cell Cardiol.* 1973;5(4):395-407.
34. Piper HM, Garcia-Dorado D, Ovize M. A fresh look at reperfusion injury. *Cardiovasc Res.* 1998;38(2):291-300.

35. Lemasters JJ, Bond JM, Chacon E, Harper IS, Kaplan SH, Ohata H, Trollinger DR, Herman B, Cascio WE. The pH paradox in ischemia-reperfusion injury to cardiac myocytes. *EXS.* 1996;76:99-114.
36. Vinten-Johansen J. Involvement of neutrophils in the pathogenesis of lethal myocardial reperfusion injury. *Cardiovasc Res.* 2004;61(3):481-497.
37. Yellon DM, Hausenloy DJ. Myocardial reperfusion injury. *N Engl J Med.* 2007;357(11):1121-1135.
38. Lima JA, Judd RM, Bazille A, Schulman SP, Atalar E, Zerhouni EA. Regional heterogeneity of human myocardial infarcts demonstrated by contrast-enhanced MRI. Potential mechanisms. *Circulation.* 1995;92(5):1117-1125.
39. Choi CJ, Haji-Momenian S, Dimaria JM, Epstein FH, Bove CM, Rogers WJ, Kramer CM. Infarct involution and improved function during healing of acute myocardial infarction: the role of microvascular obstruction. *J Cardiovasc Magn Reson.* 2004;6(4):917-925.
40. Wu KC, Zerhouni EA, Judd RM, Lugo-Olivieri CH, Barouch LA, Schulman SP, Blumenthal RS, Lima JA. Prognostic significance of microvascular obstruction by magnetic resonance imaging in patients with acute myocardial infarction. *Circulation.* 1998;97(8):765-772.
41. Duncker DJ, Klassen CL, Ishibashi Y, Herrlinger SH, Pavek TJ, Bache RJ. Effect of temperature on myocardial infarction in swine. *Am J Physiol.* 1996;270(4 Pt 2):H1189-1199.
42. Hale SL, Dave RH, Kloner RA. Regional hypothermia reduces myocardial necrosis even when instituted after the onset of ischemia. *Basic Res Cardiol.* 1997;92(5):351-357.
43. Hale SL, Kloner RA. Myocardial temperature reduction attenuates necrosis after prolonged ischemia in rabbits. *Cardiovasc Res.* 1998;40(3):502-507.
44. Miki T, Liu GS, Cohen MV, Downey JM. Mild hypothermia reduces infarct size in the beating rabbit heart: a practical intervention for acute myocardial infarction? *Basic Res Cardiol.* 1998;93(5):372-383.
45. Dae MW, Gao DW, Sessler DI, Chair K, Stillson CA. Effect of endovascular cooling on myocardial temperature, infarct size, and cardiac output in human-sized pigs. *Am J Physiol Heart Circ Physiol.* 2002;282(5):H1584-1591.
46. Grines CL. Intravascular cooling adjunctive to percutaneous coronary intervention for acute myocardial infarction. *Presented at Transcatheter Cardiovascular Therapeutics. Washington DC, USA; 2004.*
47. O'Neill WW. Cooling as an adjunct to primary PCI for myocardial infarction. . *Presented at Transcatheter Cardiovascular Therapeutics. Washington DC, USA;2004*.
48. Maeng M, Mortensen UM, Kristensen J, Kristiansen SB, Andersen HR. Hypothermia during reperfusion does not reduce myocardial infarct size in pigs. *Basic Res Cardiol.* 2006;101(1):61-68.
49. Holmes DR, Jr., Bates ER, Kleiman NS, Sadowski Z, Horgan JH, Morris DC, Califf RM, Berger PB, Topol EJ. 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.* 1995;26(3):668-674.
50. Califf RM, Bengtson JR. Cardiogenic shock. *N Engl J Med.* 1994;330(24):1724-1730.
51. Goldberg RJ, Gore JM, Alpert JS, Osganian V, de Groot J, Bade J, Chen Z, Frid D, Dalen JE. Cardiogenic shock after acute myocardial infarction. Incidence and mortality from a community-wide perspective, 1975 to 1988. *N Engl J Med.* 1991;325(16):1117-1122.
52. Hochman JS, Sleeper LA, Webb JG, Sanborn TA, White HD, Talley JD, Buller CE, Jacobs AK, Slater JN, Col J, McKinlay SM, LeJemtel TH. 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.* 1999;341(9):625-634.
53. Hochman JS, Buller CE, Sleeper LA, Boland J, Dzavik V, Sanborn TA, Godfrey E, White HD, Lim J, LeJemtel T. Cardiogenic shock complicating acute myocardial infarction--etiologies, management and outcome: a report from the SHOCK Trial Registry. SHould we emergently revascularize Occluded Coronaries for cardiogenic shocK? *J Am Coll Cardiol.* 2000;36(3 Suppl A):1063-1070.

54. Webb JG, Sanborn TA, Sleeper LA, Carere RG, Buller CE, Slater JN, Baran KW, Koller PT, Talley JD, Porway M, Hochman JS. Percutaneous coronary intervention for cardiogenic shock in the SHOCK Trial Registry. *Am Heart J.* 2001;141(6):964-970.
55. Weisser J, Martin J, Bisping E, Maier LS, Beyersdorf F, Hasenfuss G, Pieske B. Influence of mild hypothermia on myocardial contractility and circulatory function. *Basic Res Cardiol.* 2001;96(2):198-205.
56. Suga H, Goto Y, Igarashi Y, Yasumura Y, Nozawa T, Futaki S, Tanaka N. Cardiac cooling increases Emax without affecting relation between O2 consumption and systolic pressure-volume area in dog left ventricle. *Circ Res.* 1988;63(1):61-71.
57. Nishimura Y, Naito Y, Nishioka T, Okamura Y. The effects of cardiac cooling under surface-induced hypothermia on the cardiac function in the in situ heart. *Interact Cardiovasc Thorac Surg.* 2005;4(2):101-105.
58. Moriyama Y, Iguro Y, Shimokawa S, Saigenji H, Toyohira H, Taira A. Successful application of hypothermia combined with intra-aortic balloon pump support to low-cardiac-output state after open heart surgery. *Angiology.* 1996;47(6):595-599.
59. Yahagi N, Kumon K, Watanabe Y, Tanigami H, Haruna M, Hayashi H, Imanaka H, Takeuchi M, Ohashi Y, Takamoto S. Value of mild hypothermia in patients who have severe circulatory insufficiency even after intra-aortic balloon pump. *J Clin Anesth.* 1998;10(2):120-125.
60. Sharir T, Germano G, Waechter PB, Kavanagh PB, Areeda JS, Gerlach J, Kang X, Lewin HC, Berman DS. A new algorithm for the quantitation of myocardial perfusion SPECT. II: validation and diagnostic yield. *J Nucl Med.* 2000;41(4):720-727.
61. Persson E, Carlsson M, Palmer J, Pahlm O, Arheden H. Evaluation of left ventricular volumes and ejection fraction by automated gated myocardial SPECT versus cardiovascular magnetic resonance. *Clin Physiol Funct Imaging.* 2005;25(3):135-141.
62. Ugander M, Heiberg E, Soneson H, Engblom H, Pals Jvd, Erlinge D, Arheden H. A novel method for quantifying myocardial perfusion SPECT defect size by co-registration and fusion with MRI - an experimental ex vivo imaging pig heart study (Abstract). *Swedish Heart Association Scientific Sessions 2008.*
63. Kim RJ, Fieno DS, Parrish TB, Harris K, Chen EL, Simonetti O, Bundy J, Finn JP, Klocke FJ, Judd RM. Relationship of MRI delayed contrast enhancement to irreversible injury, infarct age, and contractile function. *Circulation.* 1999;100(19):1992-2002.
64. Hedström E. *Acute Myocardial Infarction. The relationship between duration of ischaemia and infarct size in humans – Assessment by MRI and SPECT.* [PhD]. Lund, University of Lund; 2005.
65. Heiberg E, Sjogren J, Ugander M, Carlsson M, Engblom H, Arheden H. Design and validation of Segment--freely available software for cardiovascular image analysis. *BMC Med Imaging.*10:1.
66. Carlsson M, Ubachs JF, Hedstrom E, Heiberg E, Jovinge S, Arheden H. Myocardium at risk after acute infarction in humans on cardiac magnetic resonance: quantitative assessment during follow-up and validation with single-photon emission computed tomography. *JACC Cardiovasc Imaging.* 2009;2(5):569-576.
67. Heiberg E, Ugander M, Engblom H, Gotberg M, Olivecrona GK, Erlinge D, Arheden H. Automated quantification of myocardial infarction from MR images by accounting for partial volume effects: animal, phantom, and human study. *Radiology.* 2008;246(2):581-588.
68. Hedstrom E, Engblom H, Frogner F, Astrom-Olsson K, Ohlin H, Jovinge S, Arheden H. Infarct evolution in man studied in patients with first-time coronary occlusion in comparison to different species - implications for assessment of myocardial salvage. *J Cardiovasc Magn Reson.* 2009;11:38.
69. Mokhtarani M, Mahgoub AN, Morioka N, Doufas AG, Dae M, Shaughnessy TE, Bjorksten AR, Sessler DI. Buspirone and meperidine synergistically reduce the shivering threshold. *Anesth Analg.* 2001;93(5):1233-1239.
70. Dixon SR, Whitbourn RJ, Dae MW, Grube E, Sherman W, Schaer GL, Jenkins JS, Baim DS, Gibbons RJ, Kuntz RE, Popma JJ, Nguyen TT, O'Neill WW. Induction of mild systemic hypothermia with endovascular cooling during primary percutaneous coronary intervention for acute myocardial infarction. *J Am Coll Cardiol.* 2002;40(11):1928-1934.

71. Mild therapeutic hypothermia to improve the neurologic outcome after cardiac arrest. *N Engl J Med.* 2002;346(8):549-556.
72. Bernard SA, Gray TW, Buist MD, Jones BM, Silvester W, Gutteridge G, Smith K. Treatment of comatose survivors of out-of-hospital cardiac arrest with induced hypothermia. *N Engl J Med.* 2002;346(8):557-563.
73. Nielsen N, Hovdenes J, Nilsson F, Rubertsson S, Stammet P, Sunde K, Valsson F, Wanscher M, Friberg H. Outcome, timing and adverse events in therapeutic hypothermia after out-of-hospital cardiac arrest. *Acta Anaesthesiol Scand.* 2009;53(7):926-934.
74. Hale SL, Kloner RA. Myocardial temperature in acute myocardial infarction: protection with mild regional hypothermia. *Am J Physiol.* 1997;273(1 Pt 2):H220-227.
75. Hale SL, Kloner RA. Ischemic preconditioning and myocardial hypothermia in rabbits with prolonged coronary artery occlusion. *Am J Physiol.* 1999;276(6 Pt 2):H2029-2034.
76. Kloner RA, Giacomelli F, Alker KJ, Hale SL, Matthews R, Bellows S. Influx of neutrophils into the walls of large epicardial coronary arteries in response to ischemia/reperfusion. *Circulation.* 1991;84(4):1758-1772.
77. Engler RL, Schmid-Schonbein GW, Pavelec RS. Leukocyte capillary plugging in myocardial ischemia and reperfusion in the dog. *Am J Pathol.* 1983;111(1):98-111.
78. Ito H, Maruyama A, Iwakura K, Takiuchi S, Masuyama T, Hori M, Higashino Y, Fujii K, Minamino T. Clinical implications of the 'no reflow' phenomenon. A predictor of complications and left ventricular remodeling in reperfused anterior wall myocardial infarction. *Circulation.* 1996;93(2):223-228.
79. Hale SL, Dae MW, Kloner RA. Hypothermia during reperfusion limits 'no-reflow' injury in a rabbit model of acute myocardial infarction. *Cardiovasc Res.* 2003;59(3):715-722.

16 Appendix: Original Papers I-IV